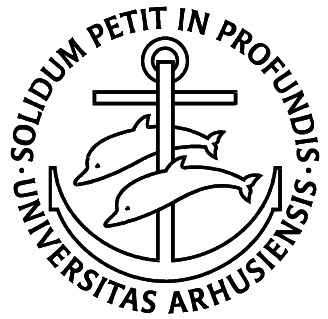
Osseointegrated implants for transfemoral amputees

Evaluation of migration, bone mineral density and bone turn-over markers

PhD thesis

Rehne Lessmann Hansen



Faculty of Health Sciences
University of Aarhus
2016

Supervisors

Main supervisor
Kjeld Søballe, Professor, MD, DMSc
Department of Orthopaedic surgery
Aarhus University Hospital, Aarhus, Denmark

Co-supervisors
Maiken Stilling, Associate Professor, MD, PhD
Department of Clinical Medicine
Aarhus University Hospital, Aarhus, Denmark

Jørgen Peter Holmberg Jørgensen, MD, DMSc
Department of Orthopaedic surgery
Aarhus University Hospital, Aarhus, Denmark

Bente Lomholt Langdahl, Professor, MD, PhD, DMSc,
Department of Endocrinology and Internal Medicine
Aarhus University Hospital, Aarhus, Denmark

Evaluation committee

Erik Fink Eriksen, Professor, MD, DMSc
Department of Endocrinology
Oslo University Hospital, Oslo, Norway

Henrik Malchau, Professor, MD, PhD
Harvard Medical School
Massachusetts General Hospital, Boston, Massachusetts, USA

Sten Rasmussen, MD, PhD (Chairman)
Orthopaedic Surgery Research Unit
Aalborg University Hospital, Aalborg, Denmark

Correspondence

Rehne Lessmann Hansen, MD
Department of Orthopaedic surgery
Aarhus University Hospital, Aarhus, Denmark
Tage-Hansens Gade 2, Building 9A
DK-8000 Aarhus C
E-mail: rehnhans@rm.dk or rehnehansen@gmail.com

Preface

My gratitude goes to the patients who generously offered their time and made this project possible. To my four supervisors, I am most indebted for your guidance given in all aspects of clinical research and inspiring me to become a better scientist. I am grateful for Kjeld Søballe's positive attitude and for giving me the liberty and working conditions needed to conduct my research. I am especially grateful for the guidance given by Maiken Stilling and her expert knowledge on the methods used and for the helpful interpretations in all studies in the thesis. The discussions with Maiken and her thorough feedback helped me to gain insight into difficult research questions and motivated me to continue forward. The precise scientific advice and reflections offered by Bente Lomholt Langdahl was helpful in understanding the complexities in clinical research. The insight in the field of osseointegration implant surgery offered by Peter Holmberg Jørgensen was invaluable. It was inspiring to work together with Peter and Klaus Kjær Petersen due to their meticulous surgical techniques and attentive patient care. I am thankful for the assistance and guidance with microdialysis given by Hanne Birke Sørensen. My gratitude goes to the research staff at the Orthopaedic Research Unit, Aarhus University Hospital. The assistance offered by Inger Krog-Mikkelsen in managing the logistics and securing that things ran smoothly were essential for the completion of the studies. Rikke Mørup, Lone Løvgren Andersen and Tina Stenumgaard were helpful with all kinds of tasks and were always great company. I wish to thank Anette Baatrup and Jane Pauli from the Orthopaedic Research Laboratory for their assistance with various tissue samples. Lars Lindgren and Mark Jensen from the Department of Radiology, Aarhus University hospital, are thanked for their skills to secure great RSA images. I am thankful to Aparna Udupi from the Biostatistical Advisory Service as her deep understanding with statistical methods was invaluable to analyse the dataset. I wish to thank my fellow PhD students Peter Bo Jørgensen for always helping out when needed and Mette Holm Hjorth for our discussions. You are both great company.And what would I have done without Jeppe Lange showing me the wonderful world of clinical research six years ago and introducing me to Kjeld Søballe.

The support from my family was needed during this marathon and thank you for helping out. Most of all, I wish to thank my wife, Signe Haltou Kristensen for her constant support and making it possible for me to achieve my goals. And to our beloved children Bjarke and Asbjørn for keeping my mind off research when at home and enjoying a wonderful playtime.

Finally, I wish to acknowledge and thank Karen Elise Jensens Fond, Spydspidspuljen, Vanførefonden, Ortopædkirurgisk Forskningsfond for supporting this study financially.

16-12-2016

List of papers

I. Bone Mineral Density Measurements around Osseointegrated Implants: A Precision Study and Validation of Scan Protocol for Transfemoral Amputees. Manuscript submitted to Journal of Clinical Densitometry.

II. Loss of periprosthetic bone mineral density and higher PTH is associated with removal of osseointegrated implants in femoral amputees. Manuscript in preparation.

III. Higher migration of removed compared with non-removed osseointegrated implants for transfemoral amputees. A prospective 2 year RSA study with 5-years clinical follow-up. Manuscript submitted to Acta Orthopaedica.

IV. The effect of denosumab on periprosthetic bone mineral density in six transfemoral amputees treated with osseointegrated implants: The observations from a terminated randomized controlled trial. Manuscript in preparation.

Contents

1. English summary 1
2. Danish summary 2
3. Introduction 4
The osseointegration concept 4
The osseointegrated implant 5
The integral leg prosthesis 6
Transfemoral amputees before and after OI surgery 6
Bone changes before and after OI surgery 7
Bone loss and implant loosening 8
Complications and implant removals 11
Bone resorption and antiresorptive therapy 12
Biochemical assessment of bone formation and resorption 13
Radiographic assessment of bone and implant stability 14
4. Aim of the thesis 17
5. Materials & methods 18
Ethical issues 18
Patients 18
Intervention and outcomes 22
Sample size 29
Statistics 30
6. Summary of results 33
Study I 33
Study II 35
Study III 39
Study IV 40
7. Conclusion 45
8. Discussion of results 46
9. Discussion of methods 51
10. Perspectives and future research 54

Abbreviations

BASP	Bone-specific alkaline phosphatase
BLUPS	Best linear unbiased predictors
BMC	Bone mineral content
BMD	Bone mineral density
Ca	Calcium
CAD	Computer-aided-design
CI	Confidence interval
CN	Condition number
CV	Coefficient of variation
CTX	C-terminal telopeptide of type I collagen
ILP	Integral leg prosthesis
LSC	Least significant change
MBL	Marginal bone loss
MD	Microdialysis
NRI	Non-removed implants
NTX	N-terminal telopeptide of type I collagen
OC	Osteocalcin
OI	Osseointegration
OR	Odds ratio
OPG	Osteoprotegerin
OPRA	Osseointegrated prostheses for the Rehabilitation of Amputees
P1CP	C-terminal Propeptide of type I Procollagen
P1NP	N-terminal Propeptide of type I Procollagen
PE	Polyethylene
PTH	Parathyroid hormone
RANK	Receptor activator of nuclear factor kB
RANKL	Receptor activator of nuclear factor kB ligand
RCT	Randomized clinical trial
RE	Reversed engineered
RI	Removed implants
RMS	Root mean square
ROI	Region of interest
RR	Relative recovery
RSA	Radiostereometric Analysis
S1	Stage 1
S2	Stage 2
SD	Standard deviation
TFA	Transfemoral amputee
TR	Total rotations
TT	Total translations

1. English summary

The osseointegration (OI) implant system is a highly-specialised treatment option for transfemoral amputees (TFAs) suffering from complications with the prosthetic socket. The system comprises a bone-anchored implant (fixture) in the residual femur connected to the external prosthetic leg via a percutaneous metal rod (abutment). Numerous papers on the subject report better mobility, improved prosthetic use and quality of life after OI implant surgery, but also find a high risk of soft tissue infection in relation to the percutaneous abutment and a risk of implant loosening. Only limited research has investigated the underlying causes leading to OI implant removal. Thus, the overall aim of this thesis was to evaluate the radiographic and biochemical changes in TFAs with OI implants using four distinct methods: dual energy x-ray absorptiometry (DXA), radiostereometric analysis (RSA), serum markers of bone turnover/metabolism and microdialysis (MD).

We validated a DXA scan protocol in order to determine the precision of periprosthetic bone mineral density (BMD) measurements (Study I). We found that *ex vivo* simulated hip flexion and rotation affected periprosthetic BMD measurements and that the *in vivo* precision was acceptable. We concluded that adhering to the DXA scan protocol secured limited movement of the amputated leg and reduced the measurement error.

We investigated the BMD in the spine, hips and adjacent to the OI implant as well as the serum bone turnover markers in a prospective TFA cohort (Study II). Eight out of 20 OI implants (5 fixtures and 3 abutments) were removed during the 30-month follow-up. We found that periprosthetic BMD loss, increase in C-terminal telopeptide of type I collagen (CTX) and elevated parathyroid hormone (PTH) was associated with OI implant removal. We concluded that biochemical deficiencies affecting bone metabolism should be corrected before and monitored after OI surgery.

The migration pattern of the OI implants were investigated in a prospective cohort using model-based RSA (Study III). We found that non-removed OI implants had a stable fixation, whereas later removed OI implants migrated continuously. Additionally, distal OI implant migration increased the odds of implant removal.

Using both DXA, model-based RSA, serum bone markers and MD, we set out to investigate the effect of antiresorptive treatment with denosumab compared to placebo in a randomised controlled design with 30-month follow-up (Study IV). The inclusion of new patients ceased after 3 years and only six patients were included in total. We had to abandon the MD-technique due to methodological difficulties. The results indicated that patients treated with denosumab had less BMD loss adjacent to the implant; however, no positive effect on implant fixation or patient reported outcomes

were observed. One patient (placebo) had the implant removed after a traumatic incident, but the other five implants stayed in situ.

There is still much to be learned about the mechanisms leading to OI implant loosening after surgery. The results of this thesis find important predictors associated with implant removal, and may inspire further studies towards improved OI implant survival.

2. Danish summary

OI protesekirurgi er et højt specialiseret behandlingstilbud til lårbensamputerede patienter, som har problemer med hylsterprotesen. OI protesesystemet består af en knogleforankret protese (fixture) og en perkutan metalstang (abutment), som kobles til en ekstern benprotese via en kliklås. Efter OI protesekirurgi anvender patienterne den eksterne benprotese hyppigere, har større mobilitet og livskvalitet end da de benyttede hylsterprotesen. Risikoen for bløddelsinfektioner er stor særligt omkring abutment og der er rapporteret flere tilfælde af aseptisk/septisk proteseløsning. De underliggende mekanismer der fører til OI protesefjernelse er kun sparsomt belyst. Derfor var det overordnede formål med afhandlingen at beskrive ændringerne i patienterne radiografisk og biokemisk indtil forsøgets afslutning eller OI protesefjernelse. Ændringerne blev målt ved brug af 4 metoder: dual energy x-ray absorptiometry (DXA), radiostereometrisk analyse (RSA), knoglemarkører målt i serum og mikrodialyse (MD).

Vi validerede en DXA skanningsprotokol for at fastslå præcisionen af knoglemineraltæthedsmålingerne (BMD) omkring OI protesen (Studie I). Vi fandt at *ex vivo* simuleret hoftefleksion og -rotation ændrede BMD og at *in vivo* præcisionen var acceptabel. Vi konkluderede, at skanningsprotokollen sikrede minimal variationen i positionering af det amputerede ben mellem skanningerne og at måleusikkerheden var lav.

I en prospektiv kohorte målte vi BMD i rygsøjlen, i hofterne og omkring OI protesen samt knoglemarkører i blodet (Studie II). Otte ud af 20 OI proteser (5 fiksturer og 3 abutments) blev fjernet i løbet af 30 måneders opfølgning. Vi fandt, at et fald i periprotetisk BMD, en stigning i C-telopeptid af type I kollagen (CTX) og forhøjet parathyreoideahormon (PTH) var associeret med OI protesefjernelse. Vi konkluderede, at knoglemarkører bør korrigeres før og monitoreres efter OI protesekirurgi.

I en prospektiv kohorte blev OI protese migrationen undersøgt med model-baseret RSA (studie III). Vi fandt at stabile proteser forblev in situ op til 5 år efter operationen, mens at kontinuerlig protese migration ind til 2 år medførte senere protesefjernelse. Distal OI protese migration var associeret med tidlig protesefjernelse.

I et randomiseret klinisk studie blev antiresorptiv behandling (denosumab) sammenlignet med placebo og effekten blev målt med DXA, model-baseret RSA, serum knoglemarkører og MD (Studie IV). Inklusionen af nye patienter ophørte efter 3 år da kun seks patienter var blevet inkluderet. Grundet metodologiske problemer måtte vi opgive MD.
Tendensen var, at tabet i BMD omkring OI protesen efter denosumab behandling var væsentligt reduceret sammenlignet med placebo. Vi fandt ingen forskel i protesefiksering eller i de patient-rapporterede spørgeskemaer. En patient i placebogruppen fik fjernet protesen efter et vridtraume.

Der er stadig meget at lære om de mekanismer, som fører til OI protesefjernelse. Resultaterne i denne afhandling finder vigtige prædiktorer, som er forbundet med protesefjernelse og kan være en hjælp for fremtidige studier rettet mod bedre OI protese overlevelsen.

3. Introduction

The osseointegration concept

The first observations of osseointegration were made by P-I Brånemark in the early 1960s during long-term observations of bone marrow response to implanted screw-shaped titanium chambers. He discovered that the surrounding bone grew into the titanium chambers and could not be removed, hence the term osseointegration (1).

It was originally defined as (R Brånemark et al., Osseointegration in skeletal reconstruction and rehabilitation p.22):
"a direct structural and functional connection between ordered living bone and the surface of a load-carrying implant" (2, 3).
This definition has since been changed to:
"...when there is no progressive relative movement between the implant and the bone with which it has direct contact" (3).

The growth of bone into titanium provides a direct bone-anchored attachment for different percutaneous devices that are used in a variety of clinical applications. The most common application of osseointegration (OI) is in the replacement of teeth or the restoration of edentulous segments of the mouth (4). More than an estimated 12 million patients have been treated with osseointegrated dental implants, and studies find an excellent implant survival of 81% at the 10-year follow-up (5). Bone-anchored implants have been applied to support the craniofacial prosthesis after the loss of an ear, nose and/or an eye (3). This is not only for the sake of cosmetic appearance, but also promotes the quality of life and social rehabilitation (6). Conductive hearing loss can be aided by a bone-anchored titanium screw inserted into the skull (7). The sound perception is gained by a bone-conducting hearing device transmitting vibrations to the skull and directly to the inner ear.

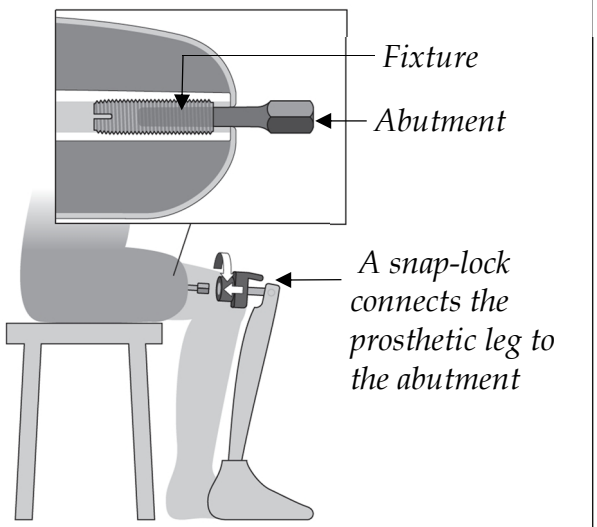
The most interesting use of OI implants is found in the field of amputation surgery. Patients missing a thumb or suffering from a transradial, humeral, or femoral amputation have been treated with OI implants since the early 1990s (8, 9).
Although osseointegrated implants have been used for several decades in orthopaedic joint replacement surgery, the use of percutaneous OI implants for amputees is a highly-specialised field, and so far, only a few papers regarding the matter have been published. Hence, this thesis refers to several applied theories on osseointegration, clinical outcomes and failures that are based upon the experience from joint replacement and dental surgery.

The osseointegrated implant

The osseointegration (OI) implant for transfemoral amputees (TFA) looks like the smaller dental implant with the screw-shaped design of the intraosseous fixture and the skin-penetrating abutment (10) (Figure 1).

During stage 1 (S1) surgery the femoral canal is reamed and thread cut using a retrograde approach. Then a slightly oversized threaded titanium fixture is screwed into the residual femur until it is 20 mm countersunk. An autologous iliac bone graft is used to close the distal opening of the femoral canal and seal the intramedullary fixture from a non-osseous environment (11). Prophylactic IV dicloxacillin is administered 1 day pre- and postoperatively. The fixture needs 6 months in an unloaded setting to sufficiently osseointegrate (healing phase) before the next operation(9, 12)

Figure 1: The osseointegration implant



During stage 2 (S2) surgery, a canal is drilled through the bone graft just big enough to allow the percutaneous titanium abutment to be connected to the fixture. Major soft tissue revision is performed, and the skin around the abutment is stripped from soft tissue and grafted onto the bone end. IV dicloxacillin is administered 1 day prior to surgery and 10 days postoperatively. After surgery, the patient is immobilised for 10 days allowing the skin to heal onto the bone and to avoid excessive swelling of the soft tissue (13). The attachment of the abutment makes it is easy to connect the external prosthetic leg with a snap-lock.

Even though the osseointegration is completed after 6 months a rapid increase in weight bearing can lead to OI implant loosening as shown by earlier clinical investigation (9). Thus, the patients follow a rehabilitation protocol that includes initial training with a short prosthesis for 12 weeks and a later training period with a long external prosthesis. After S2 surgery, gentle exercise is allowed the first 6 weeks, which is followed by weight bearing on the OI implant starting at 20 kg and increases by 10 kg/week. Twelve weeks after S2 surgery, the patients commence training with a long prosthesis and gradually increase weight bearing on the OI implant. Full mobility is normally achieved 12 months after S1 surgery (9).

The integral leg prosthesis

Another implant type is the integral leg prosthesis (ILP) (Eska Orthodynamics GmbH, Germany), which is markedly different from the OI implant. The intraosseous module is longer than the threaded titanium fixture and is made of cobalt-chrome-molybdenum with a coated macro-porous surface. The ILP system consists of two parts. First, the intraosseous component is inserted into the femoral canal and after 2 months a transdermal coupler is attached. The coupler is connected to the external leg and full weight bearing on the implant is allowed after four to six weeks (14), thus full mobility is achieved 4 months after S1 surgery. Since the introduction of the ILP in 1999 it has undergone 3 different design iterations and the surgical procedure has been changed (Figure 2). In the final design, the bone stabilising bracket and the structured surface on the transdermal coupler are removed and replaced by a smooth polished surface.

Figure 2: Design iteration of the ILP

Design iteration A, B and C (final)
(1) Bone stabilising bracket, (2) Soft tissue interface, (3) Intraosseous module
Adapted from: Juhnke et al, Fifteen years of experience with the integral-Leg-Prosthesis: Cohort study of artificial limb attachment system

The surgical procedure aims to reduce the distal soft tissue thickness to 2 cm instead of keeping a long soft-tissue-canal around the connector. These changes have proven to stimulate a rapid soft tissue healing around the percutaneous device and thereby reduce inflammation and number of infections. The final ILP design has markedly fewer infections and implant removals than the earlier implant designs (15).

Transfemoral amputees before and after OI surgery

Lower-limb amputees using a socket suspended prosthesis generally report that pain in the residual limb and/or phantom pain is a reoccurring problem (16, 17). These patients also experience a variety of problems related to the prosthetic socket; such as pain due to improper fitting, skin problems (rashes, sores) and back pain (18-20). These problems tend to reduce daily walking distance and ultimately reduce quality of life (21). The target population for OI implant surgery are patients with a unilateral transfemoral amputation caused by non-vascular diseases. This group reports that the problems leading to a reduced quality of life are heat/sweating in the prosthetic socket (72%), skin problems (62%), inability to walk on uneven terrain (61%), inability to walk quickly (59%) and pain (51%). Half of the transfemoral amputees experience pain in the residual femur, phantom pain, back pain and/or pain in the other leg (22).

The OI implant system for transfemoral amputees alleviates some of the problems associated with the socket prosthesis. After successful rehabilitation, the patients display an improved hip range of motion and better sitting comfort compared with patients using a prosthetic socket (23). The most important results after OI surgery are increased prosthetic use, improved mobility, and even though pain in the residual limb remains, the patients report an improved physical quality of life (24, 25). Compared with the socket prosthesis, the connection between the OI implant and the external prosthesis yields a better tactile sensory feedback (26). This feedback is called osseoperception and improves the amputees' perception of the terrain (3).

Bone changes before and after OI surgery

Disuse atrophy

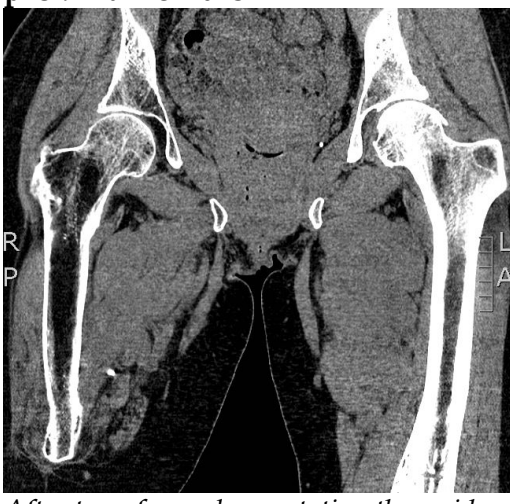
A phenomenon termed disuse atrophy is used to explain the decrease in bone mineral density (BMD) after amputation (20, 27-30). It is related to reduced weight bearing on the femoral bone, as most of the weight is transferred to the surrounding soft tissue and the tuber ischia by the prosthetic socket. Additionally, physical disability is commonly followed by a low activity level; the combined effect of reduced weight bearing and a low activity level causes demineralisation in the residual bone (31, 32).

Osteoporosis

Osteoporosis and osteopenia are conditions of low BMD that increase the fracture risk (33). It has been known for half a century that lower-limb amputees suffer from halisteresis in the residual bone and their radiographs show osteoporotic changes, such as decrease in cortical bone thickness and bone atrophy (20, 27, 34) (Figure 3).

Dual energy X-ray absorptiometry (DXA) examinations of the proximal hip on the amputated side find that BMD is reduced between 28% and 38% after lower limb amputation (28-30, 35). The level of amputation seems to be a predictor of BMD loss as patients undergoing a transfemoral amputation lose more BMD in the hip than patients undergoing transtibial amputation (29, 30, 35, 36). However, TFAs have a normal BMD in the spine and hip on the intact side compared with non-amputated controls (35). Even though a low BMD increases the fracture risk, it seems that the fracture incidence in the residual femoral bone is as low as 3% (37, 38).

Figure 3: Coronal CT scan of the proximal femurs



After transfemoral amputation the residual femur clearly demonstrates reduced cortical thickness and bone atrophy

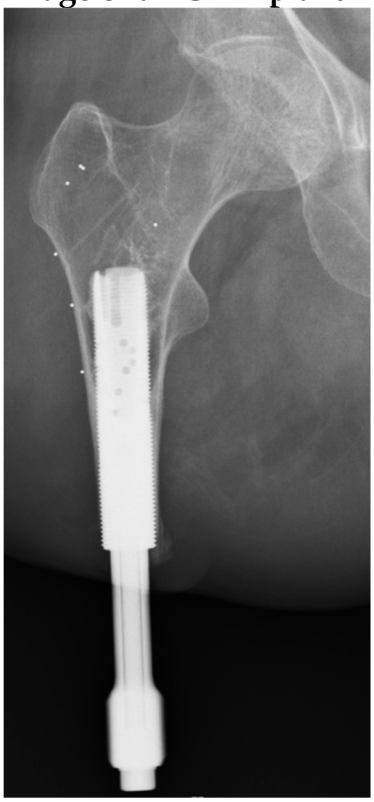
Stress shielding around the OI implant

Insertion of an implant leads to periprosthetic bone remodelling due to the new loading conditions imposed by the implant (39). This functional adaption modifies the shape and structure of the bone and is referred to as Wolff's law (Julius Wolff 1870, translated by P. Marquet et al. p. 126):

"*the law of bone remodelling is the law according to which alterations of the internal architecture clearly observed and following mathematical rules, as well as secondary alterations of the external form of the bones following the same mathematical rules, occur as a consequence of primary changes in the shape and stressing or in the stressing of the bones*" (40).

In implant surgery, these changes are referred to as stress shielding and are always related to the bone loss around the implants (39, 41). The effect of stress shielding can be altered by factors such as implant geometry, size and stiffness (39, 42, 43). The small cylindric fixture is made of titanium, which is a metal suitable for osseointegration as it is biologically inert (10), corrosion resistant and the modulus of elasticity is comparable to bone (44, 45). The comparable modulus of elasticity between bone and titanium enables a favourable load sharing relationship that theoretically should minimise the effect of stress shielding (39). However, osteoporotic bone is subjected to a greater extent of periprosthetic BMD loss than normal bone (46-48). Radiographic assessment of the bone adjacent to the OI implants (Integrum AB, Sweden) finds increased porosity, cortical thinning and distal bone resorption after 2 years (Figure 4). These changes were consistent with stress shielding and did not affect implant stability (49). This is supported by the evidence from finite-element studies, finding that bone remodelling adjacent to the implant is influenced by the distribution of stress and strain. These studies suggest that the effect of stress shielding causes the greatest bone loss in the distal region and lesser loss in the proximal regions (50-52).

Figure 4: Radiographic image of an OI implant



Example of cortical thinning and distal bone resorption five years after surgery

Bone loss and implant loosening

Papers describing the mechanism causing OI implant loosening in amputees are lacking. Thus, a short summary of aseptic loosening in ordinary orthopaedic surgery and in dental implant surgery is presented to give a preliminary understanding of the mechanisms. The dental implants were selected because of their similarities to the OI implant design and because the percutaneous abutment increases the risk of infections.

The orthopaedic implants are selected due to the many reports on aseptic implant loosening.

Orthopaedic surgery

Aseptic loosening is defined as the mechanical loosening of an implant without signs of infection and is the major cause of implant revision in joint arthroplasty (53). In the beginning, particulate debris from cement was believed to play a major role in implant loosening due to fibrous tissue formation in the bone implant interface. Later, it was discovered that particle accumulation, especially wear particles from polyethylene (PE), was strongly associated with implant loosening (54-56).

The activation of macrophages depends partly on the amount of debris and partly on the size of the debris particles. PE wear particles measure up to 10µm (57), whereas metal particles are smaller (10-400 nm); thus, the macrophages can store several metal particles, leading to fewer macrophages being activated (58). Most wear particles are found in the synovial fluid and spread to the surrounding bone and the bone-implant interface, whereas metal particles can corrode and disappear (59, 60). The macrophage-mediated wear particle response results in periprosthetic bone loss, osteolysis and predisposes to aseptic implant loosening.

Dental surgery

In dental implant surgery, loose implants are defined according to the chronological order of early and late failure. Early (primary) implant failures are caused by an insufficient osseointegration of the fixture, whereas later (secondary) implant failures happen due to a breakdown of the established osseointegration. The period defining the early failures lasts from primary surgery until the abutment is inserted (61).

There is little data on the relationship between secondary dental implant loosening and particle debris. Likely, the load on the dental implants is less than on the orthopaedic joint replacements, reducing the amount of wear particles and hence a minimal accumulation around the implant. This is supported by the fact that only a few retrieval studies have discovered phagocytosed metal particles next to the fixtures; however, peri-implant bone loss is a common observation (62, 63).

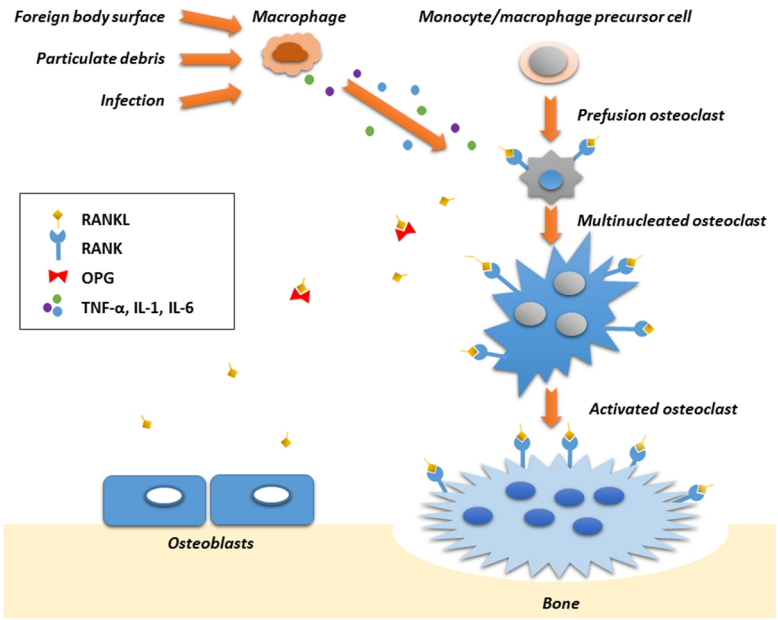
Dental implants have been thoroughly investigated because a substantial marginal bone loss (MBL) is observed the first year after implantation (4, 64, 65). The MBL refers to the bone resorption occurring in the apical bone adjacent to the implant, and it is considered a pathological sign that can lead to implant failure. A MBL <1.5 mm during the first year and thereafter <0.2 mm during the following years is considered acceptable (4). The dominant theory purports that MBL is caused by periimplantitis induced by the microorganisms in the oral cavity and that it may lead to a secondary infection (66). A new theory suggests that MBL is a reaction to the treatment and should be viewed as state of chronic inflammation due to a foreign body reaction (67). The foreign body response may follow two possible routes: a foreign body equilibrium with

a mild chronic inflammation or an early dis-balance in which bone resorption dominates the bone formation. This theory is supported by the foreign-body giant cells routinely observed adjacent to dental implants, but more studies in this area are needed (63, 68, 69).

Infection

A bone infection leads to an immune response and increased osteoclastic activity, causing localised bone loss (70). As previously described, infection is a common complication in OI implant surgery for amputees. However, some infections have a low activity and do not necessitate implant removal even though chronic fistulae may persist (71).

Figure 5: The RANKL-RANK-OPG pathway



RANKL expression is induced in osteoblast and binds to the membrane-bound receptor RANK on the osteoclast. This promotes activation and differentiation of the osteoclast lineage. OPG is produced by the osteoblasts and binds to RANKL thereby blocking the formation of osteoclasts. The activation of macrophages produces proinflammatory cytokines that potentiate osteoclastogenesis.

The cellular response leading to bone loss

Macrophages are stimulated by the presence of bacteria, foreign body surfaces and by the phagocytosis of particles (70, 72). The activation involves a cellular response comprising a complex signalling network of proinflammatory and osteoclastogenic cytokines (73-75). The most important cytokines secreted by the macrophages are TNF-α, IL-1 and IL-6, which affects the differentiation of macrophage precursors into osteoclasts (Figure 5) and enhance the RANKL-induced formation of osteoclasts (76-79). In short, this leads to an accelerated bone resorption adjacent to the implant. Depending on the resorption rate, elevated levels of bone turnover markers may be detected in serum samples.

OI implant surgery for transfemoral amputees

Papers to date have not discriminated clearly between early and late OI implant loosening. However, no papers have reported any implant instability immediately after inserting the abutment. Implant removals typically occurred after the abutment was inserted and within 2 years, indicating that the primary osseointegration was successful and implant loosening occurred due to a breakdown of the osseointegration.

Several mechanisms can lead to implant loosening as mentioned earlier. Aseptic loosening as described in orthopaedic research is mainly caused by wear particles, but since the OI implant lacks a joint this is unlikely the predominant mechanism. The substantial periprosthetic bone loss adjacent to the OI implant seems to be primarily caused by stress shielding as described by radiographs and CT finite-element analysis. However, considering the MBL as osteolytic changes, dental research shows that bone loss around the implant is caused by microorganisms or a state of chronic inflammation. There is evidence that the bone canal around the abutment is colonized with bacteria, as 27 out of 30 TFAs had a positive sample taken (80).
Thus, the bone changes around the OI implants are a complex interaction between the surgically induced trauma, stress shielding, microorganisms, wear particles and periprosthetic inflammation/foreign body reaction leading to implant loosening.

Complications and implant removals

All published studies to date reporting the number of implant removals and their causes are shown in Table 1.

Table 1: The number of patients treated and implants removed

Implant	Country	Period	Patients in cohort	Implants removed	Cause of implant removal
OI implant					
	(9) Sweden	1990 - 2008	100	20	Unknown
	(81) Sweden(OPRA)*	1999 - 2007	51	4	3 aseptic and 1 deep infection within two years
	(12) UK	1997 - 2003	11	2	2 deep infections within one year
Integral leg prosthesis					
	(85) Germany	2003 - 2014	86	8	2 aseptic, 3 deep infections, 2 chronic tissue infections and 1 implant failure
	(83) Two-center study from The Netherlands and Australia	2009-2013	86	3	1 aseptic and 2 intramedullary breakage of the component

**The OPRA cohort is a subpopulation from the first 100 Swedish OI implant patients*

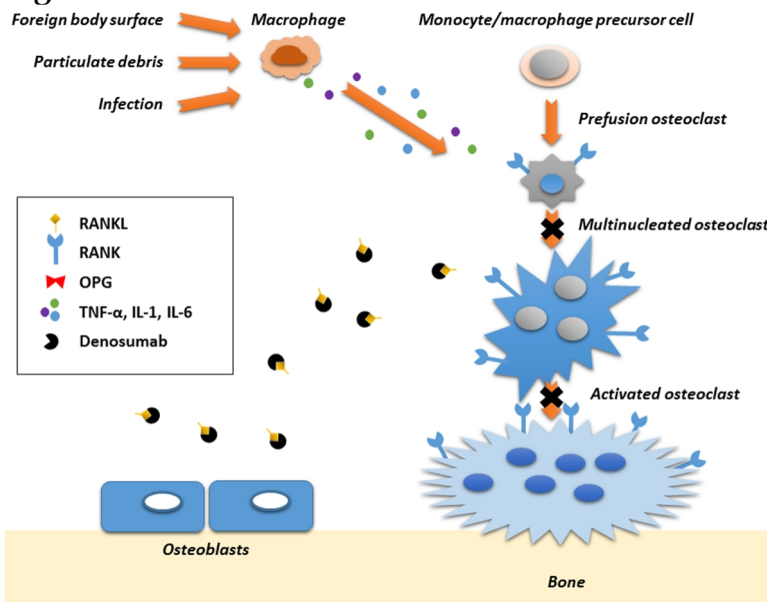
The largest OI implant study to date has not reported the cause of implant removals or the complications. However, the Swedish subpopulation originating from that cohort, reports that superficial infections occurred 41 times in 28 patients during a 2-year follow-up and periprosthetic infections occurred in four patients of which three were successfully treated with IV antibiotics (81). The German Osseointegration Team reports more than 100 unplanned surgical interventions in 29 patients and the majority are due to soft tissue infections in patients treated with the early implant design (15).

The two-center study from the Netherlands and Australia reports 47 low-grade soft tissue infections in 29 patients during the median 34-month follow-up (82-84). The risk of fracture may increase after OI surgery due to increased prosthetic use and mobility. The OPRA cohort had three ipsilateral hip fractures, the Australian cohort sustained four periprosthetic fractures and the German cohort counted six hip fractures and one periprosthetic fracture (81, 84, 85). Most of these infectious complications are resolved with oral antibiotics, but a substantial number of patients also require IV antibiotics or surgical debridement of infected soft tissue. Approximately 2/3 of patients treated with an OI implant can expect an infectious complication during a 3-year follow-up period (71). Other complications involve breakage of the percutaneous device requiring replacement and redundant distal soft tissue or hypergranulation around the percutaneous device requiring surgical revision.

Bone resorption and antiresorptive therapy

Osteoclasts are recruited via the differentiation of monocytes/macrophages precursor cells near the bone. The final link in the osteoclastogenesis pathway is the binding of RANKL to the RANK receptor on the precursor cells and the binding to mature osteoclasts in a dose-dependent manner (Figure 5). This leads to recruitment and activation of the osteoclasts and thus an increase in bone resorption (86). This formation is especially regulated by the osteoblast production of OPG or RANKL. OPG serves as a decoy receptor for RANKL and can neutralise both osteoclastogenesis and osteoclast activation (87, 88). The interaction between osteoblasts and osteoclasts explains the catabolic effect of continuous elevated PTH concentrations; it suppresses OPG production and stimulates RANKL production in osteoblasts and thereby increases osteoclast activity (86, 89). Denosumab is a human monoclonal RANKL antibody and acts like OPG by inhibiting the recruitment and activation of osteoclasts (Figure 6). The FREEDOM-, DECIDE- and STAND-trial finds a significant increase in BMD and a reduced fracture risk after treatment with denosumab compared to placebo and alendronate (90-92).

Figure 6: The effect of denosumab



Denosumab binds to RANKL and reduces the osteoclast differentiation and activity. The proinflammatory cytokines from the macrophages no longer enhances the osteoclastogenesis.

Denosumab is used in the treatment of postmenopausal osteoporotic women, bone metastasis (93) and giant cell tumour of bone (94). Animal studies indicate that anti-RANKL treatment may improve implant fixation (95) and reduce periprosthetic bone loss (96). Clinical trials examining the antiresorptive effect around implants after denosumab treatment are lacking. A study on the effect of denosumab compared to placebo in the treatment of wear-induced periprosthetic osteolysis has recently been initiated (97).

Biochemical assessment of bone formation and resorption

Bone remodelling and turnover markers

Bone remodelling is a dynamic process of bone formation by the osteoblasts and resorption by the osteoclasts. Most of the organic matrix consists of type-I collagen and small amounts of osteocalcin, glycoproteins and proteoglycans. The rate of bone remodelling can be determined by bone turnover markers (BTMs), which are released during the formation/degradation of type-I collagen and the mineralisation of bone (98). Fragments of collagen can be evaluated in samples collected from blood and urine. Increased concentrations of bone turnover markers are associated with accelerated bone loss, which may lead to a low BMD and an increased fracture risk (99-101). The osteoclastic degradation of type-I collagen releases C-terminal telopeptide of type I collagen (CTX) and N-terminal telopeptide of type I collagen (NTX), whereas the osteoblastic formation releases N-terminal propeptide of type I procollagen (P1NP) and C-terminal propeptide of type I procollagen (P1CP) during the synthesis of type-I collagen. Bone-specific alkaline phosphatase (BASP) is associated with osteoblast activity, whereas osteocalcin (OC) is involved in the mineralisation of bone (98).

An increase in BTMs may also be detected following an increase in parathyroid hormone (PTH) and a decrease in vitamin D concentrations. PTH stimulates bone remodelling by direct effect on the osteoblasts and indirect effect on the osteoclast. The outcome is either anabolic or catabolic depending on the dose and frequency of PTH signals. Continuous exposure of PTH modulates the osteoblastic lineage and increases the RANKL production, which is a strong activator of osteoclasts. Intermittent low doses of PTH results in an anabolic effect by the promotion of osteoblastogenesis (102).

BTMs have proven useful in monitoring the treatment effect of antiresorptive therapy, metabolic disease or metastatic cancer (103, 104). In orthopaedic surgery, the BTM response has been investigated after fracture (105), implant surgery (106) and in aseptic implant loosening (107-109). BTMs may be useful to detect periprosthetic osteolysis although no markers have been validated for this purpose (110). Osteolysis leads to an increase in bone resorption and release of CTX and NTX (111). CTX concentrations are elevated in animal models with particle-induced osteolysis (112, 113) and in patients

with a potential unstable fixation of the tibial implant component (107). Similar, serum and urine CTX and NTX show a trend toward increased concentration in aseptic loosening (108, 109, 114, 115).

Microdialysis

Microdialysis was developed in early 1960s to monitor and quantify the metabolism in the central nervous system of animals (116). Today, it is used to investigate the pharmacokinetics of drugs, ischemic changes and the metabolism in a wide range of human tissues. In the musculoskeletal tissue, microdiaslysis has primarily been used to monitor ischaemic changes in bone and muscle following surgery or fracture (117-120). However, microdialysis has also been used in range of different purposes, such as determination of the dose-dependent penetration of antibiotics in bone (121-124). Additionally, type-I collagen synthesis in the peritendinous tissue has been monitored by sampling the collagen fragments (PICP, ICTP) before and after exercise (125, 126). Bone resorption may also be monitored with microdialysis, because some fragments are small enough to diffuse across the 100kDa probe after type-I collagen degradation.

The microdialysis technique consists of a double lumen catheter with a semipermeable membrane inserted into the target tissue using a minimally invasive procedure. A pump is used to deliver the perfusion fluid (perfusate) to the probe at a slow and constant flow rate. The probe seeks to reach equilibrium between the solution in the extracellular space and the perfusate in the catheter across the semipermeable membrane. Hence, the sampling of unbound extracellular molecules occurs as diffusion depending on the concentration gradient. As the probe is continuously flushed it will never reach a concentration-equilibrium with the extracellular molecules, which is referred to as the relative recovery (RR). The RR depends on temperature, type of perfusate, flow rate, membrane length, pore-size and the molecular size. Thus, if the RR is not determined the true concentration of extracellular molecules is unknown. Most studies aim to achieve a maximal recovery, hence a slow flow rate and a long membrane length with an optimal pore-size are needed (127, 128).

Radiographic assessment of bone and implant stability

Dual energy X-ray absorptiometry

The gold standard for measuring BMD is DXA (129). This method uses two low-dose X-ray beams with different energy levels. The energies are absorbed in bone and soft tissue; the high and low energies generate different attenuations that are measured with the DXA detector. Based on these measurements the DXA software can segment and quantify the mass of the bone, fat and muscle. The DXA system is a well-validated technique that provides an accurate and precise assessment of the bone and soft tissue. DXA is mostly used in the diagnosis of osteoporosis and in the assessment of fracture

risk. The diagnosis is based on the young adult reference population: osteopenia is diagnosed when the BMD is less than 1 standard deviation below the young adult reference population (T-score <-1) and osteoporosis when it is less than or equal to -2.5 standard deviations (T-score <-2.5) (130, 131).

In orthopaedic research, DXA scans are predominantly used to measure the periprosthetic BMD changes after joint arthroplasty. These changes can be evaluated on radiographs; however, this is an inferior method since bone loss recognition is not reproducible until 70% of the bone is gone (132).
The precision of periprosthetic BMD measurements is acceptable (133-137), but still lower than the precision of the proximal hip and spine scans. The most common factors affecting the precision are the patient positioning and the scan analysis (138, 139).
To accurately interpret serial measurements, estimation of the least significant change (LSC) is recommended, because BMD measurements above the LSC are likely caused by a true bone loss (138). A common observation after joint replacement is an accelerated bone loss around the implant that stabilises after a few months (106, 140, 141). A substantial bone resorption is observed around the OI implants on radiographs, but the amount of bone loss during the unloaded phase and during weight bearing phase remains to be quantified with DXA.

Radiostereometric analysis

Radiostereometric analysis (RSA) for orthopaedic purposes was developed by Göran Selvik in 1974 and it is regarded as the gold standard for determining implant migration (142, 143). The method is based on two simultaneous radiographs of the implant in relation to a calibration box, thus creating a three-dimensional (3D) coordinate system. The 3D system creates two rigid bodies based on the implant and bone position, thereby allowing a computer to calculate micromotions between the rigid bodies at follow-up examinations.
The core of RSA is to determine implant migration as it is strongly associated with implant loosening (144, 145). The system can determine micromotions with high accuracy and precision, which makes it possible to conduct investigations with a small study population (146, 147). Two approaches are used to determine implant micromotion: marker-based and model-based RSA.

Marker-based RSA is based on tantalum beads welded onto the implant and tantalum beads surgically inserted into the bone adjacent to the implant, thus forming two rigid bodies (142). In comparison, model-based RSA is a method to determine migration without tantalum beads attached on the implant, but only via surgically inserted tantalum bone markers. This method requires a 3D model of the implant, which is digitally fitted to the implant contours on the radiographs (148, 149). This is handled by the computer software repeatedly moving the model until it fits the contour of the actual implant with minimal differences.

The 3D model can be created from a reversed engineered (RE) model or a CAD model provided from the implant manufacturer (149). The CAD model differs more from the actual implant than the RE model; however, the precision is still high and CAD models have been used in numerous RSA studies (150-152). Marker-based RSA is considered more precise than model-based RSA, albeit model-based RSA is often preferred as problems with implant rectification and occluded implant markers are eliminated (153).

Translations are used to describe implant migration along the axis in an orthogonal coordinate system. The coordinate system is associated with the anatomical directions; hence the X-axis describes medial/lateral, the Y-axis describes proximal/distal, and the Z-axis describes anterior/posterior migration. Positive directions along the X, Y and Z-axis are medial, proximal and anterior, respectively (154, 155).

Given the predictive power of RSA to detect implant loosening based on the early migration pattern, it has been recommended to implement RSA as a tool for a stepwise introduction of new implants on the market. A stepwise introduction consists of three steps: first preclinical tests followed by large scale tests (multicentre or randomized) and finally surveillance in registries (156). It has since been proposed to add an intermediary test (2a) and conduct 2-year RSA trials after the preclinical tests (step 1) (157). A prospective (step 2a) RSA examination of the OI implant fixation in the OPRA cohort was conducted and found a stable fixation at 2-year follow-up (n = 40) and at 7-year follow-up (n = 12). However, randomised trials and multicentre trials (step 2b) are lacking, as well as surveillance in registries (step 3).

4. Aim of the thesis

Reports regarding patient mobility, prosthetic use and quality of life after OI implant surgery in transfemoral amputees is promising. Little is known about the changes in the bone adjacent to the OI implant or the mechanisms leading to removal of OI implant. The primary research question concerned the association between the periprosthetic bone quality, bone remodelling and implant fixation as prognostic factors for long-term OI implant survival. During the course of this study period, we were surprised by the high number of implant removals and secondary set off to investigate the differences between removed implants (RI) and non-removed implants (NRI). This thesis is based on the work of four studies with the following designs and specific aims:

Study I

Design: A methodological study based on a repeated measurement design, comprising an *ex vivo* investigation and an *in vivo* investigation.

Aim: To determine the feasibility and precision of the clinical DXA scan protocol using two approaches. First, the change in periprosthetic BMD was examined by simulated hip flexion and rotation in an ex vivo study. Second, the precision was tested by double examinations in a clinical study.

Study II

Design: Patients from a consecutive prospective observational cohort with 30-month follow-up were examined using different methods in Studies II and III.

Aim: To investigate the BMD and BTM changes compared with baseline during the 30-months follow-up, and investigate whether these changes were different between the removed and non-removed implants.

Study III

Design: As in Study II

Aim: To examine the migration of OI implants with RSA and investigate whether there was a difference in migration between the removed and non-removed implants. Finally, to examine whether the OI implant migration correlated with preoperative BMD.

Study IV

Design: A double-blinded randomised clinical controlled trial with 30-months follow-up.

Aim: To compare the effect of two denosumab injections to saline injections on periprosthetic BMD, BTM, bone metabolism and implant migration. Furthermore, to investigate the CTX concentrations in the femoral bone and pelvic bone after first stage surgery with microdialysis.

5. Materials & methods

Ethical issues

The work of this thesis was performed in accordance with the ethical principles of the Helsinki declaration. The investigations conducted in **Studies I-III** did not require a formal approval from the Central Denmark Region Committees on Biomedical Research Ethics (inquiry number 135/2016) or the Danish Data Protection Agency (approval of 2012-28-005) since all examinations were performed according to an established quality assurance protocol. **Study IV** was approved from the Central Denmark Region Committees on Biomedical Research Ethics (1-10-72-444-12), the Danish Data Protection Agency (1-16-02-32-13), the Danish Department of Health (2013081593) and registered at EudraCT (2012-003574-66).

Patients

Study I

The population consisted of 20 patients already enrolled in Studies II-IV. The precision of DXA scans was tested by double-examinations after S2 surgery from 2014 to 2015.

Studies II and III

A total of 20 patients were prospectively enrolled in the observational cohort study from 2010 to 2013 by two senior consultants based on the eligibility criteria presented in Table 2.

Table 2: Eligibility criteria Studies II and III

Inclusion criteria
▪ Age between 18-70 years ▪ BMI<30 ▪ A bone structure suitable for OI implant surgery
Exclusion criteria
▪ Diabetes ▪ Atherosclerosis ▪ Smoking ▪ Treatment with bisphosphonates, NSAID or cytostatic medicine ▪ Active cancer ▪ Kidney or liver insufficiency ▪ Dementia ▪ Pregnancy ▪ Weight >100kg

The demographic characteristics are presented in Table 3. The majority had undergone transfemoral amputation due to trauma (n = 9), tumour (n = 4) and infection (n = 2), other causes leading to amputation were compartment syndrome, severe pain after knee-alloplastic surgery and knee arthrodesis.

The patients had blood samples collected and were examined with DXA and RSA. The follow-up interval between each examination, the number of patients analysed at each follow-up and the number of removed implants are presented in Table 4.

Table 3: Patient demographics Studies II and III

OI cohort baseline values	Mean (range)
Gender (M/F)	13/7
Mean age at surgery (years)	48 (30 to 66)
Mean time since amputation (years)	4 (0 to 39)
Mean femur length (cm)	26.3 (12 to 36)
Amputation side (R/L)	10/10
L1-L4 spine BMD (g/cm^2)	1.13 (0.89 to 1.68)
T-score (L1-L4)	-0.63 (-2.74 to 4.21)
Total hip BMD amputated side (g/cm^2)	0.69 (0.33 to 1.33)
T-score hip	-2.78 (-5.3 to 2.53)
Total hip BMD intact side (g/cm^2)	1.03 (0.78 to 1.44)
T-score hip	-0.26 (-1.94 to 3.44)

Table 4: Number of patients analysed at each follow-up study II and III

Follow-up interval (Months after S1 surgery)	Patients in the cohort	Implants removed	Patients analysed		
			DXA	Blood samples	RSA
Preoperative (0)	20		20	16	
Stage 1 surgery					
1	20		16	16	
3	20		16	16	
6	20		16	15	
Stage 2 surgery					
7	20		18	16	16
9	20		19	20	15
12	20		19	18	16
18	17	3	16	16	12
24	14	3	14	14	10
30	12	2 (3) *	11	11	10
60	9	2			

Five fixtures were removed; two at 18-months and three at 24-months follow-up.
Five abutments were removed; one at 18-months, two at 30-months and two before 60-months follow-up.
**One patient could not use the OI implant and counted as an implant removal in the analysis.*

Study IV

Six out of 16 planned patients were included in this RCT study (Figure 7). Eligibility criteria are presented in Table 5 and demographics in Table 6. The reasons for amputation were: tumour (n = 2), trauma (n = 2), infection (n = 1) and thrombosis (n = 1). The inclusion of new patients ceased in June 2016 since too few patients were enrolled in the study after three years and a new biohelix™ coated OI implant was introduced from the implant manufacturer (Integrum AB, Sweden).

Randomisation

The Hospital Pharmacy, Central Denmark Region conducted the bloc randomisations of four groups in a 1:1 ratio to placebo or denosumab and delivered the test drug in sealed boxes. To maintain blinding, the test drug was handled by an independent nurse otherwise not involved with the project. All personnel were asked to leave the room before the seal was broken and the patient was instructed not to look at the syringe.

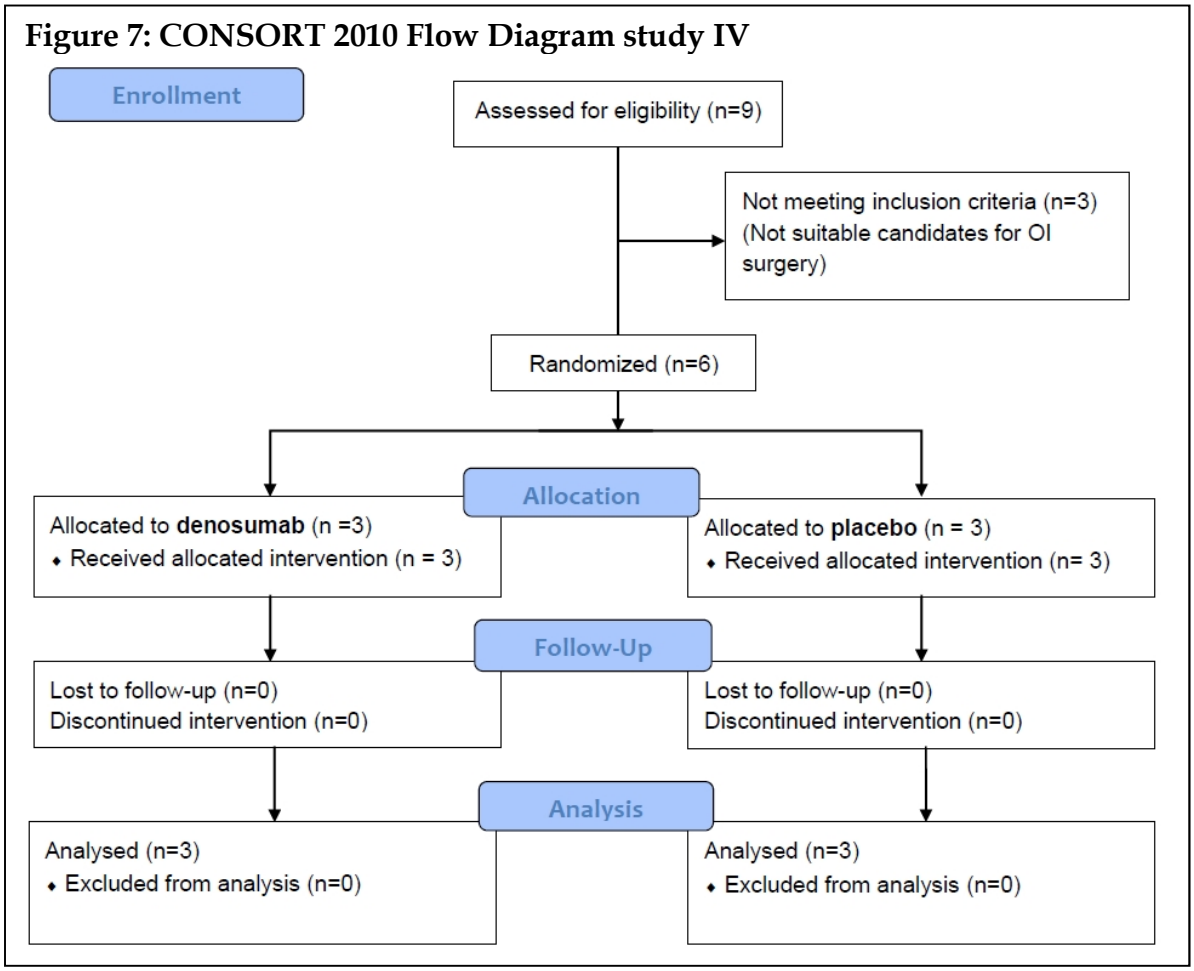
Table 5: Eligibility criteria Study IV

Inclusion criteria
▪ Age between 18-70 years
▪ Scheduled for OI-implant surgery
▪ Body mass index <30
▪ Female patients of childbearing age must produce a negative pregnancy test and use effective contraception
▪ Informed consent
Exclusion criteria
▪ Diabetes with complications
▪ Atherosclerosis
▪ Smoking
▪ Drug abuse
• Treatment with NSAID or cytostatic medicine
▪ Active cancer
▪ Liver or kidney insufficiency
▪ Dementia
• Hip flexion contracture on the affected side >10 degrees
▪ Body weight <100kg
▪ Hypocalcaemia
▪ Contraindications to denosumab

Table 6: Demographics Study IV

Baseline values	**Mean (range)**
Gender (M/F)	6/0
Mean age at surgery (years)	55.5 (36 to 68)
Mean time since amputation (years)	15 (2 to 40)
Amputation side (R/L)	2/4
L1-L4 spine BMD (g/cm^2)	1.32 (0.94 to 1.52)
T-score (L1-L4)	0.9 (-2.4 to 2.6)
Total hip BMD amputated side (g/cm^2)	0.72 (0.57 to 0.91)
T-score hip	-2.4 (-3.5 to -1.3)
Total hip BMD intact side (g/cm^2)	1.02 (0.84 to 1.21)
T-score hip	-0.1 (-1.4 to 1.6)

Figure 7: CONSORT 2010 Flow Diagram study IV



Administration of test drug

The patients were injected subcutaneously in the shoulder with a syringe containing 1ml of denosumab solution 60 mg/ml (Prolia, Amgen) or 1 ml of saline solution 9 mg/ml (Takeda pharma). The drug was administered twice 6 months apart, 1 month before S1 surgery and one month before S2 surgery. Daily intake of 800 calcium with 38µg vitamin D was subscribed for 1 year.

Follow-up

The patients are examined according to the intervals given in Table 7. Microdialysis was conducted at S1 surgery.

Table 7: Follow-up examinations in Study IV

Follow-up (months)	0	1	3	6	7	9	12	18	30
DXA	X	X	X	X	X	X	X	X	X
RSA					X	X	X	X	X
Blood tests	X	X	X	X	X	X	X	X	X

Follow-up interval (in months) after S1-surgery

Intervention and outcomes

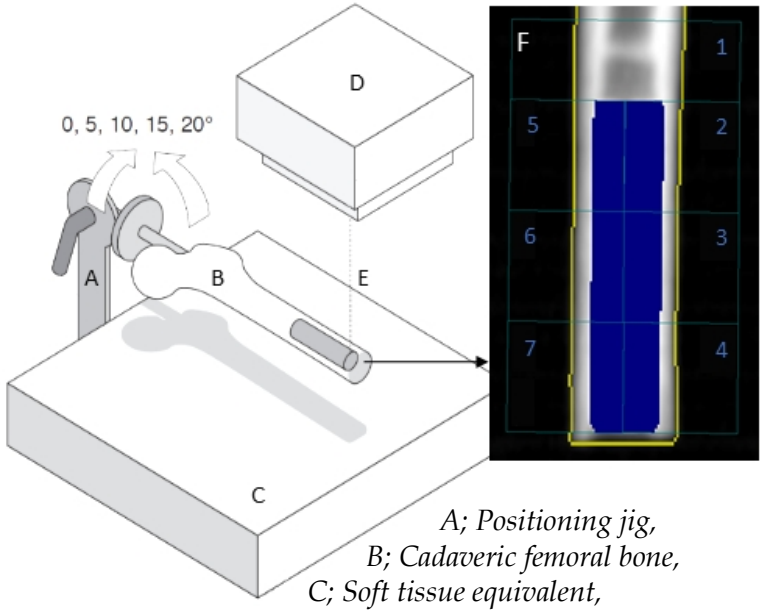
Dual energy X-ray absorptiometry

Ex vivo DXA scan setup - Study I

Two steel fixtures (OI implant, Integrum AB, Sweden) with diameters of 16 mm and 16.5 mm were surgically inserted into two human cadaveric femoral bones. The femurs were cut through the diaphysis and the proximal parts were reamed and thread cut with surgical equipment for OI implant surgery.

Due to anatomical variations, the intramedullary implant position along the femoral midline differed between the two specimens. In specimen A (male, 43 years), the fixture was inserted laterally to the femoral midline resulting in a thin lateral cortical bone, whereas the fixture was inserted along the midline in specimen B (female, 98 years). The bones were tightly fixed to a positioning jig (135) that allowed adjustment of flexion and rotation within 1°. The jig was placed in the middle of the densitometer table and the outlines were marked to secure the same position throughout the experiment. Pilot scans were conducted to determine the optimal soft tissue thickness equivalent (5 cm nylon plate, 3 cm acrylic plate) for the software to detect the different tissue edges (bone, soft tissue, and artefact) correctly.

Figure 8: Ex vivo DXA setup



A; Positioning jig, B; Cadaveric femoral bone, C; Soft tissue equivalent, D; DXA X-ray arm, E; Start of scan F; DXA scan view. The bone edges are marked with the yellow line and the fixture is marked as metal artefact (blue). ROI 1 is placed proximal to the fixture, ROI 2-4 lateral, and ROI 5-7 medial to the fixture.

Scans were performed according to a protocol with cadaveric femoral bone positions resembling the movement of the amputated leg (Figure 8). Neutral position was defined as the femoral bone situated parallel to the densitometer table. Five scans were repeated in increments of 5° from neutral position to 20° of simulated flexion and to 20° of external rotation.

A 7-region of interest (ROI) template (Figure 8) was designed to measure the BMD (g/cm^2) adjacent to the OI implant. The cadaveric femoral bones were scanned on the

GE Lunar Prodigy Advance densitometer (General Healthcare, Madison WI, USA) using the scan mode "ortho hip" with standard settings. The scans started from the distal part of the fixture and was discontinued three sweeps proximal to the implant. The scans were acquired in the anterior–posterior (AP) plane with a scan-window 25.2 cm long and 15 cm wide.
One technician performed all clinical scans and the data was analysed with the enCORE 14.10.022 software (General Healthcare, Madison WI).

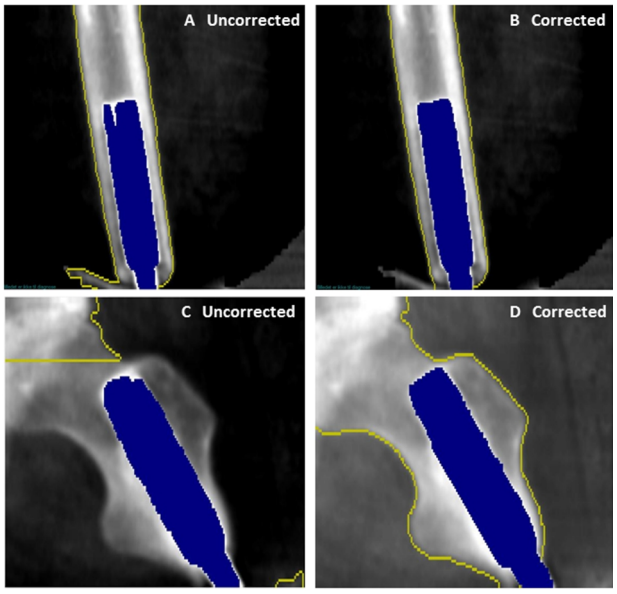
Clinical DXA double examinations - Study I

Double examinations were performed 2 years (SD 1.4) after second -stage surgery to determine the precision of the DXA system. The patients (n = 20) had two sets of DXA scans within a few minutes using the same setup as in Study II. Before the second scan, the patient was repositioned by either sitting or standing before returning to the supine position on the densitometer table.

Any difference in BMD between the first and second scans was assumed to be caused by measurement error, as both scans were performed within a few minutes.

The software uses a dynamic tissue detection algorithm and identifies bone, tissue, air and artefact automatically. In some cases, bone and artefact edges were incorrectly detected and demanded manual adjustment. Out of 40 scans (double examinations), 27 scans needed minor manual corrections of the implant and 21 scans needed either minor (n = 9), intermediate (n = 10), or major (n = 2) adjustment of the bone (Figure 9). All edge detection errors were corrected before analysis.

Figure 9: Edge detection errors.



(A-B): The typical site for minor artefact corrections was the proximal part of the implant. (C-D): A correction of a major bone and a minor artefact edge detection error.

Clinical DXA setup - Studies II, IV

It was no easy task to measure periprosthetic BMD in TFAs. The amputated leg could not be kept in the same position using a device. This was due to the patients having different stump lengths, various soft tissue thickness and/or pain after surgery. The best approach was for each patient to maintain the amputated leg in a still and relaxed position on the densitometer table. Rice bags were placed around the stump to imitate

the expected soft tissue volume, to yield support and to avoid air in the scan field. If individual measures were needed to position the patient, it was documented with a photo and written in the scan protocol.
Hip scans were conducted with the patient in a relaxed position without a foot brace rotating the hips inward. Spine scans and total body scans were conducted according to recommendations (158, 159).

DXA scan acquisition – Studies II, IV

In Study II, scans were acquired with the GE Lunar Prodigy Advance densitometer, except in one patient who was scanned on the iDXA scanner.
In Study IV, the GE Healthcare Lunar iDXA densitometer (General Healthcare, Madison WI, USA) was used.
Both instruments had three scan settings that adjusted the X-ray attenuation depending on the soft tissue thickness of each patient: thin (<13 cm), standard (13–25 cm) and thick (>25 cm). The scan modes and follow-up intervals are presented in Table 8. Double examinations were conducted for all regions. The follow-up analysis was conducted by positioning the ROI on the first scan and copying it to subsequent scans on the same patient to allow for easier template fitting. All DXA scans were analysed with the enCORE 14.10.022 software (General Healthcare, Madison WI, USA).

Table 8: DXA scan follow-up examinations in Studies II and IV

Follow-up (months)	0	1	3	6	7	9	12	18	24*	30
Hip (total)	X	X	X	X	X	X	X	X	X*	X
Spine (l1-l4)	X						X	X	X*	X
Periprosthetic (7-ROI template)		X	X	X	X	X	X	X	X*	X

Follow-up interval (in months) after S1-surgery
**No patients are examined in Study IV at 24-month follow-up.*

Analysis of bone turnover - Studies II, IV

Venous blood samples were drawn between 10AM and 15PM in non-fasting patients. Blood samples were allowed to clot at room temperature for 20 minutes and were centrifuged at 4000 RPM at 4 °C for 10 minutes. Blood serum was distributed in tubes and stored at −80 °C. All biochemical measurements were conducted as a batch analysis at the Department of Biochemistry, Aarhus University Hospital.

Blood serum samples were analysed using the following methods: PTH, P1NP, CTX, OC were determined by electrochemiluminescence analysis (Cobas 6000 modul e601, Roche Diagnostic A/S); vitamin D by high-performance liquid chromatography (API 5500, AB Sciex); Calcium was determined by absorption spectrophotometry; bone specific alkaline phosphatase by ELISA. The detection limit and precision is displayed in Table 9.

Table 9: Detection limit, reference interval and precision of blood tests

Blood tests	Detection limit	Reference interval*	Lower precision		Upper precision	
			Mean	± 2 SD	Mean	± 2 SD
Ca (mmol/L)	0.31	2.2 - 2.55	2.161	0.064	3.134	0.094
Vitamin D (nmol/L)	10	50 – 160	36.2	7.2	121.3	24.2
BASP (U/L)	1.2	Adjusted*	15	3	68	14
PTH (pmol/L)	0.4	1.6 - 6.9	2	0.4	10	2.0
P1NP (µg/L)	13	Adjusted*	30	2.2	205	15.2
CTX (µg/L)	0.03	Adjusted*	0.26	0.03	0.59	0.06
OC (µg/L)	2	Adjusted*	19	1.1	92	5.5

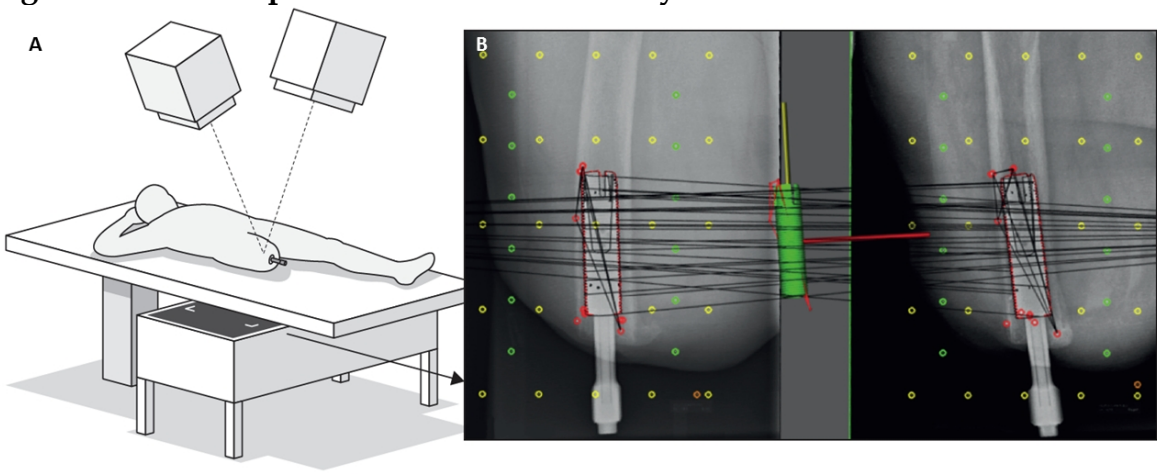
**The reference interval is adjusted by gender and age.*
The precision of each blood test is determined by the laboratory as 2 standard deviations of an upper and lower mean.

Radiostereometric analysis

RSA setup - Studies III, IV

A standardised RSA setup (Figure 10) was used to obtain stereoradiographs of the OI implant (155). The patients were positioned supine on the X-ray table and two ceiling-fixed, synchronised roentgen tubes (Acro-Ceil/Medira; Santax Medico; Denmark) pointed directly towards the OI implant and crossed the centre in a 40º angle of convergence. An unfocussed uniplanar carbon calibration box (Box 24; Medis Specials, Leiden, the Netherlands) was placed under the patient.

Figure 10: The setup of radiostereometric analysis.



A; The roentgen tubes point directly at the OI implant.
B; Screen view from the analysis displaying the cage markers (yellow and green), bone markers (red), and the CAD-fixture model (green model) fitted to the outlines of the implant on the stereoradiographs (red lines). The Y-axis (yellow line) is aligned with the model.

The roentgen dose depended on the size of the patient, but the standard setting was 90 kV and 6.3 mAs. A plastic container with 1 litre saline solution was placed under the abutment to compensate for differences in density on the stereoradiograph. All stereoradiographs were digitised images (Fuji CR (ST-VI IP), 200 µm pixels pitch).
Due to a hardware upgrade in 2014, the equipment was replaced with an automated RSA system (Adora RSA; NRT, Denmark) with ceiling-fixed and synchronised roentgen tubes (Varian Medical Systems, USA). We continued to use the same RSA setup, roentgen tube position, patient position, calibration box and exposure setting. The quality of the images improved as the stereoradiographs were direct digital with better resolution (Canon CXDI-70C, 125 µm pixel pitch).

During first-stage surgery, 6-10 tantalum beads (sized 1.0 mm) were inserted into the femoral cortical bone using a bead gun (Wennbergs Finmek AB, Sweden). The beads were dispersed proximally and distally around the OI implant to achieve the lowest condition number as possible.

Eleven CAD models (diameter 16 mm to 23 mm) matching the size of the inserted fixtures were acquired from the manufacturer (Integrum AB, Sweden). The models were created using 10,000 triangles to preserve a precise threaded surface of the fixture and they were implemented into the model-based RSA software by the software provider (RSAcore, Leiden, the Netherlands).

Follow-up RSA examination – Studies III, IV

Study III: Stereoradiographs were obtained at 7 (1 month after S2 surgery), 9, 12, 18, 24 and 30 months after S1 surgery.

Study IV: Stereoradiographs were obtained at 7, 9, 12, 18 and 30 months after S1 surgery.

RSA double examination – Studies III, IV

Study III: Double examinations were performed 6 months after second -stage surgery to determine the precision of the model-based RSA system. The same patient (n = 12) had two sets of stereoradiographs obtained within an interval of a few minutes. Between the examinations, the patient changed position by either standing or sitting before returning to the supine position on the X-ray table. The difference between the two examinations should be close to zero since the OI implant was not expected to move within such a short period.

Study IV: Four patients had tantalum markers welded onto the OI implant, thus a comparison between marker-based RSA (gold standard) and model-based RSA was performed to investigate the difference in clinical precision.

RSA analysis - Studies III, IV

The migration pattern is based on the X, Y, and Z translations and total translations (TT) of the OI implant. The Y-axis was placed in the centre of the implant aligned along the longitudinal direction. A positive motion along the Y-axis specified a proximal implant migration (subsidence). Total translation was calculated using the 3D Pythagorean Theorem ($TT = \sqrt{X^2 + Y^2 + Z^2}$).

The same stable bone markers on each stereoradiograph were selected for all follow-up analyses to avoid loose tantalum beads and to ensure a similar rigid body reference. The cut-off for stable markers was 0.35 mm (rigid body error).

Study III: The mean rigid body error was 0.14 (range 0.024 to 0.35). Six patients had a high condition number (CN >120), which resulted in a mean CN = 138.4 (range 37 to 406). High CNs (>120) were accepted as the translations were solely used to estimate implant migration.

Study IV: The mean rigid body error was 0.14 mm (range 0.03 to 0.32 mm) and the mean CN was 79.3 (range 39.3 to 113.9).

One observer analysed all stereoradiographs using the model-based RSA 4.0 (RSAcore, Leiden, the Netherlands) software.

Microdialysis

In vitro setup - Study IV

First, we investigated if CTX and P1NP could be detected using microdialysis. After transfemoral amputation a drain was placed near the surgical wound. The blood was collected from the drain into a measuring cylinder. The cylinder was placed on a magnetic stirrer and the blood was slowly stirred during the experiment.

A double lumen catheter (71 High Cut-Off Catheter, MDialysis AB, Sweden), with a 10 mm long semipermeable membrane and 100,000 Dalton cut-off was placed in the blood. A 107 microdialysis syringe pump was connected to the catheter and delivered the isotonic perfusate (T1, MDialysis AB, Sweden) at a constant flow rate. The theory was that the unbound metabolites (CTX and P1NP) would diffuse into the catheter seeking equilibrium with the perfusate. The dialysate was stored in small vials that could contain up to 250 µL.

The *in vitro* experiment lasted 24 hours. The flow rate was set at 2 µl/min and the vials were collected after 2, 4, 6 and 8 hours. Then, the flow rate was set on 0.5 µl/min and two additional vials were collected after 16 and 24 hours.

Blood (5 ml) was collected from the cylinder after 8 and 24 hours and centrifuged at 1500 g at 20 °C for 10 minutes. The blood serum and the dialysate were immediately stored in the freezer (-20 °C) until analysis.

In vivo setup – Study IV

After the fixture was implanted, two microdialysis (MD) catheters were placed under visual guidance. A 2 cm canal was drilled with a 2 mm drill in the iliac bone crest and 3-5 cm proximally from the fixture in the femoral bone. The catheters were tunnelled through the skin and carefully placed in the canals. Both MD catheters were fixed to the soft tissue and to the skin with sutures. The same setup was used (pump, catheter and perfusate) as in the *in vitro* study. The flow rate was set to 0.5 µl/min, sampling started 30 min after insertion and every 8 hours for 3 days. The vials were immediately stored in a -20°C freezer.

The gold tip at the end of the probe could be visualised on radiographs. Before removing the MD catheters, the patients had radiographs taken to determine the position of the gold tip.

Due to problems with displacement of the MD catheters the fixation technique was improved. After testing the technique on phantom bones and cadavers the following approach was used: An absorbable bone-anchor size 4/0 with polyester sutures (Mitek, DePuy Synthes) was inserted in the femoral bone. The MD catheter was pre-

pared with a small custom-made sleeve, made by fibrillar absorbable hemostat (Surgicel, Ethicon) tied together with small pouch sutures (Vicryl suture rapid 4/0, Ethicon). After the MD catheter was placed in the bone canal, the sleeve was tied to the bone-anchor without disrupting the flow in the catheter.

Sample size

Study I

A sample size was not calculated due to the study design.

Studies II, III

Studies II and III were designed as observational cohort studies and no sample sizes were calculated a priori.

Study IV

An a priori sample size was calculated. The primary outcome parameter assumed a two-sided alpha of 0.05, a power of 80% and a 1:1 ratio. We expected that bone mineral density would increase 0.2 g/cm^2 (SD = 0.1) in the denosumab group and 0.05 g/cm^2 (SD = 0.1) in the control group. Assuming a 10% dropout, a total of 16 patients would be needed.

Statistics

Considerations

It was possible to analyse the data from the observational cohort studies in many ways (160). The repeated measures design allowed us to monitor changes on an individual, group and/or cohort level at several time points. However, incomplete data due patients missing follow-up examinations could lead to an unbalanced result (161).

To handle the missing data and keep all patients in the analysis, we used a linear mixed model. In comparison, a traditional repeated-measures ANOVA would exclude the entire patient from the analysis if just a single time point was missing (162, 163). The linear mixed model analysis was based on the entire dataset and allowed for fixed and random factors. Fixed factors included constant covariates (such as gender) referring to population, whereas random factors referred to individual variations (162). The model assumptions were visually evaluated by two methods: First, the residuals were examined on qq plots, and second, the best linear unbiased predictors (BLUPS) or fitted values of the model were examined on a residuals vs BLUPS plot.
The results were reported as absolute or relative values at specific time points, and could be calculated as a difference between groups and/or time points.

However, another approach was needed to investigate the change from baseline until implant removal, since the latter happened at separate time points. The best approach was to gather the data at the last follow-up examination and create two new groups: the RI and NRI group. The NRI group was followed until the end of study period. The results depended on the normality of data and the use of parametric or non-parametric analyses (164).
To identify predictors possibly related to implant loosening a univariate logistic regression model was used. The independent variables (predictors) were measurements from the preoperative examination and changes at the last follow-up examination, whereas RI and NRI was the binary dependent variable. The odds was defined as the probability of implant removal divided by the probability of non-removal. The odds of implant removal increased by each unit of change in the predictor variable if odds ratio (OR) > 1 (164).

Data was analysed using the following approach: Normal distribution was assessed on qq plots. Parametric data were described as means with standard deviation (SD) or range (min-max) and tested using a two-sample *t* test. Non-parametric data were reported as medians with range (min-max) and tested using a ranksum or a Mann Whitney U test. Correlations were examined with a Spearman's rank test. All statistical analysis was performed using Stata software 13.1 (STATA corp., TX, USA). A *p*-value <0.05 was considered significant.

Study I

Ex vivo

A log linear mixed model with random effects (independent) for femoral bones and positions was used to calculate BMD in each position. The results were reported as percent change from neutral position and the difference between specimen A and B. The coefficient of variation was calculated in neutral and outermost positions to estimate the precision of the *ex vivo* study.

Figure 11: Precision

$$\text{RMS SD} = \sqrt{\frac{\sum_{i=1}^{m}(SD^2)}{m}}$$

$$\text{RMS \%CV} = \left(\sqrt{\frac{\sum_{i=1}^{m}(CV^2)}{m}}\right) x100$$

m =Number of patients; SD= Standard deviation; CV= Coefficient of variation

Adapted from: Bonnick SL et al. Importance of precision in bone density measurements. J Clin Densitom. 2001;4(2):105-10

Clinical study

Normal distribution was evaluated on qq plots. Short-term BMD precision was calculated according to the algorithms described in Figure 11 (165). The LSC was determined by multiplying RMS SD or RMS%CV by 2.77 (165). The LSC (with 95% statistical confidence level) represented the least significant BMD change at follow-up that was statistically significant.

Study II

A log linear mixed model with random effects for patients (with exponential variance between time points) was used. The primary outcome was reported as percent change with 95% confidence interval (CI) compared to baseline values. Changes in total hip BMD, spine BMD and blood tests were calculated using a preoperative baseline, whereas changes in periprosthetic BMD were calculated from a postoperative baseline (1 month after S1 surgery).

The subgroup analysis of the RI and NRI group was performed in a time-series analysis using the log linear mixed model and in a last follow-up analysis using parametric and non-parametric tests. OR was calculated using the preoperative BTMs and the change in periprosthetic BMD as predictors to estimate the odds of implant removal during 3 years of follow-up. Precision of spine and hip BMD was calculated as in Study I.

Study III

Implant migration was analysed with a linear mixed model with random effects for patients (with exponential variance between time points).

Translations were assessed along the X-, Y- and Z-axis. Total translation (TT) was calculated using the 3D Pythagoras theorem $T = \sqrt{X^2 + Y^2 + Z^2}$.

The primary outcome was the mean Y translation and TT with 95%CI. Continuous Y-migration and TT was defined as the difference in translation between 3 months and up to 24 months of follow-up.
Implant removal was reported if it occurred during the 60 months of follow-up. The migration pattern of the RI and NRI groups was calculated in a linear mixed model time-series analysis and in a last follow-up analysis. OR was used to determine the predictors of implant removal during 5 years of follow-up using the Y- and total translations at the last follow-up examinations.
The precision of the model-based RSA system was estimated by double examinations. The standard deviation represented the precision. The measurement error/precision limit was expressed as ±1.96 x SD according to the RSA ISO standard (155). Implant migration above the precision limit represented "true" implant migration.

Study IV
The DXA, RSA and blood test data were reported as mean (SD) and analysed using *t* tests at 18-month follow-up. The outcome values were presented as Δ difference (Δ_{diff}) with 95%CI between the groups using unadjusted p-values. Due to a limited number of patients the results should focus on the 95%CI and not the significance level. Only the allocation letter (A or B) of each group was known during the statistical analysis and writing of the manuscript.

Precision of marker-based RSA versus model-based RSA
The precision of the systems was presented as the standard deviation and was compared using Levene's test for equality of variances

Microdialysis
The degree of equilibration between the concentration in the dialysate and in the extracellular tissue was calculated as:
Relative recovery (RR) = $(C_{dialysate}-C_{perfusate})/(C_{tissue}-C_{perfusate})$ (128).
$C_{perfusate}$ = Concentration of perfusate, inflow to probe; $C_{dialysate}$ = Concentration of perfusate, outflow from probe; C_{tissue} = Extracellular tissue concentration.

6. Summary of results

Study I

The ex vivo study

Two cadaveric femoral bones with different thickness of the cortical bone lateral to the implant were scanned in different positions. A significant change in average periprosthetic BMD was found in most positions compared to the neutral position (Figure 12, $p < 0.04$). Average BMD changed up to 9.9% ($p < 0.041$).

An interaction was observed between the specimens and an effect of changing the bone position on BMD ($p < 0.001$). The relative BMD difference between specimen A and B on the lateral side (ROI 2, 3 and 4) changed up to 27.8% (flexion) and 11% (rotation) ($p < 0.001$). On the medial side (ROI 5, 6 and 7), the relative difference between the specimens changed up to 5.3% (flexion) and 10% (rotation) ($p < 0.036$). The precision of the *ex vivo* study ranged from 0.31% to 5.93% CV depending on ROI.

The in vivo study

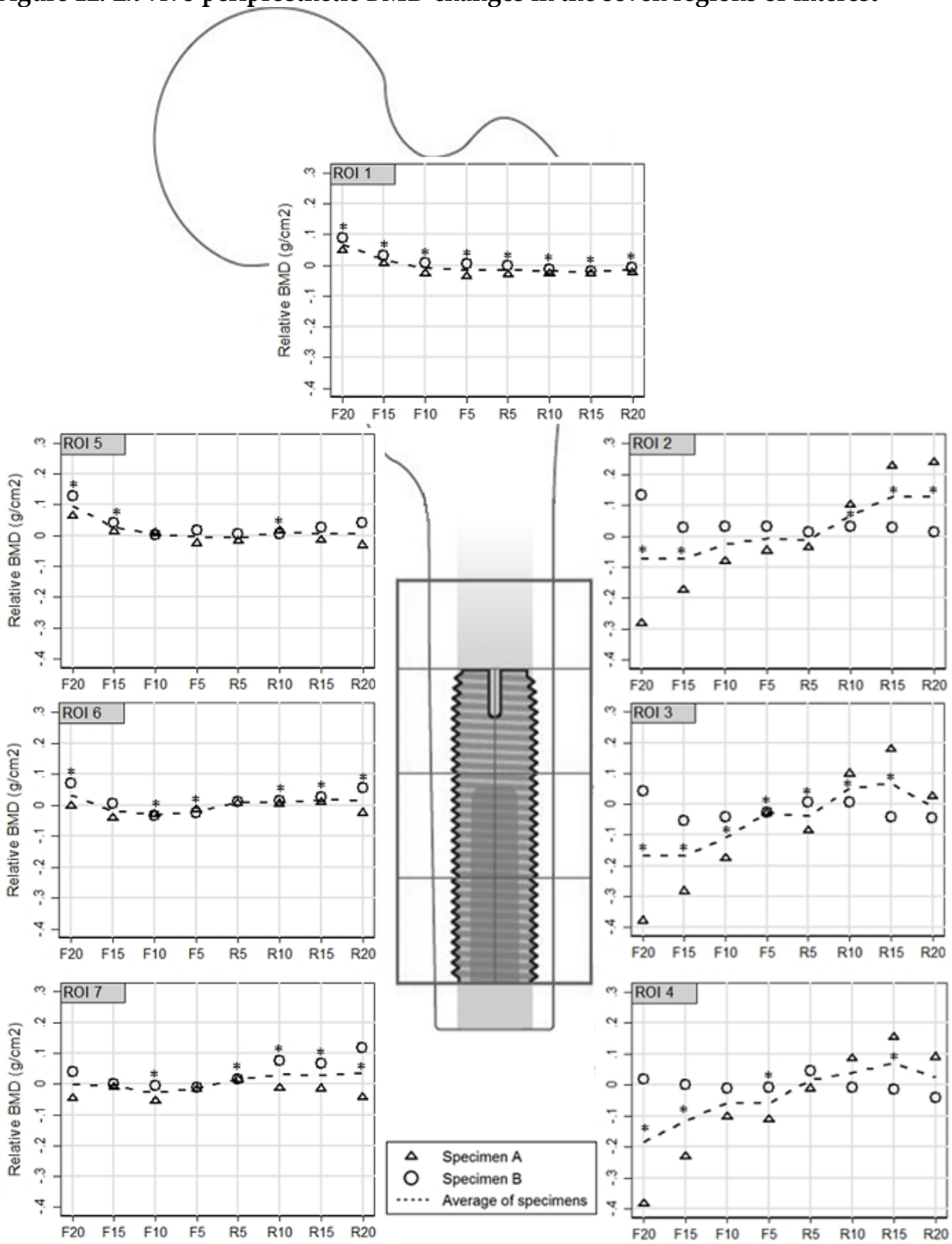
Twenty patients were scanned twice to determine the precision of the periprosthetic BMD measurements. The clinical precision RMS %CV ranged from 3.12% to 6.57% (Table 10).

Table 10: Precision BMD measurements

ROI	Mean BMD* (g/cm^2)	± SD (g/cm^2)	Range (g/cm^2)	RMS SD (g/cm^2)	RMS%CV	LSC-SD (g/cm^2)	LSC%CV
1	1.145	0.570	0.456 2.227	0.031	4.66	0.085	12.91
2	1.181	0.469	0.505 2.027	0.034	3.12	0.093	8.65
3	1.081	0.490	0.210 1.913	0.041	4.58	0.115	12.68
4	0.870	0.608	0.465 1.777	0.040	6.57	0.111	18.21
5	1.302	0.457	0.587 2.041	0.047	4.33	0.129	12.00
6	1.201	0.385	0.412 1.885	0.037	3.88	0.103	10.76
7	1.095	0.356	0.453 1.781	0.043	4.61	0.118	12.77

**Mean bone mineral density from double examinations (± standard deviation, range)*
Abbreviation: RMS, root mean square; LSC, least significant change

Figure 12: Ex vivo periprosthetic BMD changes in the seven regions of interest



*Changes in BMD from neutral position as a function of flexion (F5 to F20) or external rotation (R5 to R20). *Significant change of average BMD compared to neutral ($p < 0.04$).*

Study II

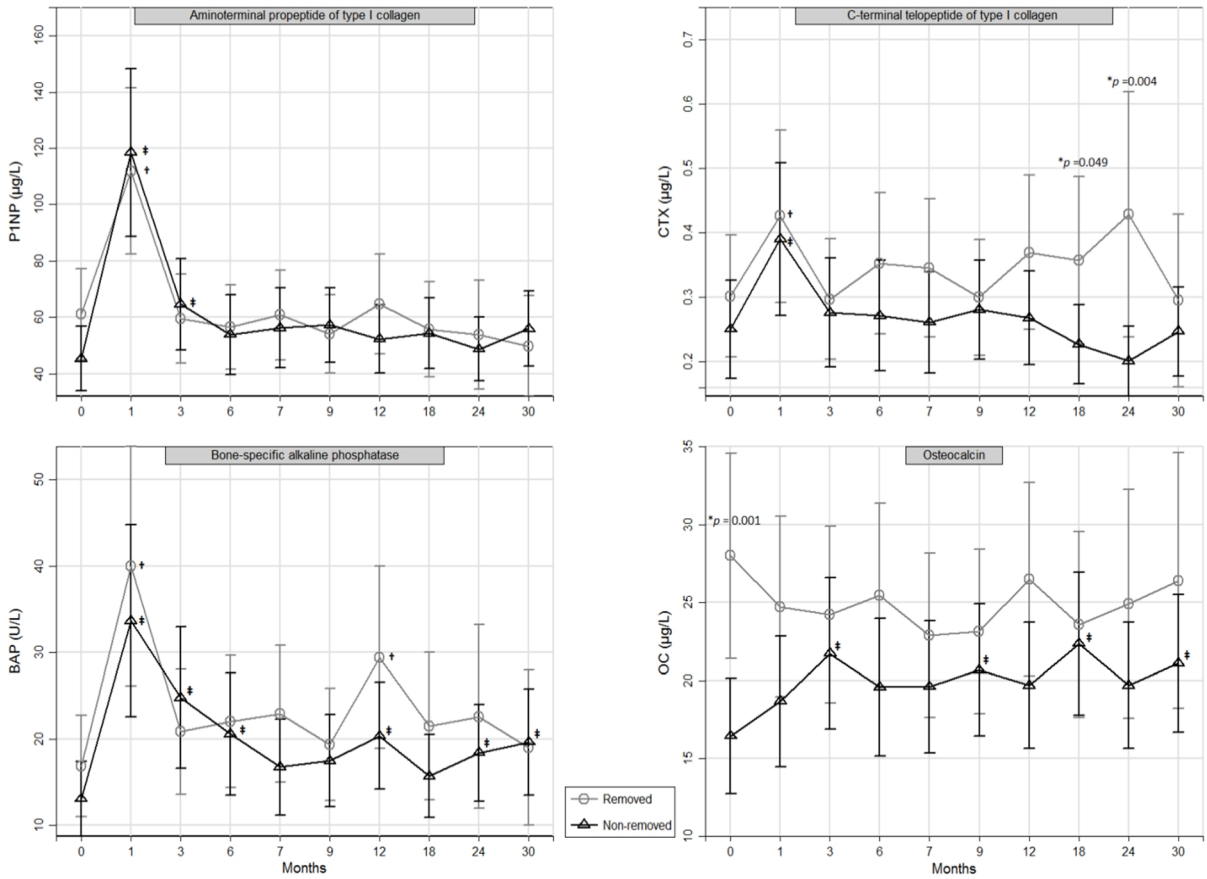
Periprosthetic BMD changes

No difference in periprosthetic BMD was found between the RI and NRI group in the time series analysis (Figure 13, p >0.12) or in the last follow-up analysis (p > 0.07). Compared to baseline, BMD decreased by 26% CI (3;44) to 40% CI (23;54) in the RI group (Figure 13, p < 0.03), whereas BMD in the NRI group regained baseline values at 30-month follow-up (Figure 13, p > 0.083).

Changes in bone turnover markers

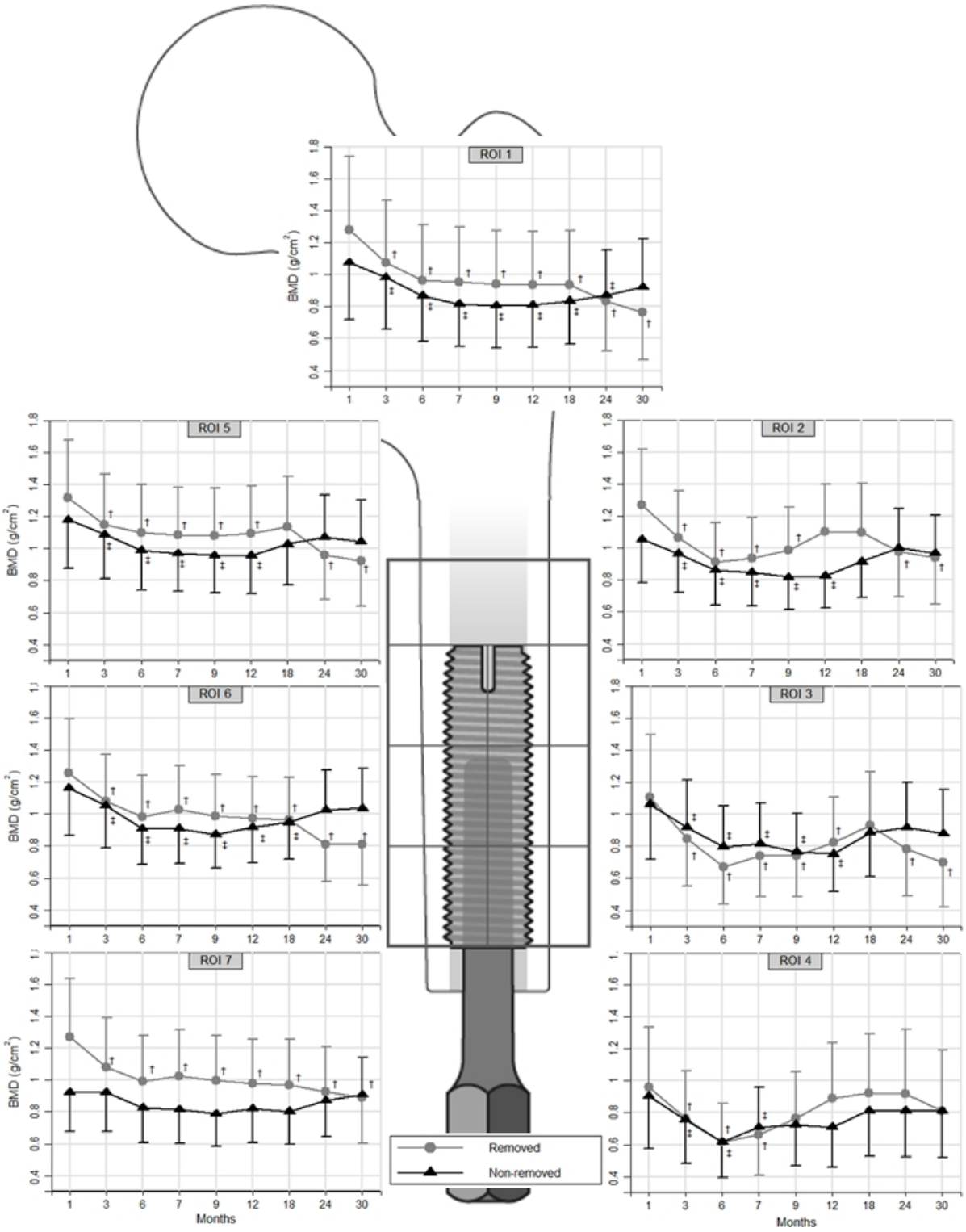
In the time series analysis, a significant difference was found in the OC concentrations between the groups at baseline (Figure 14, p = 0.001) and in the CTX concentrations at 18 and 24 months (Figure 14, p < 0.049), whereas no group difference was found in OC or CTX at the last follow-up analysis (p > 0.15).

Figure 14: BTMs (95%CI) in the RI and NRI group during 30 months of follow-up



Significant changes from the preoperative baseline in the removed (RI) group (†) and the non-removed (NRI) group (‡); Significant group difference (*)

Figure 13: BMD (95%CI) in the RI and NRI group during 30 months of follow-up

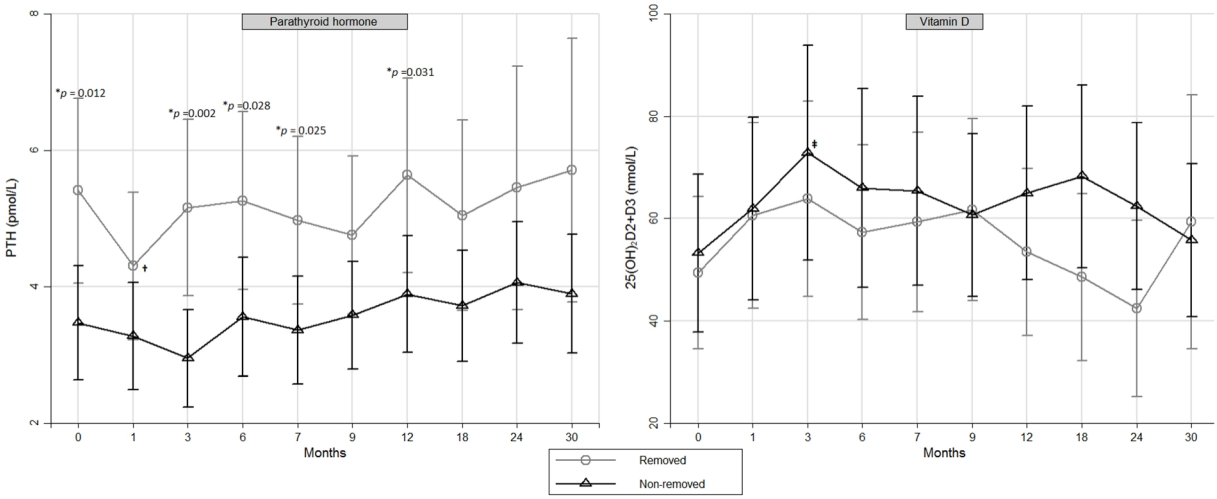


Statistically significant BMD changes from the postoperative baseline are labelled as (†) in the RI group and (‡) in the non-removed NRI group

Changes in bone metabolism

PTH concentrations were higher in the RI group during the first year compared to the NRI group (Figure 15, p <0.031). In the last follow-up analysis, PTH was 5.8 pmol/L 95%CI (4.6; 7.3) in the RI group and 4.0 pmol/L 95%CI (3.7; 4.5) in the NRI group (p = 0.01). There was no significant group difference in vitamin D concentrations (Figure 15, p > 0.08). There was an association between PTH and the odds of implant removal (Table 11)

Figure 15: PTH and vitamin D (95%CI) concentrations in the RI and NRI group.



Significant group difference (*); Significant changes from the preoperative baseline in the removed (RI) group (†) and the non-removed (NRI) group (‡)

Precision

The precision of periprosthetic BMD measurements was calculated in Study I. The precision (RMS %CV) of spine (L1-L4) BMD was 1.2%, the proximal hip (amputated side) was 2.1% and the intact proximal hip was 1.1%.

Table 11: Univariate logistic regression determining the OR of implant removal

Predictors	OR	95% CI lower	95% CI upper	Unadjusted p-value*
Preoperative data				
Age (year)	1.03	0.95	1.15	0.50
Femur length (cm)	1.11	0.95	1.30	0.20
Time since amputation (year)	0.99	0.92	1.07	0.80
PTH (pmol/L)	2.40	0.92	6.29	0.07
Vitamin D (nmol/L)	0.99	0.95	1.03	0.63
BASP (µg/L)	1.09	0.90	1.31	0.39
OC (µg/L)	1.09	0.98	1.21	0.13
CTX (µg/L)†	.	.	.	.
T-score hip amputated leg	0.86	0.56	1.34	0.52
T-score hip intact leg	0.63	0.26	1.52	0.31
T-score spine	0.85	0.51	1.41	0.52
BMD change‡				
ROI 1	0.86	0.18	4.17	0.85
ROI 2	1.32	0.16	11.06	0.80
ROI 3	0.63	0.08	4.87	0.66
ROI 4	0.66	0.08	5.33	0.69
ROI 5	0.93	0.14	6.12	0.94
ROI 6	0.57	0.07	4.60	0.60
ROI 7	1.64	0.20	13.23	0.64

** The p-values were not adjusted for multiple comparison; thus, the interpretation should focus on the 95% CI.*

† Not possible to calculate OR.

‡ Change in BMD from baseline last follow-up examination

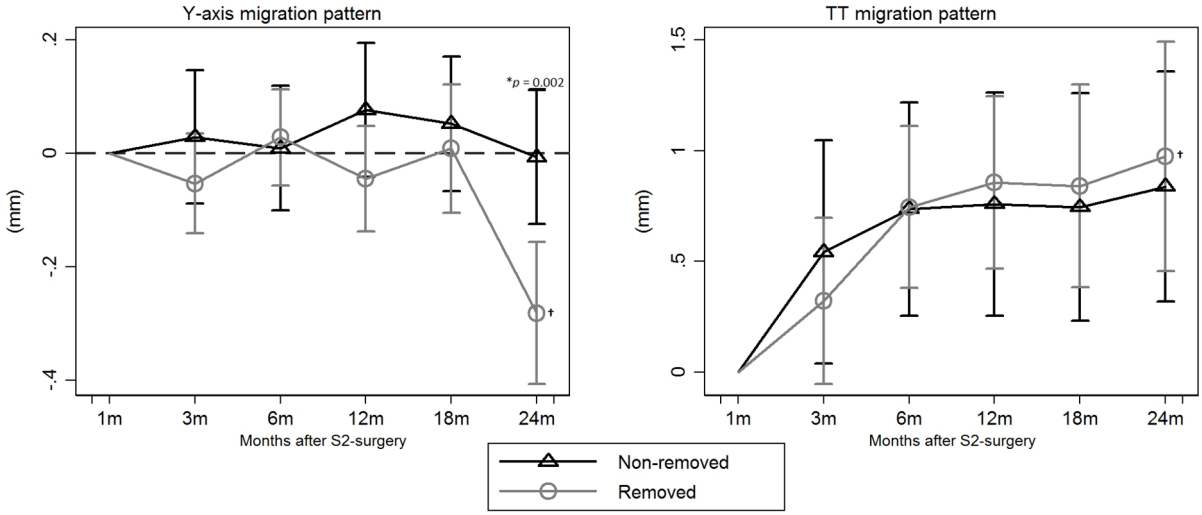
Study III

The migration pattern

The OI implants in the RI group migrated -0.28 mm 95%CI(-0.41; -0.16) along the Y-axis (Figure 16), which was more distal than -0.01 mm 95%CI(-0.12; 0.11) migration in the NRI group at 24-month follow-up (p = 0.002).

The RI group (Figure 16) migrated continuously -0.23 mm 95%CI(-0.36; -0.09, p = 0.001) in Y translations and 0.65 mm 95%CI(0.10; 1.21, p = 0.021) in TT.

Figure 16: Proximal/distal and TT migration pattern in the RI and NRI group



(*) Significant difference between the groups.
(†) Significant changes compared with early migration (3 month) indicating continuous migration.

At the last follow-up analysis, there was no difference between the groups in Y translations (p = 0.16) or TT (p = 0.76), but the RI group migrated continuously 0.55 mm 95%CI(-0.03; 1.14, p = 0.009) in TT. The OR of implant removal was 22.5 95%CI (1.6; 314) if the implants migrated distally and 0.04 95%CI (0.01; 0.62) if the implants migrated proximally (p = 0.021).

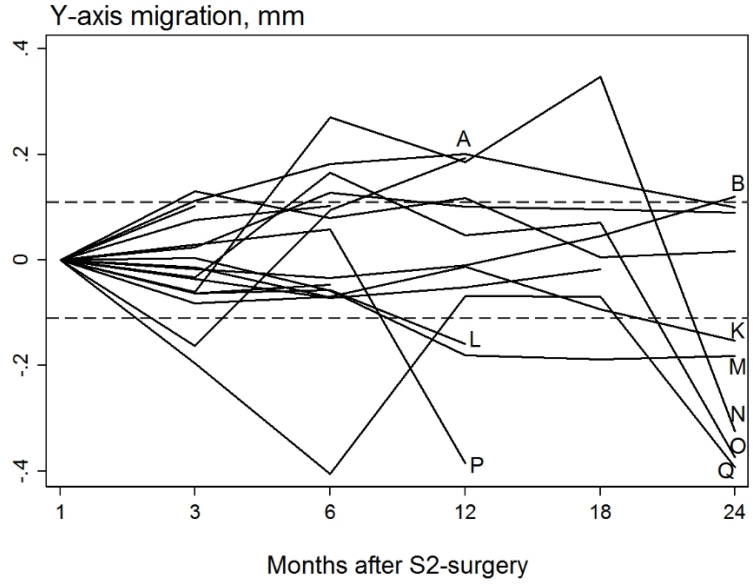
BMD effect on implant migration

There was no correlation between the OI implant migration (Y- and total translation) and the T-scores in the hips or lumbar spine (p > 0.45)

Migration pattern along the Y-axis

The precision measured along the y-axis was SD = 0.06 mm and the precision limit was 0.11 mm. Seven out of nine OI implants migrating above the y-axis precision limit were removed (Figure 17).

Figure 17: Individual implant migration pattern



OI-implants migrating above the precision limit (dashed line) are labelled according to the patient ID in paper III.

Precision

Twelve patients were scanned twice in the same day to determine the precision of model-based RSA (Table 12).

Table 12: Precision of implant migration

Double examination (n=12)				
Axis of translation	x	y	z	TT*
Mean (mm)	0.06	0.002	-0.06	0.51
SD (mm)	0.20	0.06	0.25	0.28
Precision limit**	0.39	0.11	0.49	0.56

* $T = \sqrt{X^2 + Y^2 + Z^2}$

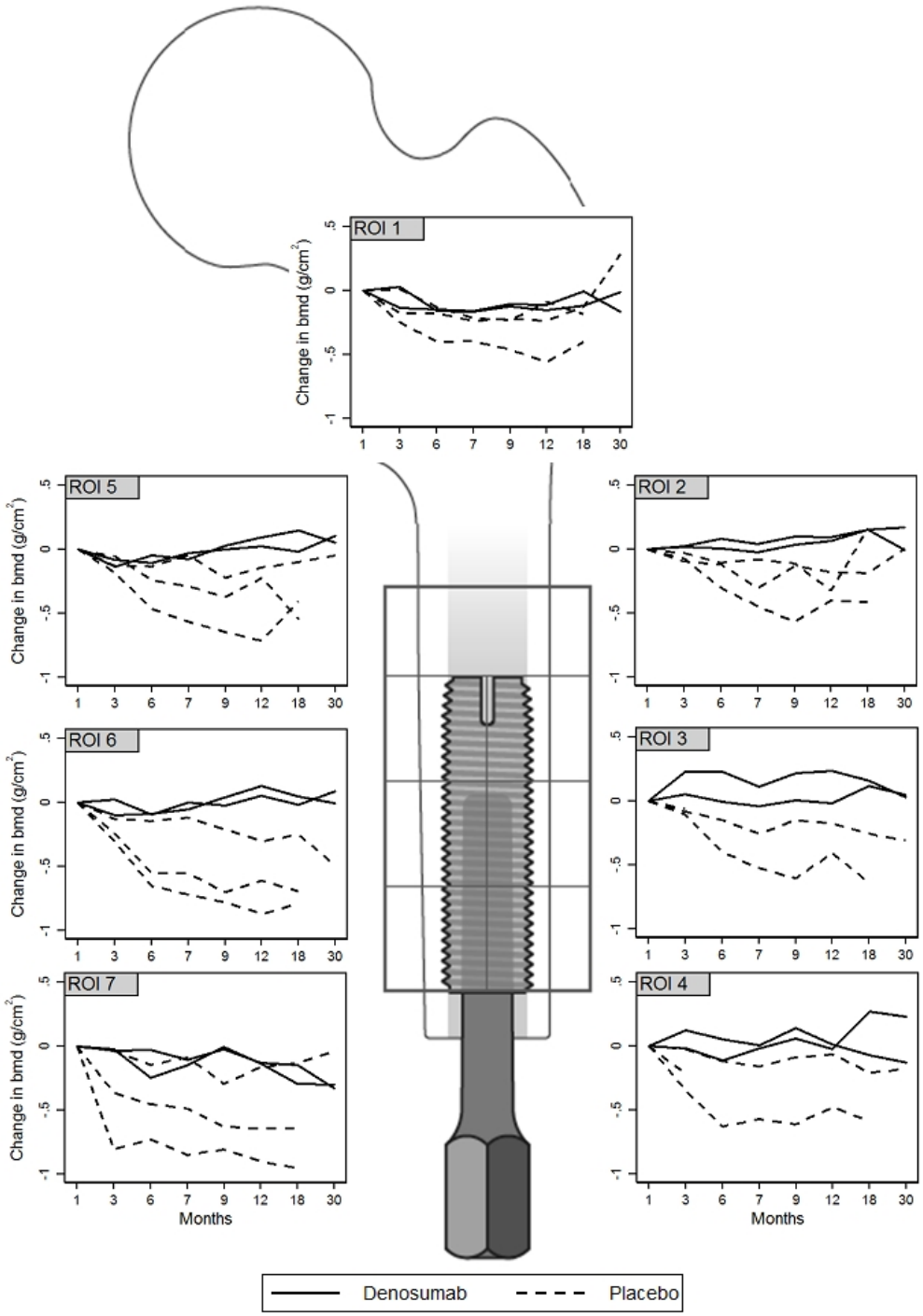
***Precision limit = SDx1.96.*

Study IV

BMD changes

At 18-month follow-up (Figure 18), the mean (SD) change in BMD (ROI 1-7) ranged from -0.22 (0.1) to 0.155 (0.01) g/cm² in the denosumab group), -0.578 (0.29) to -0.145 (0.29) g/cm² in the placebo group and the largest difference between groups was 0.59 g/cm² 95%CI(-0.09; 1.28, p = 0.07).

Figure 18: Change in periprosthetic BMD after denosumab or placebo treatment

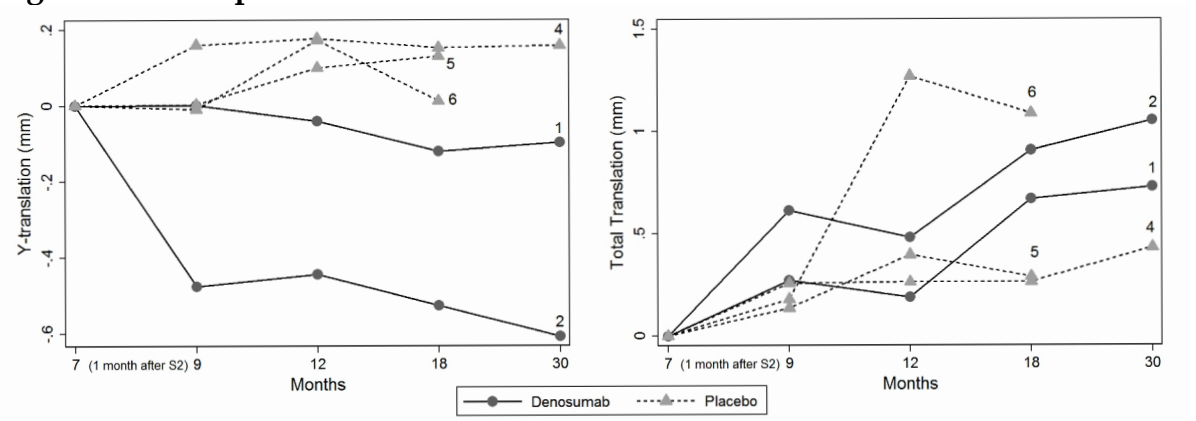


The change in BMD is presented as relative to the BMD value at 1-month follow-up.

The mean (SD) proximal hip BMD of the amputated leg increased by 0.03 g/cm² (0.01) in the denosumab group, decreased by -0.11 g/cm² (0.05) in the placebo group and the difference between groups was 0.14 g/cm² 95%CI (0.01; 0.26, p = 0.045) at 18-month follow-up.
The precision (RMS %CV) of BMD measurements (n = 5) was: 1.6 to 4.1% (ROI 1 to 7), 0.7% (spine), 3.6% (proximal hip amputated side) and 1.1% (proximal hip intact side).

Radiostereometric analysis
There was no difference between the mean (SD) -0.32 mm (0.29) distal OI implant migration along the Y-axis in the denosumab group compared with 0.1 mm (0.08) subsidence in the placebo-group at 18-month follow-up (p = 0.08). The mean (SD) TT was 0.79 mm (0.17) in the denosumab group and 0.54 mm (0.47) in the placebo group at 18-month follow-up (p = 0.55). (Figure 19).
One patient (id = 6) had a traumatic implant loosening that migrated 1.63 mm in TT between the last two follow-up examinations

Figure 19: OI implant Y translation and total translation



Each curve is labelled with patient id (1-6). Migration patterns after dmab treatment are presented as a solid line and as dashed line after placebo treatment.

The precision of model-based and marker-based RSA is presented in Table 13.

Table 13: Precision of marker-based and model based RSA implant migration (n=4)

	Marker-based RSA translations (mm)				**Marker-based RSA rotations (°)**			
	X	Y	Z	TT*	rX	rY	rZ	TR**
Mean	-0.01	-0.02	-0.10	0.11	-0.02	0.13	0.05	0.33
SD	0.03	0.03	0.06	0.06	0.22	0.35†	0.08	0.25†

	Model based RSA translations (mm)				**Model based RSA rotations (°)**			
	X	Y	Z	TT*	rX	rY	rZ	TR**
Mean	0.05	0.00	0.12	0.19	0.35	-2.09	-0.17	2.55
SD	0.09	0.04	0.17	0.11	0.21	1.92†	0.28	1.08†

*T T $= \sqrt{X^2 + Y^2 + Z^2}$

** T R $= \sqrt{rX^2 + rY^2 + rZ^2}$

† $p<0.05$ between the precisions determined around rY and TR

Blood tests

The largest difference between the groups was measured in CTX at 6-month follow-up: The mean (SD) CTX was 0.1 µg/L (0.07) in the denosumab group and 0.33 µg/L (0.09) in the placebo group (p = 0.052).

Microdialysis

In vitro study

CTX could be sampled with microdialysis, whereas P1NP could not be detected in the dialysate. The best relative recovery (35%) was achieved with a flow rate set at 0.5 µl/min. (Table 14)

Table 14: The relative recovery of CTX and P1NP

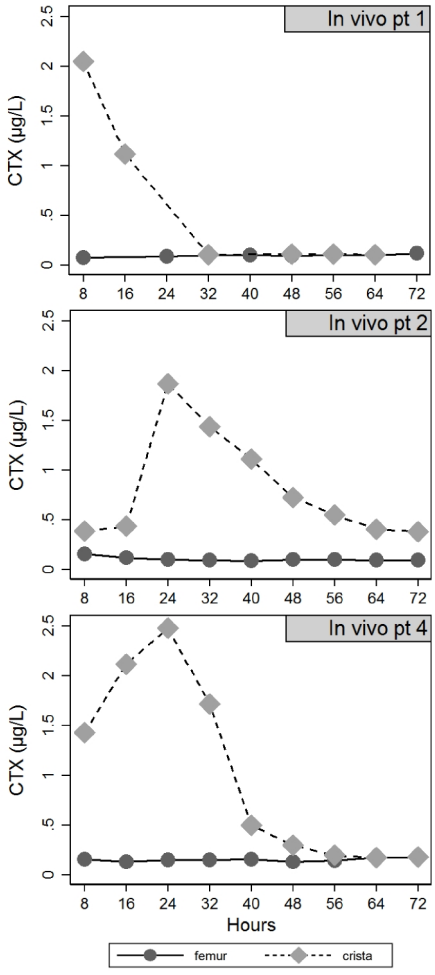
Time (hours)	Flow-rate	Blood serum		MD dialysate	
		CTX	P1NP	CTX	P1NP
2	2 µl/min			0.492	<5.0
4	2 µl/min			0.448	<5.0
6	2 µl/min			0.403	<5.0
8	2 µl/min	2.47	90.03	0.371	<5.0
16	0.5 µl/min			0.887	<5.0
24	0.5 µl/min	2.4	91.43	0.828	<5.0

In vivo study

Five out of six patients underwent microdialysis during S1 surgery and three patients had their samples analysed.

Microdialysis was discontinued in the clinical trial due to complications with the femoral (displacement n = 4, membrane rupture n = 1) and the pelvic MD catheters (displacement n = 2).
The femoral CTX concentrations ranged from 0.07 to 0.17µg/L during the 72-hour sampling period. The pelvic CTX concentrations ranged from 0.38 to 2.48 µg/L within the first 24 hours and decreased to 0.176 to 0.379 µg/L at 72 hours (Figure 20).

Figure 20: In vivo microdialysis of CTX



All femoral MD catheters were displaced.
The pelvic MD catheter was displaced into subcutis in patient 1

7. Conclusion

Study I

Knowing the precision of periprosthetic BMD measurements adjacent to the OI implants is essential to interpret clinical results. We found that changing the amputated leg position even 5° affects the periprosthetic BMD measurements. We proved that the clinical precision of periprosthetic DXA scans is acceptable (< 6.6%) and that adhering to the scan protocol causes minimal variations in leg position.

Study II

A drastic periprosthetic BMD loss is observed during the healing period between first-stage and second-stage surgery. The periprosthetic BMD remains low in implants that are later removed, whereas BMD returns to baseline values in surviving OI implants. An increase in CTX concentrations is measured in implants removed at the 18- and 24-month follow-up and is possibly related to implant loosening. Elevated PTH concentrations are measured in patients with removed implants. Low vitamin D level is found in one out of three patients.

Our recommendation is that elevated PTH and low vitamin D levels are corrected before surgery.

Study III

The removed OI implants migrate continuously (TT), whereas the non-removed OI implants are stable. We found that implant removal can be predicted if the implants migrated distally. The implant migration does not correlate with bone mineral density. Model-based RSA proved to be a precise method for monitoring the OI implant migration.

Study IV

The trend is that denosumab treatment preserves the periprosthetic BMD adjacent to the OI implant and in the ipsilateral proximal hip. We find no difference in implant migration between the denosumab group and the placebo group.

Microdialysis catheters are often displaced due to soft tissue motion in the anatomical region, but we proved that this method can detect CTX in patients treated with OI implants.

8. Discussion of results

Precision

The precision of the DXA and RSA system should be estimated to distinguish between the measurement error and the "true" effect (138, 155).

Proper patient positioning was one of the most important factors affecting BMD measurements. Therefore, the magnitude of the effect was investigated in an *ex vivo* study **(Study I)**. We found that changing the positions of the cadaveric femoral bones even 5° affected the BMD measurements adjacent to the OI implants significantly. This was in agreement with tibial cadaver studies showing that simulated flexion and rotation changed the periprosthetic BMD measurements around the tibial components up to 14.5% (135, 136). An earlier femoral cadaver study found that periprosthetic BMD measurements around hip stems varied within 5% between 15° of internal and 15° of external rotation (166). In our study, changing the femoral cadaver positions (20° flexion) affected the average BMD adjacent to the OI implants up to 9.9%. However, the cadaveric femoral bones with OI implants interacted differently when changing positions, which caused up to 28% difference in the periprosthetic BMD relative to the neutral position. The largest change in relative BMD was measured in the thin lateral cortical bone due to the inherent imprecision in small bone areas (167). This effect was also observed in clinical studies examining the BMD in small bone areas adjacent to acetabular cups, hip resurfacing and hip stem implants (137, 166, 168). These studies suggested using larger ROIs covering more bone to increase precision; however, detailed information about specific bone areas would be lacking.

In the clinical study, we estimated the precision of each periprosthetic ROI and found that RMS %CV was <6.6%. No studies have previously determined the precision of BMD measurements adjacent to OI implants, but %CV has been estimated around numerous hip stems using a similar 7-ROI model (Gruen zones). In these studies, the hip stem precision ranged from 3.4 to 5.7%CV depending on implant design (48, 169, 170). The precision was somewhat lower around knee implants than around hip stems with %CV ranging from 3.7 to 6% (135, 136, 171). Adjacent to the acetabular implant %CV ranged between 4.5 and 8.4% (168) The reason for the lower precision determined in periprosthetic bone around OI implants compared to hip and knee implants is that the leg was not fixated during DXA scans. However, the precision of BMD measurements adjacent to the OI implants was acceptable and comparable to the precision estimated in other implant studies.

In **Studies II and IV**, the precision of spine and intact proximal hip BMD corresponded with the precision determined by other fan-beam densitometers. The published precision of spine (L1-L4) BMD ranged from 1.1 to 1.45 %CV and proximal hip (total) BMD ranged from 0.67 to 1.0 %CV (138, 172, 173). The amputated proximal hip BMD had a

lower precision (RMS %CV = 2.1) owing to difficulties in reproducing the same position during double examinations (139).
In **Studies III and IV**, using model-based RSA the highest precision (SD) along the translational axis was determined along the y-axis (Study III; SD = 0.06 mm, Study IV; SD = 0.04 mm), which was superior to that determined in hip stems (SD = 0.17) (174) and similar to knee implants (SD = 0.058) (150).

Preoperative results

BMD

In Study II and IV, the BMD measured in the proximal hip on the amputated leg was markedly lower than the BMD in the intact hip. The estimated T-score in the hip of the amputated leg indicated osteopenia/osteoporosis, whereas the T-score in the contralateral hip and spine indicated normal bone. The BMD in the hip of the intact leg and the spine (L1-L4) were similar to BMD measured in hips and spine (L1-L4) of an age- and gender-matched European population (175). In study II, the BMD changes measured after amputation were located in the amputated leg and these results corresponds those reported in earlier studies concerning the BMD in lower-limb amputees (28-30, 35).
Animal studies reported that osteopenic/osteoporotic bone could delay the osseointegration process (176, 177); however, we were not able to demonstrate a correlation between the OI implant migration and the preoperative T-scores (hip, spine) in **Study III**. Clinical studies reported that a low systemic BMD reduced the initial cup and hip stem stability (178, 179). Our results may be explained by the fact that OI implant migration was more affected by infection or aseptic loosening than by a low BMD.

Vitamin D and PTH

In **Study II**, six patients (30%) had low vitamin D (<50 nmol/L) and three patients had elevated PTH concentrations (>6.9 pmol/L) before S1 surgery. Only one previous study described the vitamin D concentrations in lower-limb amputees and found that an overwhelming number (80%) had vitamin D insufficiency. This result was explained by the fact that the blood samples were collected during the winter months on the Northern hemisphere (180). In comparison, a survey of a general adult population (n = 6784) in Denmark found that 52.2% had low vitamin D (181). Animal studies indicated that vitamin D deficiency impaired the osseointegration process, whereas vitamin D supplementation improved implant osseointegration (182-184); however, even though several patients in **Study II** had low vitamin D, there was no difference in vitamin D concentrations between the NRI group and the RI group.

Factors associated with implant loosening

BMD

CT finite-element studies suggest that bone remodelling around the OI implants is caused by stress shielding imposed by the new loading conditions (51, 52). In **Study II**, the periprosthetic BMD decreased in all ROIs during the healing phase between S1 and S2 surgery. The changes in BMD were at this point not caused by stress shielding, since the OI-implants were not subjected to weight bearing. These changes may partly be explained by the disuse atrophy described after amputation (20, 27-30); however, the patients were already amputated and had a low BMD in the residual femur bone. Consequently, additional catabolic periprosthetic bone remodelling occurred after S1 surgery until the patient commenced weight bearing on the OI implant. Interestingly, a similar observation was made around dental implants with the greatest MBL occurring during the healing phase and a lesser amount of MBL after insertion of the abutment (4). We found that periprosthetic BMD in the NRI group returned to the baseline values at 30-month follow-up, whereas the BMD remained below baseline values in the RI group. The finding in the RI group was consistent with the excessive BMD loss found adjacent to clinically loose hip stems (aseptic loosening) previously described (109). Earlier studies reported that even severe stress shielding adjacent to hip stems did not cause more adverse events (like aseptic loosening or infection) and did not affect the clinical outcome (185-188). The logistic regression analysis showed that periprosthetic BMD changes did not increase the odds of implant loosening. This rather contradictory result may be explained by the BMD decrease in the RI group being caused by the osteolytic changes after septic/aseptic loosening and disuse osteoporosis due to pain (189).

CTX

Several studies have investigated the changes in CTX in aseptic implant loosening (109, 114, 115) as the osteolytic changes may lead to an increase in serum CTX. We found that the CTX concentrations in the RI group increased at 18- and 24-month follow-up compared with the NRI group. A similar trend was found by two case-control studies, reporting elevated serum CTX concentrations in patients with aseptic loosening (109, 115).

PTH

Surprisingly, the preoperative PTH concentrations were higher in the RI group than in the NRI group and remained elevated during the first year. We found a trend that the odds of implant loosening increased with elevated PTH concentrations. This association may be explained by continuous exposure of PTH increasing the osteoblastic RANKL production, which has proven to be a strong activator of osteoclastic bone resorption (102).

Increased osteoclastic activity and bone resorption around the OI implant would likely have affected the osseointegration negatively and resulted in early removal of the OI

implant. Interestingly, intermittent treatment with a daily low-dose recombinant PTH causes a peak in PTH concentrations that stimulates the osteoblastic activity in humans and promotes bones formation (190, 191). Animal studies have found that intermittent PTH treatment improved osseointegration (192); however, the effect of continuous PTH exposure on osseointegration in humans are lacking.

Implant migration

In **Study III**, the OI implants migrated continuously in the RI group, whereas the implants in the NRI group were stable. Ryd et al concluded that a continuous migration >0.2 mm (maximum total point of motion) at any follow-up one year after total knee arthroplasty could identify implants at risk of revision with a probability of 85% (145). This was supported by animal studies showing that continuous micromotion (0.15 mm) caused fibrous tissue formation at the bone-implant interface (193). Nebergall et al. found that in the OPRA cohort the OI implants were stable after 2, 5 and 7 years. The proximal/distal OI implant migration pattern in the OPRA cohort was 0.00 mm 95%CI (-0.53 to 0.13, n = 40) at 24 months (49). This migration pattern was similar to that of the NRI group in our study -0.01 mm 95%CI(-0.12; 0.11, n = 6) in distal direction at 24 months. Kärrholm et al. described that the probability of hip stem revision was greater than 50% if the stem subsided (distal direction) ≥1.2 mm at 2 years (144). Our removed OI implants, however, migrated -0.13 mm 95%CI(-0.25; -0.01) in distal direction (out of the bone) at the last follow-up examination (in 9 out of 11 removed implants), which was a strong predictor of implant removal (OR=22.5). Seven out of eleven removed OI implants migrated above the precision of the RSA system (y-axis precision limit = 0.11 mm). This supports the current understanding that early implant migration increases the risk of implant revision (194).

OI implant removals

In **Study III**, six fixtures (5 infections, 1 trauma) and 4 abutments (pain) out of 17 implants were removed within 5 years of follow-up. This was surprising as we were expecting similar implant survival as in the OPRA cohort. They reported that the OI implant survival at 2-years follow-up was 92%(81), whereas we experienced a similar number of implant removals as reported by the Swedish Osseointegration Team during the first 4 years. They removed 10 out of 15 OI implants (Integrum AB, Sweden) due to a learning curve of treatment development, but the cause of implant removal was not reported (9). The Swedish, Dutch and Australian Osseointegration Teams thoroughly reported that soft tissue infections around the abutment remained a recurring problem (81, 82, 84). The abutment is made of pure titanium, which has antiinfectious properties (195, 196), but still 90% of bacterial swaps from bone canal adjacent to the abutment were positive (80). The challenge with the percutaneous abutment was that it provided a permanent open access to the bone and thereby increased the risk of an ascending intramedullary infection. During S2 surgery, the tissue thickness around

the abutment was surgically reduced and the skin was grafted onto the distal bone. This technique allowed for soft tissue down-growth facing the abutment (197), but even with a soft-tissue-seal around the abutment, deep infections remain an unresolved problem (71). All implant designs attempting to seal off the intramedullary implant and eliminate the infection risk have failed so far (15, 198). However, lower infection risk is observed after reducing the soft tissue thickness around the percutaneous abutment, using biomaterials with antiinfectious properties and instructing the patients to follow a defined wound-hygiene protocol.

Effect of denosumab

In **Study IV**, patients treated with denosumab showed a trend towards less periprosthetic BMD loss than the placebo group. This trend was supported by a significant BMD increase in the proximal hip of the amputated leg in the denosumab group at 18-month follow-up. No clinical trials have investigated the effect of denosumab on periprosthetic BMD; however, several trials have investigated the antiresorptive effect of bisphosphonates around hip stems. A single dose of pamidronate administered 5 days after total hip surgery reduced the periprosthetic femoral bone loss in the calcar region compared with placebo, but it did not affect the acetabular cup migration (199). This effect was matched by a weekly dose of risedronate without affecting the implant migration or outcome (200). Even a daily low-dose of risedronate reduced the periprosthetic BMD loss in most ROIs around the hip stems (201). Compared with placebo, bisphosphonates had only a minor effect on periprosthetic BMD as the relative difference compared with baseline values ranged from 4 to 8%.

Assuming that the antiresorptive effect of denosumab corresponds to the effect of bisphosphonates, we found that denosumab may be even more potent as the mean BMD (SD) relative to baseline ranged from -0.22 (0.1) to 0.16 (0.01) g/cm^2 (denosumab group) and -0.58 (0.29) to -0.15 (0.29) g/cm^2 (placebo group) at 18-month follow-up.

A previous RCT study investigated the migration of tibial components and found that a daily dose of clodronate reduced prosthetic migration at one-year follow-up (202). We found no difference in OI implant migration between the groups, which corresponded with the hip stem migration reported in the bisphosphonate trials (200, 203). The serum CTX concentration was lower in patients receiving denosumab, which was expected since denosumab reduced the osteoclast activity and type-I collagen degradation (92).

We also wanted to measure the CTX concentration in the bone proximal to the OI implant using microdialysis. Following several attempts and method optimisation we concluded that this was not possible due to MD catheter displacements; however, some interesting trends were observed. In the *in vitro* study, we detected CTX and found that the RR could be expected to range from 16% to 35% depending on the flow-rate. A slower flow-rate improved the RR, which corroborates extant knowledge (127,

128). Another observation was that the CTX concentration in the iliac bone crest peaked within the first 24 hours after surgery. In comparison, the change in serum CTX concentrations after sustaining a fracture remained unchanged during the first 3 days and increased during the following months (105, 204).

9. Discussion of methods

The design of the studies

Study I was a repeated measure design and used two cadaveric femoral bones to examine the effect of changing positions on periprosthetic BMD. If changes in periprosthetic BMD could be demonstrated in this *ex vivo* study, the same problems could likely occur in the clinical study.

Studies II and III were originally designed as observational cohort studies, but due to the high numbers of implant removals, the statistical analysis focussed on differences between the RI and NRI group. The study population was small, but all patients treated with an OI implant in Denmark were included, which strengthens the generalizability of the results. The Danish cohort represented approximately 15% of all transfemoral amputated patients treated with an OI implant in the world (based on the published papers). We acknowledge, that the small sample size may increase the risk of type-II error; thus, interpretation of the results should focus on the 95% CI (205, 206). These studies were exploratory and the estimates were not adjusted for potential confounders between the groups for the following reasons: the study design was not truly comparative, the statistical model would likely fail to produce a sensible result as the sample size was small (206) and, finally, adjustment of confounding was not believed to contribute to the conclusions.

We chose to evaluate the predictors of implant removals using a simple univariate logistic regression model, since implant removal occurred at different time points and due to different causes. The results were severely limited by the small sample size; thus, we focused on the trends and the 95% CIs.

Study IV was designed as a Level 1 prospective, randomised study, but only six out of 16 planned patients were included before the enrolment of new patients ceased. The same challenges with a small sample size and an increased risk of type-II error pertained to this population.

Dual energy X-ray absorptiometry

The cadaveric femoral bones investigated in **Study I** differed with regard to age, BMD and cortical thickness, which represented the differences found around the OI implants *in vivo*. The average BMD of the femoral bones represented an inaccurate estimation of the BMD change, but provided the best model of interpretation to describe the overall effect of changing position. Subsequently, we clarified the inaccuracies by reporting the differences between the cadaveric femoral bones at each position.

(Studies II and IV) It was not possible to evaluate the change in periprosthetic BMD by comparing the postoperative DXA scans with the preoperative DXA scan. No landmarks on the preoperative scans could help determine the level of implantation or ROI location; thus, we used the first scan after S1 surgery as a baseline for all follow-up examinations. After S2 surgery, we could have used the abutment to lock the position of the leg and we could thereby possibly have improved the precision of the scans. However, the greatest variation in leg position occurred between S1 surgery and up to 3 months after S2 surgery due to common post-surgical complications (pain and swelling). Additionally, some patients experienced pain and could not use the abutment due to septic/aseptic loosening. Therefore, we found that the best approach was to keep the amputated leg in a relaxed and comfortable position at every follow-up examination, and the clinical double examinations proved this to be feasible (precision <6.6%). We calculated the precision for all regions investigated, even though the precision estimation may be biased by the small population. The actual biological change around the OI implants must be measured individually by determining if the BMD change in each ROI exceed the LSC.

Blood tests

Bone markers undergo a circadian rhythm of variation; therefore, blood samples were drawn during daytime (207). To further minimise this variation, a narrower time window to collect the blood samples would be needed. However, this was not possible due to the logistics of the study. The patients were non-fasting for the same reason (some had a very long transportation time), which may have increased the measured CTX concentration (111). Vitamin D undergoes seasonal variance resulting in a lower concentration during winter (208, 209). Additionally, the biochemical vitamin D precision was low; therefore, this analysis must be interpreted with caution. By conducting a batch analysis, the individual samples were analysed using the same method on the same day. This reduces the imprecision caused by inter-assay variation affecting the results. The BTM concentrations were markedly elevated after surgery, osteolysis and aseptic loosening; thus, the BTM changes in **Studies II and IV** were likely to represent a bone related incident.

Model-based radiostereometric analysis

It has been recommended that the accuracy of RSA is determined with a method considerably better than that of RSA (155). This could have been performed with a phantom model capable of determining the positions with an error of a few microns; however, this was not the aim of the thesis. We compared the clinical precision of model-based RSA to marker-based RSA (gold standard) (153) in **Study IV**. The estimated precision using model-based RSA was high; however, as expected the precision of marker-based RSA was superior along all translational and rotational axes, as it has been the case with hip and knee implants (150, 174). However, the precision was almost the same for marker-based and model-based RSA in Y translations. We reason, that the y-symmetrical geometric configuration and the detailed surface of the threads along the CAD-model probably fitted the model more precisely. Yet, this symmetrical shape was likely the cause for a reduced precision of the rotations about the y-axis when model-based RSA was used. Due to the high precision of RSA, implant stability can be assessed with even a small study population (154).

In **Study III,** patients with high condition numbers (CN >120) were included in the analysis. Thus, we focused solely on the translations (210).

Microdialysis

The challenge was to avoid displacement of the MD catheter and to estimate the RR. It was not a simple task to keep the MD catheter fixated in the bone for 3 days because the patients were mobilised and could move the amputated stump after surgery. A previous clinical trial experienced similar challenges of MD catheter displacement in patients mobilised after hip surgery. They reported that 11 out of 35 MD catheters were displaced during a 3-day follow-up period (119). In **Study IV**, six out of ten microdialysis catheters were displaced. We tried to improve the fixation technique with a resorbable bone-anchor and sleeve, but were unsuccessful. All catheters were placed in the bone under visual guidance during surgery. We did not get daily x-rays to visualise the catheter position, and thus we do not know exactly when the MD catheters were displaced during the 3-day observation period. This was a bias in the study as we had no certain valid CTX measurements from the bone.

We estimated the RR in an *in vitro* experiment before the trial and found the RR was 35% when it was sampled in blood from a surgical drain.

We do not know the RR of each individual MD catheter placed in each of the patients. However, we used the same MD catheter type and the same setting during surgery (same size of the bone canals and dead space around the catheters). Thus, it was acceptable to assume the RR would be same in each catheter even though the true extracellular concentration was unknown.

10. Perspectives and future research

Future research should focus on improving the knowledge of osseointegration and how to reduce the risk of implant failure. Based on the results described in this thesis, there seems to be a need for more research in this area.

1. We need to improve our understanding of the mechanisms involved in the loss of osseointegration.
2. We need greater knowledge on preoperative prognostic factors predicting the implant survival.
3. We need to identify more risk factors leading to septic/aseptic loosening.

Further research may benefit from deploying the methods described in this thesis:
As shown in Study II, the changes in BMD, CTX and PTH may be involved in the process leading to implant loosening. We recommend that a larger study with sufficient power be conducted, and for this a multicentre collaboration will be needed.
In Study III, we demonstrated that model-based RSA can detect implant loosening. Existing implants should be monitored closely with RSA and a stepwise introduction of all new implants for transfemoral amputees should be considered because the mechanisms leading to implant removal needs further investigation.
In Study IV, we found that denosumab may preserve BMD around the OI implants, but more patients were needed to reach a definite conclusion. We tried to use a novel method (microdialysis) to investigate the CTX changes in the bone near the OI implant, but had technical challenges. We recommend further investigations with antiresorptive treatment to investigate if preserving BMD around the OI implant positively affects implant migration and survival.

At the Orthopaedic Research Department, we plan to continue our investigation of OI implants. The six patients in Study IV will be followed for 2.5 years to investigate the effect of denosumab.
We are currently examining the BMD change around OI implants in upper limb amputation in relation to patient performance.
We are currently planning to perform a dynamic RSA investigation to examine the effect of stress-related inducible displacement during weight bearing on the OI implant.

References

1. Branemark PI. Osseointegration and its experimental background. The Journal of Prosthetic Dentistry. 1983;50(3):399-410.

2. Branemark PI, Hansson BO, Adell R, Breine U, Lindstrom J, Hallen O, et al. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. Scand J Plast Reconstr Surg Suppl. 1977;16:1-132.

3. Branemark R, Branemark PI, Rydevik B, Myers RR. Osseointegration in skeletal reconstruction and rehabilitation: a review. J Rehabil Res Dev. 2001 Mar-Apr;38(2):175-81.

4. Adell R, Lekholm U, Rockler B, Branemark PI. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. Int J Oral Surg. 1981 Dec;10(6):387-416.

5. Adell R, Eriksson B, Lekholm U, Brånemark P, Jemt T. Long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws. Int J Oral Maxillofac Implants. 1990;5(4):347-59.

6. Schoen PJ, Raghoebar GM, van Oort RP, Reintsema H, van der Laan, Bernard FAM, Burlage FR, et al. Treatment outcome of bone□anchored craniofacial prostheses after tumor surgery. Cancer. 2001;92(12):3045-50.

7. Tjellstrom A, Lindstrom J, Hallen O, Albrektsson T, Branemark PI. Osseointegrated titanium implants in the temporal bone. A clinical study on bone-anchored hearing aids. Am J Otol. 1981 Apr;2(4):304-10.

8. Jonsson S, Caine-Winterberger K, Branemark R. Osseointegration amputation prostheses on the upper limbs: methods, prosthetics and rehabilitation. Prosthet Orthot Int. 2011 Jun;35(2):190-200.

9. Hagberg K, Branemark R. One hundred patients treated with osseointegrated transfemoral amputation prostheses--rehabilitation perspective. J Rehabil Res Dev. 2009;46(3):331-44.

10. Albrektsson T, Branemark PI, Hansson HA, Lindstrom J. Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. Acta Orthop Scand. 1981;52(2):155-70.

11. Integrum AB. OPRA Stage 1 Surgical Technique. 2013.

12. Sullivan J, Uden M, Robinson KP, Sooriakumaran S. Rehabilitation of the transfemoral amputee with an osseointegrated prosthesis: the United Kingdom experience. Prosthet Orthot Int. 2003 Aug;27(2):114-20.

13. Integrum AB. OPRA Stage 2 Surgical Technique. 2013.

14. Aschoff HH, Kennon RE, Keggi JM, Rubin LE. Transcutaneous, distal femoral, intramedullary attachment for above-the-knee prostheses: an endo-exo device. J Bone Joint Surg Am. 2010 Dec;92 Suppl 2:180-6.

15. Juhnke DL, Beck JP, Jeyapalina S, Aschoff HH. Fifteen years of experience with Integral-Leg-Prosthesis: Cohort study of artificial limb attachment system. J Rehabil Res Dev. 2015;52(4):407-20.

16. Ephraim PL, Wegener ST, MacKenzie EJ, Dillingham TR, Pezzin LE. Phantom pain, residual limb pain, and back pain in amputees: results of a national survey. Arch Phys Med Rehabil. 2005 Oct;86(10):1910-9.

17. Ehde DM, Czerniecki JM, Smith DG, Campbell KM, Edwards WT, Jensen MP, et al. Chronic phantom sensations, phantom pain, residual limb pain, and other regional pain after lower limb amputation. Arch Phys Med Rehabil. 2000 Aug;81(8):1039-44.

18. Hoaglund FT, Jergesen HE, Wilson L, Lamoreux LW, Roberts R. Evaluation of Problems and Needs of Veteran Lower-Limb Amputees in the San Francisco Bay Area during the Period 1977–1980. Journal of Rehabilitation R&D. 1983;20(1):57-71.

19. Legro MW, Reiber G, del Aguila M, Ajax MJ, Boone DA, Larsen JA, et al. Issues of importance reported by persons with lower limb amputations and prostheses. J Rehabil Res Dev. 1999 Jul;36(3):155-63.

20. Burke MJ, Roman V, Wright V. Bone and joint changes in lower limb amputees. Ann Rheum Dis. 1978 Jun;37(3):252-4.

21. Sinha R, van den Heuvel WJ, Arokiasamy P, van Dijk JP. Influence of adjustments to amputation and artificial limb on quality of life in patients following lower limb amputation. Int J Rehabil Res. 2014 Mar;37(1):74-9.

22. Hagberg K, Branemark R. Consequences of non-vascular trans-femoral amputation: a survey of quality of life, prosthetic use and problems. Prosthet Orthot Int. 2001 Dec;25(3):186-94.

23. Hagberg K, Haggstrom E, Uden M, Branemark R. Socket versus bone-anchored trans-femoral prostheses: hip range of motion and sitting comfort. Prosthet Orthot Int. 2005 Aug;29(2):153-63.

24. Hagberg K, Hansson E, Branemark R. Outcome of percutaneous osseointegrated prostheses for patients with unilateral transfemoral amputation at two-year follow-up. Arch Phys Med Rehabil. 2014 Nov;95(11):2120-7.

25. Hagberg K, Branemark R, Gunterberg B, Rydevik B. Osseointegrated trans-femoral amputation prostheses: prospective results of general and condition-specific quality of life in 18 patients at 2-year follow-up. Prosthet Orthot Int. 2008 Mar;32(1):29-41.

26. Jacobs R, Branemark R, Olmarker K, Rydevik B, Van Steenberghe D, Branemark PI. Evaluation of the psychophysical detection threshold level for vibrotactile and pressure stimulation of prosthetic limbs using bone anchorage or soft tissue support. Prosthet Orthot Int. 2000 Aug;24(2):133-42.

27. Benichou C, Wirotius JM. Articular cartilage atrophy in lower limb amputees. Arthritis Rheum. 1982 Jan;25(1):80-2.

28. Rush PJ, Wong JS, Kirsh J, Devlin M. Osteopenia in patients with above knee amputation. Arch Phys Med Rehabil. 1994 Jan;75(1):112-5.

29. Flint JH, Wade AM, Stocker DJ, Pasquina PF, Howard RS, Potter BK. Bone mineral density loss after combat-related lower extremity amputation. J Orthop Trauma. 2014 Apr;28(4):238-44.

30. Kulkarni J, Adams J, Thomas E, Silman A. Association between amputation, arthritis and osteopenia in British male war veterans with major lower limb amputations. Clin Rehabil. 1998 Aug;12(4):348-53.

31. Smeltzer SC, Zimmerman V, Capriotti T. Osteoporosis risk and low bone mineral density in women with physical disabilities. Arch Phys Med Rehabil. 2005 Mar;86(3):582-6.

32. Ramirez JF, Isaza JA, Mariaka I, Velez JA. Analysis of bone demineralization due to the use of exoprosthesis by comparing Young's modulus of the femur in unilateral transfemoral amputees. Prosthet Orthot Int. 2011 Dec;35(4):459-66.

33. Kanis JA, Johnell O, Oden A, Jonsson B, De Laet C, Dawson A. Risk of hip fracture according to the World Health Organization criteria for osteopenia and osteoporosis. Bone. 2000 Nov;27(5):585-90.

34. Sevastikoglou JA, Eriksson U, Larsson SE. Skeletal changes of the amputation stump and the femur on the amputated side. A clinical investigation. Acta Orthop Scand. 1969;40(5):624-33.

35. Sherk VD, Bemben MG, Bemben DA. BMD and bone geometry in transtibial and transfemoral amputees. J Bone Miner Res. 2008 Sep;23(9):1449-57.

36. Tugcu I, Safaz I, Yilmaz B, Goktepe AS, Taskaynatan MA, Yazicioglu K. Muscle strength and bone mineral density in mine victims with transtibial amputation. Prosthet Orthot Int. 2009 Dec;33(4):299-306.

37. Gonzalez EG, Mathews MM. Femoral fractures in patients with lower extremity amputations. Arch Phys Med Rehabil. 1980 Jun;61(6):276-80.

38. Denton JR, McClelland SJ. Stump fractures in lower extremity amputees. J Trauma. 1985 Nov;25(11):1074-8.

39. Huiskes R. The various stress patterns of press-fit, ingrown, and cemented femoral stems. Clin Orthop Relat Res. 1990 Dec;(261)(261):27-38.

40. Brand R, Claes L. The law of bone remodelling: Lulius Wolff, Translated by P. Maquet and R. Furlong, Springer, 1986, DM 188, 126 pp. J Biomech. 1989;22(2):185-7.

41. Oh I, Harris WH. Proximal strain distribution in the loaded femur. An in vitro comparison of the distributions in the intact femur and after insertion of different hip-replacement femoral components. J Bone Joint Surg Am. 1978 Jan;60(1):75-85.

42. Engh CA, Bobyn JD. The influence of stem size and extent of porous coating on femoral bone resorption after primary cementless hip arthroplasty. Clin Orthop Relat Res. 1988 Jun;(231)(231):7-28.

43. Bobyn JD, Mortimer ES, Glassman AH, Engh CA, Miller JE, Brooks CE. Producing and avoiding stress shielding. Laboratory and clinical observations of noncemented total hip arthroplasty. Clin Orthop Relat Res. 1992 Jan;(274)(274):79-96.

44. Williams DF. Titanium as a metal for implantation. Part 1: physical properties. J Med Eng Technol. 1977 Jul;1(4):195,8, 202 contd.

45. Williams DF. Titanium as a metal for implantation. Part 2: biological properties and clinical applications. J Med Eng Technol. 1977 Sep;1(5):266-70.

46. Engh CA, McGovern TF, Bobyn JD, Harris WH. A quantitative evaluation of periprosthetic bone-remodeling after cementless total hip arthroplasty. J Bone Joint Surg Am. 1992 Aug;74(7):1009-20.

47. Maloney WJ, Sychterz C, Bragdon C, McGovern T, Jasty M, Engh CA, et al. The Otto Aufranc Award. Skeletal response to well fixed femoral components inserted with and without cement. Clin Orthop Relat Res. 1996 Dec;(333)(333):15-26.

48. Alm JJ, Makinen TJ, Lankinen P, Moritz N, Vahlberg T, Aro HT. Female patients with low systemic BMD are prone to bone loss in Gruen zone 7 after cementless total hip arthroplasty. Acta Orthop. 2009 Oct;80(5):531-7.

49. Nebergall A, Bragdon C, Antonellis A, Karrholm J, Branemark R, Malchau H. Stable fixation of an osseointegated implant system for above-the-knee amputees. Acta Orthop. 2012 Apr;83(2):121-8.

50. Xu W, Robinson K. X-ray image review of the bone remodeling around an osseointegrated trans-femoral implant and a finite element simulation case study. Ann Biomed Eng. 2008 Mar;36(3):435-43.

51. Tomaszewski PK, van Diest M, Bulstra SK, Verdonschot N, Verkerke GJ. Numerical analysis of an osseointegrated prosthesis fixation with reduced bone failure risk and periprosthetic bone loss. J Biomech. 2012 Jul 26;45(11):1875-80.

52. Tomaszewski PK, Verdonschot N, Bulstra SK, Rietman JS, Verkerke GJ. Simulated bone remodeling around two types of osseointegrated implants for direct fixation of upper-leg prostheses. J Mech Behav Biomed Mater. 2012 Nov;15:167-75.

53. Annual Report 2011 [Internet].; 2011 [].

54. Willert HG, Semlitsch M. Reactions of the articular capsule to wear products of artificial joint prostheses. J Biomed Mater Res. 1977 Mar;11(2):157-64.

55. Mirra JM, Marder RA, Amstutz HC. The pathology of failed total joint arthroplasty. Clin Orthop Relat Res. 1982 Oct;(170)(170):175-83.

56. Howie DW, Vernon-Roberts B, Oakeshott R, Manthey B. A rat model of resorption of bone at the cement-bone interface in the presence of polyethylene wear particles. J Bone Joint Surg Am. 1988 Feb;70(2):257-63.

57. Benz EB, Federman M, Godleski JJ, Bierbaum BE, Thornhill TS, Spector M. Transmission electron microscopy of intracellular particles of polyethylene from joint replacement prostheses: size distribution and cellular response. Biomaterials. 2001 Nov;22(21):2835-42.

58. Doorn PF, Campbell PA, Worrall J, Benya PD, McKellop HA, Amstutz HC. Metal wear particle characterization from metal on metal total hip replacements: transmission electron microscopy study of periprosthetic tissues and isolated particles. J Biomed Mater Res. 1998 Oct;42(1):103-11.

59. Schmalzried TP, Jasty M, Harris WH. Periprosthetic bone loss in total hip arthroplasty. Polyethylene wear debris and the concept of the effective joint space. J Bone Joint Surg Am. 1992 Jul;74(6):849-63.

60. Amstutz HC, Campbell P, Kossovsky N, Clarke IC. Mechanism and clinical significance of wear debris-induced osteolysis. Clin Orthop. 1992;276:7-18.

61. Esposito M, Hirsch JM, Lekholm U, Thomsen P. Biological factors contributing to failures of osseointegrated oral implants. (I). Success criteria and epidemiology. Eur J Oral Sci. 1998 Feb;106(1):527-51.

62. Takeshita F, Kuroki H, Yamasaki A, Suetsugu T. Histopathologic observation of seven removed endosseous dental implants. Int J Oral Maxillofac Implants. 1995 May-Jun;10(3):367-72.

63. Takeshita F, Ayukawa Y, Iyama S, Suetsugu T, Kido MA. A histologic evaluation of retrieved hydroxyapatite-coated blade-form implants using scanning electron, light, and confocal laser scanning microscopies. J Periodontol. 1996 Oct;67(10):1034-40.

64. Pikner SS, Grondahl K. Radiographic analyses of "advanced" marginal bone loss around Branemark dental implants. Clin Implant Dent Relat Res. 2009 Jun;11(2):120-33.

65. Quirynen M, Naert I, van Steenberghe D. Fixture design and overload influence marginal bone loss and fixture success in the Branemark system. Clin Oral Implants Res. 1992 Sep;3(3):104-11.

66. Rosen P, Clem D, Cochran D, Froum S, McAllister B, Renvert S, et al. Peri-implant mucositis and peri-implantitis: a current understanding of their diagnoses and clinical implications. J Periodontol. 2013 Apr;84(4):436-43.

67. Albrektsson T, Dahlin C, Jemt T, Sennerby L, Turri A, Wennerberg A. Is marginal bone loss around oral implants the result of a provoked foreign body reaction? Clin Implant Dent Relat Res. 2014 Apr;16(2):155-65.

68. Donath K, Laass M, Gunzl HJ. The histopathology of different foreign-body reactions in oral soft tissue and bone tissue. Virchows Arch A Pathol Anat Histopathol. 1992;420(2):131-7.

69. Trindade R, Albrektsson T, Tengvall P, Wennerberg A. Foreign Body Reaction to Biomaterials: On Mechanisms for Buildup and Breakdown of Osseointegration. Clin Implant Dent Relat Res. 2016 Feb;18(1):192-203.

70. Lew DP, Waldvogel FA. Osteomyelitis. Lancet. 2004 Jul 24-30;364(9431):369-79.

71. Tillander J, Hagberg K, Hagberg L, Branemark R. Osseointegrated titanium implants for limb prostheses attachments: infectious complications. Clin Orthop Relat Res. 2010 Oct;468(10):2781-8.

72. Murray DW, Rushton N. Mediators of bone resorption around implants. Clin Orthop. 1992;281:295-304.

73. Murray DW, Rushton N. Macrophages stimulate bone resorption when they phagocytose particles. J Bone Joint Surg Br. 1990 Nov;72(6):988-92.

74. Ingham E, Fisher J. The role of macrophages in osteolysis of total joint replacement. Biomaterials. 2005;26(11):1271-86.

75. Redlich K, Smolen JS. Inflammatory bone loss: pathogenesis and therapeutic intervention. Nature reviews Drug discovery. 2012;11(3):234-50.

76. Neale SD, Athanasou NA. Cytokine receptor profile of arthroplasty macrophages, foreign body giant cells and mature osteoclasts. Acta Orthop Scand. 1999 Oct;70(5):452-8.

77. CHIBA J, RUBASH HE, KANG JUNG K, IWAKI Y. The characterization of cytokines in the interface tissue obtained from failed cementless total hip arthroplasty with and without femoral osteolysis. Clin Orthop. 1994;300:304-12.

78. Devlin RD, Reddy SV, Savino R, Ciliberto G, Roodman GD. IL□6 Mediates the Effects of IL□1 or TNF, but Not PTHrP or 1, 25 (OH) 2D3, on Osteoclast□like Cell Formation in Normal Human Bone Marrow Cultures. Journal of bone and mineral research. 1998;13(3):393-9.

79. Itonaga I, Sabokbar A, Murray DW, Athanasou NA. Effect of osteoprotegerin and osteoprotegerin ligand on osteoclast formation by arthroplasty membrane derived macrophages. Ann Rheum Dis. 2000 Jan;59(1):26-31.

80. Lenneras M, Tsikandylakis G, Trobos M, Omar O, Vazirisani F, Palmquist A, et al. The clinical, radiological, microbiological and molecular profile of the skin-penetration site of transfemoral amputees treated with bone-anchored prostheses. J Biomed Mater Res A. 2016 Oct 17.

81. Branemark R, Berlin O, Hagberg K, Bergh P, Gunterberg B, Rydevik B. A novel osseointegrated percutaneous prosthetic system for the treatment of patients with transfemoral amputation: A prospective study of 51 patients. Bone Joint J. 2014 Jan;96-B(1):106-13.

82. Van de Meent H, Hopman MT, Frolke JP. Walking ability and quality of life in subjects with transfemoral amputation: a comparison of osseointegration with socket prostheses. Arch Phys Med Rehabil. 2013 Nov;94(11):2174-8.

83. Al Muderis M, Khemka A, Lord SJ, Van de Meent H, Frolke JP. Safety of Osseointegrated Implants for Transfemoral Amputees: A Two-Center Prospective Cohort Study. J Bone Joint Surg Am. 2016 Jun 1;98(11):900-9.

84. Muderis MA, Tetsworth K, Khemka A, Wilmot S, Bosley B, Lord SJ, et al. The Osseointegration Group of Australia Accelerated Protocol (OGAAP-1) for two-stage osseointegrated reconstruction of amputated limbs. Bone Joint J. 2016 Jul;98-B(7):952-60.

85. Aschoff HH, Juhnke DL. Endo-exo prostheses : Osseointegrated percutaneously channeled implants for rehabilitation after limb amputation. Unfallchirurg. 2016 May;119(5):421-7.

86. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature. 2003 May 15;423(6937):337-42.

87. Burgess TL, Qian Y, Kaufman S, Ring BD, Van G, Capparelli C, et al. The ligand for osteoprotegerin (OPGL) directly activates mature osteoclasts. J Cell Biol. 1999 May 3;145(3):527-38.

88. Fuller K, Murphy C, Kirstein B, Fox SW, Chambers TJ. TNFalpha potently activates osteoclasts, through a direct action independent of and strongly synergistic with RANKL. Endocrinology. 2002 Mar;143(3):1108-18.

89. Li J, Sarosi I, Yan XQ, Morony S, Capparelli C, Tan HL, et al. RANK is the intrinsic hematopoietic cell surface receptor that controls osteoclastogenesis and regulation of bone mass and calcium metabolism. Proc Natl Acad Sci U S A. 2000 Feb 15;97(4):1566-71.

90. Cummings SR, San Martin J, McClung MR, Siris ES, Eastell R, Reid IR, et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N Engl J Med. 2009 Aug 20;361(8):756-65.

91. Brown JP, Prince RL, Deal C, Recker RR, Kiel DP, de Gregorio LH, et al. Comparison of the effect of denosumab and alendronate on BMD and biochemical markers of bone turnover in postmenopausal women with low bone mass: a randomized, blinded, phase 3 trial. J Bone Miner Res. 2009 Jan;24(1):153-61.

92. Kendler DL, Roux C, Benhamou CL, Brown JP, Lillestol M, Siddhanti S, et al. Effects of denosumab on bone mineral density and bone turnover in postmenopausal women transitioning from alendronate therapy. J Bone Miner Res. 2010 Jan;25(1):72-81.

93. Fizazi K, Carducci M, Smith M, Damiao R, Brown J, Karsh L, et al. Denosumab versus zoledronic acid for treatment of bone metastases in men with castration-resistant prostate cancer: a randomised, double-blind study. Lancet. 2011 Mar 5;377(9768):813-22.

94. Sorensen AL, Hansen RL, Jorgensen PH. Denosumab may be a supplement to the surgical treatment of giant cell tumours of bone. Ugeskr Laeger. 2016 Sep 5;178(36):V03160204.

95. Bernhardsson M, Sandberg O, Aspenberg P. Anti-RANKL treatment improves screw fixation in cancellous bone in rats. Injury. 2015 Feb 19.

96. Aspenberg P, Agholme F, Magnusson P, Fahlgren A. Targeting RANKL for reduction of bone loss around unstable implants: OPG-Fc compared to alendronate in a model for mechanically induced loosening. Bone. 2011 Feb;48(2):225-30.

97. Skoldenberg O, Rysinska A, Eisler T, Salemyr M, Boden H, Muren O. Denosumab for treating periprosthetic osteolysis; study protocol for a randomized, double-blind, placebo-controlled trial. BMC Musculoskelet Disord. 2016 Apr 23;17:174,016-1036-5.

98. Medical biochemistry [Internet]. [Edinburgh]: Mosby Elsevier; 2005 [].

99. Garnero P, Sornay□Rendu E, Chapuy M, Delmas P. Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis. Journal of Bone and Mineral Research. 1996;11(3):337-49.

100. Garnero P, Hausherr E, Chapuy M, Marcelli C, Grandjean H, Muller C, et al. Markers of bone resorption predict hip fracture in elderly women: the EPIDOS Prospective Study. Journal of Bone and Mineral Research. 1996;11(10):1531-8.

101. Garnero P, Sornay□Rendu E, Duboeuf F, Delmas P. Markers of bone turnover predict postmenopausal forearm bone loss over 4 years: the OFELY study. Journal of Bone and Mineral Research. 1999;14(9):1614-21.

102. Silva BC, Bilezikian JP. Parathyroid hormone: anabolic and catabolic actions on the skeleton. Curr Opin Pharmacol. 2015 Jun;22:41-50.

103. Alvarez L, Guanabens N, Peris P, Vidal S, Ros I, Monegal A, et al. Usefulness of biochemical markers of bone turnover in assessing response to the treatment of Paget's disease. Bone. 2001 Nov;29(5):447-52.

104. Seibel MJ. Clinical use of markers of bone turnover in metastatic bone disease. Nat Clin Pract Oncol. 2005 Oct;2(10):504,17; quiz 1 p following 533.

105. Veitch SW, Findlay SC, Hamer AJ, Blumsohn A, Eastell R, Ingle BM. Changes in bone mass and bone turnover following tibial shaft fracture. Osteoporos Int. 2006;17(3):364-72.

106. Arabmotlagh M, Sabljic R, Rittmeister M. Changes of the biochemical markers of bone turnover and periprosthetic bone remodeling after cemented hip arthroplasty. J Arthroplasty. 2006 Jan;21(1):129-34.

107. Li MG, Thorsen K, Nilsson KG. Increased bone turnover as reflected by biochemical markers in patients with potentially unstable fixation of the tibial component. Arch Orthop Trauma Surg. 2004 Jul;124(6):404-9.

108. von Schewelov T, Carlsson A, Dahlberg L. Cross-linked N-telopeptide of type I collagen (NTx) in urine as a predictor of periprosthetic osteolysis. J Orthop Res. 2006 Jul;24(7):1342-8.

109. Wilkinson JM, Hamer AJ, Rogers A, Stockley I, Eastell R. Bone mineral density and biochemical markers of bone turnover in aseptic loosening after total hip arthroplasty. J Orthop Res. 2003 Jul;21(4):691-6.

110. Sumner DR, Ross R, Purdue E. Are there biological markers for wear or corrosion? A systematic review. Clin Orthop Relat Res. 2014 Dec;472(12):3728-39.

111. Hlaing TT, Compston JE. Biochemical markers of bone turnover - uses and limitations. Ann Clin Biochem. 2014 Mar;51(Pt 2):189-202.

112. Liu S, Virdi AS, Sena K, Hughes WF, Sumner DR. Bone turnover markers correlate with implant fixation in a rat model using LPS-doped particles to induced implant loosening. J Biomed Mater Res A. 2012 Apr;100(4):918-28.

113. Ross RD, Virdi AS, Liu S, Sena K, Sumner DR. Particle-induced osteolysis is not accompanied by systemic remodeling but is reflected by systemic bone biomarkers. J Orthop Res. 2014 Jul;32(7):967-73.

114. Streich NA, Gotterbarm T, Jung M, Schneider U, Heisel C. Biochemical markers of bone turnover in aseptic loosening in hip arthroplasty. Int Orthop. 2009 Feb;33(1):77-82.

115. Landgraeber S, Loer F, Heep H, Classen T, Grabellus F, Totsch M, et al. Tartrate-resistant acid phosphatase 5b and C-terminal telopeptides of type I collagen as markers for diagnosis of aseptic loosening after total hip replacement. Arch Orthop Trauma Surg. 2010 Apr;130(4):441-5.

116. Bito LZ, Davson H. Local variations in cerebrospinal fluid composition and its relationship to the composition of the extracellular fluid of the cortex. Exp Neurol. 1966 Mar;14(3):264-80.

117. Bogehoj M, Emmeluth C, Overgaard S. Blood flow and microdialysis in the human femoral head. Acta Orthop. 2007 Feb;78(1):56-62.

118. Ren G, Eiskjaer S, Kaspersen J, Christensen FB, Rasmussen S. Microdialysis of paraspinal muscle in healthy volunteers and patients underwent posterior lumbar fusion surgery. Eur Spine J. 2009 Nov;18(11):1604-9.

119. Lorenzen ND, Stilling M, Ulrich-Vinther M, Trolle-Andersen N, Pryno T, Soballe K, et al. Increased post-operative ischemia in the femoral head found by microdialysis by the posterior surgical approach: a randomized clinical trial comparing surgical approaches in hip resurfacing arthroplasty. Arch Orthop Trauma Surg. 2013 Dec;133(12):1735-45.

120. Forster Y, Gao W, Demmrich A, Hempel U, Hofbauer LC, Rammelt S. Monitoring of the first stages of bone healing with microdialysis. Acta Orthop. 2013 Feb;84(1):76-81.

121. Tottrup M, Bibby BM, Hardlei TF, Bue M, Kerrn-Jespersen S, Fuursted K, et al. Continuous versus short-term infusion of cefuroxime: assessment of concept based

on plasma, subcutaneous tissue, and bone pharmacokinetics in an animal model. Antimicrob Agents Chemother. 2015 Jan;59(1):67-75.

122. Tottrup M, Hardlei TF, Bendtsen M, Bue M, Brock B, Fuursted K, et al. Pharmacokinetics of cefuroxime in porcine cortical and cancellous bone determined by microdialysis. Antimicrob Agents Chemother. 2014 Jun;58(6):3200-5.

123. Stolle LB, Arpi M, Holmberg-Jorgensen P, Riegels-Nielsen P, Keller J. Application of microdialysis to cancellous bone tissue for measurement of gentamicin levels. J Antimicrob Chemother. 2004 Jul;54(1):263-5.

124. Stolle LB, Arpi M, Jorgensen PH, Riegels-Nielsen P, Keller J. In situ gentamicin concentrations in cortical bone: an experimental study using microdialysis in bone. Acta Orthop Scand. 2003 Oct;74(5):611-6.

125. Langberg H, Skovgaard D, Petersen LJ, Bulow J, Kjaer M. Type I collagen synthesis and degradation in peritendinous tissue after exercise determined by microdialysis in humans. J Physiol. 1999 Nov 15;521 Pt 1:299-306.

126. Langberg H, Rosendal L, Kjaer M. Training-induced changes in peritendinous type I collagen turnover determined by microdialysis in humans. J Physiol. 2001 Jul 1;534(Pt 1):297-302.

127. Ungerstedt U. Microdialysis--principles and applications for studies in animals and man. J Intern Med. 1991 Oct;230(4):365-73.

128. Chaurasia CS, Muller M, Bashaw ED, Benfeldt E, Bolinder J, Bullock R, et al. AAPS-FDA workshop white paper: microdialysis principles, application and regulatory perspectives. Pharm Res. 2007 May;24(5):1014-25.

129. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. World Health Organ Tech Rep Ser. 1994;843:1-129.

130. Prevention and management of osteoporosis: report of a WHO scientific group. . Geneva 2003.

131. Lewiecki EM, Watts NB, McClung MR, Petak SM, Bachrach LK, Shepherd JA, et al. Official positions of the international society for clinical densitometry. J Clin Endocrinol Metab. 2004 Aug;89(8):3651-5.

132. Engh CA,Jr, McAuley JP, Sychterz CJ, Sacco ME, Engh CA S. The accuracy and reproducibility of radiographic assessment of stress-shielding. A postmortem analysis. J Bone Joint Surg Am. 2000 Oct;82-A(10):1414-20.

133. Cohen B, Rushton N. Accuracy of DEXA measurement of bone mineral density after total hip arthroplasty. J Bone Joint Surg Br. 1995 May;77(3):479-83.

134. Kroger H, Miettinen H, Arnala I, Koski E, Rushton N, Suomalainen O. Evaluation of periprosthetic bone using dual-energy x-ray absorptiometry: precision of the method and effect of operation on bone mineral density. J Bone Miner Res. 1996 Oct;11(10):1526-30.

135. Stilling M, Soballe K, Larsen K, Andersen NT, Rahbek O. Knee flexion influences periprosthetic BMD measurement in the tibia. Suggestions for a reproducible clinical scan protocol. Acta Orthop. 2010 Aug;81(4):463-70.

136. Tjornild M, Soballe K, Bender T, Stilling M. Reproducibility of BMD measurements in the prosthetic knee comparing knee-specific software to traditional DXA software: a clinical validation. J Clin Densitom. 2011 Apr-Jun;14(2):138-48.

137. Penny JO, Ovesen O, Brixen K, Varmarken JE, Overgaard S. Bone mineral density of the femoral neck in resurfacing hip arthroplasty. Acta Orthop. 2010 Jun;81(3):318-23.

138. Baim S, Wilson CR, Lewiecki EM, Luckey MM, Downs RW,Jr, Lentle BC. Precision assessment and radiation safety for dual-energy X-ray absorptiometry: position paper of the International Society for Clinical Densitometry. J Clin Densitom. 2005 Winter;8(4):371-8.

139. Lekamwasam S, Lenora RS. Effect of leg rotation on hip bone mineral density measurements. J Clin Densitom. 2003 Winter;6(4):331-6.

140. Soininvaara TA, Harju KA, Miettinen HJ, Kroger HP. Periprosthetic bone mineral density changes after unicondylar knee arthroplasty. Knee. 2013 Mar;20(2):120-7.

141. Nysted M, Benum P, Klaksvik J, Foss O, Aamodt A. Periprosthetic bone loss after insertion of an uncemented, customized femoral stem and an uncemented anatomical stem. A randomized DXA study with 5-year follow-up. Acta Orthop. 2011 Aug;82(4):410-6.

142. Selvik G. Roentgen stereophotogrammetric analysis. Acta Radiol. 1990 Mar;31(2):113-26.

143. Karrholm J, Gill RH, Valstar ER. The history and future of radiostereometric analysis. Clin Orthop Relat Res. 2006 Jul;448:10-21.

144. Karrholm J, Borssen B, Lowenhielm G, Snorrason F. Does early micromotion of femoral stem prostheses matter? 4-7-year stereoradiographic follow-up of 84 cemented prostheses. J Bone Joint Surg Br. 1994 Nov;76(6):912-7.

145. Ryd L, Albrektsson BE, Carlsson L, Dansgard F, Herberts P, Lindstrand A, et al. Roentgen stereophotogrammetric analysis as a predictor of mechanical loosening of knee prostheses. J Bone Joint Surg Br. 1995 May;77(3):377-83.

146. Valstar ER, Vrooman HA, Toksvig-Larsen S, Ryd L, Nelissen RG. Digital automated RSA compared to manually operated RSA. J Biomech. 2000 Dec;33(12):1593-9.

147. Borlin N, Thien T, Karrholm J. The precision of radiostereometric measurements. Manual vs. digital measurements. J Biomech. 2002 Jan;35(1):69-79.

148. Valstar ER, de Jong FW, Vrooman HA, Rozing PM, Reiber JH. Model-based Roentgen stereophotogrammetry of orthopaedic implants. J Biomech. 2001 Jun;34(6):715-22.

149. Kaptein BL, Valstar ER, Stoel BC, Rozing PM, Reiber JH. A new model-based RSA method validated using CAD models and models from reversed engineering. J Biomech. 2003 Jun;36(6):873-82.

150. Kaptein BL, Valstar ER, Stoel BC, Reiber HC, Nelissen RG. Clinical validation of model-based RSA for a total knee prosthesis. Clin Orthop Relat Res. 2007 Nov;464:205-9.

151. Lorenzen ND, Stilling M, Jakobsen SS, Gustafson K, Soballe K, Baad-Hansen T. Marker-based or model-based RSA for evaluation of hip resurfacing arthroplasty? A clinical validation and 5-year follow-up. Arch Orthop Trauma Surg. 2013 Nov;133(11):1613-21.

152. Baad-Hansen T, Kold S, Kaptein BL, Soballe K. High-precision measurements of cementless acetabular components using model-based RSA: an experimental study. Acta Orthop. 2007 Aug;78(4):463-9.

153. Kaptein BL, Valstar ER, Stoel BC, Rozing PM, Reiber JH. A new type of model-based Roentgen stereophotogrammetric analysis for solving the occluded marker problem. J Biomech. 2005 Nov;38(11):2330-4.

154. Valstar ER, Gill R, Ryd L, Flivik G, Borlin N, Karrholm J. Guidelines for standardization of radiostereometry (RSA) of implants. Acta Orthop. 2005 Aug;76(4):563-72.

155. RSA ISO standard. Implants for surgery — Roentgen stereophotogrammetric analysis for the assessment of migration of orthopaedic implants. 2013. Report No.: ISO 16087.

156. Malchau H. On the importance of stepwise introduction of new hip implant technology : assessment of total hip replacement using clinical evaluation, radiostereometry, digitised radiography and a national hip registry.[dissertation]. University of Gothenburg; 1995.

157. Nelissen RG, Pijls BG, Karrholm J, Malchau H, Nieuwenhuijse MJ, Valstar ER. RSA and registries: the quest for phased introduction of new implants. J Bone Joint Surg Am. 2011 Dec 21;93 Suppl 3:62-5.

158. Watts NB. Fundamentals and pitfalls of bone densitometry using dual-energy X-ray absorptiometry (DXA). Osteoporos Int. 2004 Nov;15(11):847-54.

159. Libber J, Binkley N, Krueger D. Clinical observations in total body DXA: technical aspects of positioning and analysis. J Clin Densitom. 2012 Jul-Sep;15(3):282-9.

160. Morshed S, Tornetta P,3rd, Bhandari M. Analysis of observational studies: a guide to understanding statistical methods. J Bone Joint Surg Am. 2009 May;91 Suppl 3:50-60.

161. Goldstein H. Tutorial in Biostatistics□Longitudinal data analysis (repeated measures) in clinical trials by P.□S. Albert, Statistics in Medicine, 1999, 18, 1707–1732. Stat Med. 2000;19(13):1821-.

162. West BT, Welch KT, Galecki AT. LINEAR MIXED MODELS A Practical Guide Using Statistical Software. 1st ed. Chapman and Hall/CRC; 2007.

163. Cnaan A, Laird NM, Slasor P. Using the general linear mixed model to analyse unbalanced repeated measures and longitudinal data. Stat Med. 1997 Oct 30;16(20):2349-80.

164. Kirkwood BR, Sterne JAC. Essential medical statistics. 2nd ed. Blackwell; 2003.

165. Bonnick SL, Johnston CC,Jr, Kleerekoper M, Lindsay R, Miller P, Sherwood L, et al. Importance of precision in bone density measurements. J Clin Densitom. 2001 Summer;4(2):105-10.

166. Mortimer ES, Rosenthall L, Paterson I, Bobyn JD. Effect of rotation on periprosthetic bone mineral measurements in a hip phantom. Clin Orthop Relat Res. 1996 Mar;(324)(324):269-74.

167. Engelke K, Gluer CC, Genant HK. Factors influencing short-term precision of dual X-ray bone absorptiometry (DXA) of spine and femur. Calcif Tissue Int. 1995 Jan;56(1):19-25.

168. Jayasuriya RL, Wilkinson JM. Pelvic periprosthetic bone mineral density measurement around cemented vs cementless acetabular prostheses. J Clin Densitom. 2014 Jan-Mar;17(1):116-20.

169. Digas G, Karrholm J. Five-year DEXA study of 88 hips with cemented femoral stem. Int Orthop. 2009 Dec;33(6):1495-500.

170. Lazarinis S, Mattsson P, Milbrink J, Mallmin H, Hailer NP. A prospective cohort study on the short collum femoris-preserving (CFP) stem using RSA and DXA. Primary stability but no prevention of proximal bone loss in 27 patients followed for 2 years. Acta Orthop. 2013 Feb;84(1):32-9.

171. Gilchrist N, Hooper G, Frampton C, Maguire P, Heard A, March RL, et al. Measurement of bone density around the Oxford medial compartment knee replacement using iDXA. A precision study. J Clin Densitom. 2013 Apr-Jun;16(2):178-82.

172. Henzell S, Dhaliwal S, Pontifex R, Gill F, Price R, Retallack R, et al. Precision error of fan-beam dual X-ray absorptiometry scans at the spine, hip, and forearm. J Clin Densitom. 2000 Winter;3(4):359-64.

173. Krueger D, Vallarta-Ast N, Checovich M, Gemar D, Binkley N. BMD measurement and precision: a comparison of GE Lunar Prodigy and iDXA densitometers. J Clin Densitom. 2012 Jan-Mar;15(1):21-5.

174. Kaptein BL, Valstar ER, Spoor CW, Stoel BC, Rozing PM. Model-based RSA of a femoral hip stem using surface and geometrical shape models. Clin Orthop Relat Res. 2006 Jul;448:92-7.

175. GE Healthcare Lunar. enCORE-baseret røntgen-knogledensitometer Brugermanual. In: ; 2015. p. 315.

176. Fini M, Giavaresi G, Torricelli P, Krajewski A, Ravaglioli A, Belmonte MM, et al. Biocompatibility and osseointegration in osteoporotic bone. J Bone Joint Surg Br. 2001 Jan;83(1):139-43.

177. Mori H, Manabe M, Kurachi Y, Nagumo M. Osseointegration of dental implants in rabbit bone with low mineral density. J Oral Maxillofac Surg. 1997 Apr;55(4):351,61; discussion 362.

178. Finnila S, Moritz N, SvedstroM E, Alm JJ, Aro HT. Increased migration of uncemented acetabular cups in female total hip arthroplasty patients with low systemic bone mineral density. A 2-year RSA and 8-year radiographic follow-up study of 34 patients. Acta Orthop. 2016 Feb;87(1):48-54.

179. Aro HT, Alm JJ, Moritz N, Makinen TJ, Lankinen P. Low BMD affects initial stability and delays stem osseointegration in cementless total hip arthroplasty in women: a 2-year RSA study of 39 patients. Acta Orthop. 2012 Apr;83(2):107-14.

180. Smith E, Comiskey C, Carroll A, Ryall N. A study of bone mineral density in lower limb amputees at a national prosthetics center. Journal of Prosthetics and Orthotics. 2011;23(1):14-20.

181. Thuesen B, Husemoen L, Fenger M, Jakobsen J, Schwarz P, Toft U, et al. Determinants of vitamin D status in a general population of Danish adults. Bone. 2012 Mar;50(3):605-10.

182. Zhou C, Li Y, Wang X, Shui X, Hu J. 1,25Dihydroxy vitamin D(3) improves titanium implant osseointegration in osteoporotic rats. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012 Nov;114(5 Suppl):S174-8.

183. Kelly J, Lin A, Wang CJ, Park S, Nishimura I. Vitamin D and bone physiology: demonstration of vitamin D deficiency in an implant osseointegration rat model. J Prosthodont. 2009 Aug;18(6):473-8.

184. Dvorak G, Fugl A, Watzek G, Tangl S, Pokorny P, Gruber R. Impact of dietary vitamin D on osseointegration in the ovariectomized rat. Clin Oral Implants Res. 2012 Nov;23(11):1308-13.

185. Bugbee WD, Culpepper WJ,2nd, Engh CA,Jr, Engh CA S. Long-term clinical consequences of stress-shielding after total hip arthroplasty without cement. J Bone Joint Surg Am. 1997 Jul;79(7):1007-12.

186. Kronick JL, Barba ML, Paprosky WG. Extensively coated femoral components in young patients. Clin Orthop Relat Res. 1997 Nov;(344)(344):263-74.

187. Keisu KS, Mathiesen EB, Lindgren JU. The uncemented fully textured Lord hip prosthesis: a 10- to 15-year followup study. Clin Orthop Relat Res. 2001 Jan;(382)(382):133-42.

188. Engh CA,Jr, Young AM, Engh CA S, Hopper RH,Jr. Clinical consequences of stress shielding after porous-coated total hip arthroplasty. Clin Orthop Relat Res. 2003 Dec;(417)(417):157-63.

189. Rosenbaum TG, Bloebaum RD, Ashrafi S, Lester DK. Ambulatory activities maintain cortical bone after total hip arthroplasty. Clin Orthop Relat Res. 2006 Sep;450:129-37.

190. Lindsay R, Nieves J, Formica C, Henneman E, Woelfert L, Shen V, et al. Randomised controlled study of effect of parathyroid hormone on vertebral-bone mass and fracture incidence among postmenopausal women on oestrogen with osteoporosis. Lancet. 1997 Aug 23;350(9077):550-5.

191. Eriksen EF, Keaveny TM, Gallagher ER, Krege JH. Literature review: The effects of teriparatide therapy at the hip in patients with osteoporosis. Bone. 2014 Oct;67:246-56.

192. Daugaard H, Elmengaard B, Andreassen TT, Lamberg A, Bechtold JE, Soballe K. Systemic intermittent parathyroid hormone treatment improves osseointegration of press-fit inserted implants in cancellous bone. Acta Orthop. 2012 Aug;83(4):411-9.

193. Soballe K, Brockstedt-Rasmussen H, Hansen ES, Bunger C. Hydroxyapatite coating modifies implant membrane formation. Controlled micromotion studied in dogs. Acta Orthop Scand. 1992 Apr;63(2):128-40.

194. Karrholm J. Radiostereometric analysis of early implant migration - a valuable tool to ensure proper introduction of new implants. Acta Orthop. 2012 Dec;83(6):551-2.

195. Cordero J, Munuera L, Folgueira MD. Influence of metal implants on infection. An experimental study in rabbits. J Bone Joint Surg Br. 1994 Sep;76(5):717-20.

196. Arens S, Schlegel U, Printzen G, Ziegler WJ, Perren SM, Hansis M. Influence of materials for fixation implants on local infection. An experimental study of steel versus titanium DCP in rabbits. J Bone Joint Surg Br. 1996 Jul;78(4):647-51.

197. Stenlund P, Trobos M, Lausmaa J, Branemark R, Thomsen P, Palmquist A. Effect of load on the bone around bone-anchored amputation prostheses. J Orthop Res. 2016 Jun 24.

198. Pitkin M. Design features of implants for direct skeletal attachment of limb prostheses. J Biomed Mater Res A. 2013 Nov;101(11):3339-48.

199. Wilkinson JM, Eagleton AC, Stockley I, Peel NF, Hamer AJ, Eastell R. Effect of pamidronate on bone turnover and implant migration after total hip arthroplasty: a randomized trial. J Orthop Res. 2005 Jan;23(1):1-8.

200. Skoldenberg OG, Salemyr MO, Boden HS, Ahl TE, Adolphson PY. The effect of weekly risedronate on periprosthetic bone resorption following total hip arthroplasty: a randomized, double-blind, placebo-controlled trial. J Bone Joint Surg Am. 2011 Oct 19;93(20):1857-64.

201. Yamasaki S, Masuhara K, Yamaguchi K, Nakai T, Fuji T, Seino Y. Risedronate reduces postoperative bone resorption after cementless total hip arthroplasty. Osteoporos Int. 2007 Jul;18(7):1009-15.

202. Hilding M, Ryd L, Toksvig-Larsen S, Aspenberg P. Clodronate prevents prosthetic migration: a randomized radiostereometric study of 50 total knee patients. Acta Orthop Scand. 2000 Dec;71(6):553-7.

203. Friedl G, Radl R, Stihsen C, Rehak P, Aigner R, Windhager R. The effect of a single infusion of zoledronic acid on early implant migration in total hip arthroplasty. A randomized, double-blind, controlled trial. J Bone Joint Surg Am. 2009 Feb;91(2):274-81.

204. Ivaska KK, Gerdhem P, Akesson K, Garnero P, Obrant KJ. Effect of fracture on bone turnover markers: a longitudinal study comparing marker levels before and after injury in 113 elderly women. J Bone Miner Res. 2007 Aug;22(8):1155-64.

205. Altman DG, Bland JM. Absence of evidence is not evidence of absence. BMJ. 1995 Aug 19;311(7003):485.

206. Hackshaw A. Small studies: strengths and limitations. Eur Respir J. 2008 Nov;32(5):1141-3.

207. Hannon R, Eastell R. Preanalytical variability of biochemical markers of bone turnover. Osteoporos Int. 2000;11 Suppl 6:S30-44.

208. Krall EA, Sahyoun N, Tannenbaum S, Dallal GE, Dawson-Hughes B. Effect of vitamin D intake on seasonal variations in parathyroid hormone secretion in postmenopausal women. N Engl J Med. 1989 Dec 28;321(26):1777-83.

209. Woitge HW, Scheidt-Nave C, Kissling C, Leidig-Bruckner G, Meyer K, Grauer A, et al. Seasonal variation of biochemical indexes of bone turnover: results of a population-based study. J Clin Endocrinol Metab. 1998 Jan;83(1):68-75.

210. Ryd L, Yuan X, Lofgren H. Methods for determining the accuracy of radiostereometric analysis (RSA). Acta Orthop Scand. 2000 Aug;71(4):403-8.

Paper I

Bone Mineral Density Measurements around Osseointegrated Implants: A Precision Study and Validation of Scan-Protocol for Transfemoral Amputees.

List of authors and affiliations:

1) Hansen, Rehne Lessmann[1,4], MD
2) Bente Lomholt Langdahl[2,4], Professor, MD, PhD, DMSc
3) Jørgensen, Peter Holmberg[3], MD, DMSc
4) Petersen, Klaus Kjær[3], MD
5) Søballe, Kjeld[1,3,4], Professor, MD, DMSc
6) Stilling, Maiken[1,4], MD, PhD

1: Orthopaedic Research Unit, University Hospital of Aarhus, Aarhus, Denmark

2: Department of Endocrinology and Internal Medicine, University Hospital of Aarhus, Aarhus, Denmark

3: Department of Orthopaedic Surgery, University Hospital of Aarhus, Aarhus, Denmark

4. Department of Clinical Medicine, Aarhus University, Denmark

Abstract

Introduction and Background: Visual evaluation of bone changes around an osseointegration (OI) implant in femoral amputees examined on plain radiographs shows that periprosthetic bone resorption takes place during the first years after OI surgery, but the bone mineral density (BMD) change has not previously been quantified by dual energy X-ray absorptiometry (DXA). Precision is vital when monitoring BMD changes around implants, and thus the aim of this study was to evaluate the precision and feasibility of a scan protocol for BMD measurements in proximity of OI implants.

Methods: The proximal part of two human cadaveric femoral bones (specimens A and B) with OI implants were mounted in a positioning jig and DXA scans were repeated 5 times in increments of 5° from neutral (0°) to 20° flexion and rotation. BMD changes as a result of change in leg position were evaluated. Repeated patient examinations (n = 20) were conducted in a clinical setting and the precision error was calculated for each of seven periprosthetic custom made regions of interest (ROI).

Results: The precision of ex vivo BMD measurements ranged from 0.31 to 5.93% CV depending on the examined ROI. In all regions, changing positions affected the BMD measurements relative to the value at neutral position up to 9.9% ($p < 0.041$). Along the three ROI (ROI 2, 3, and 4) on the lateral side, the relative difference between specimens A and B varied up to 27.8% ($p < 0.001$), whereas along the medial side (ROI 5, 6, and 7) the relative difference varied up to 10% ($p < 0.036$). The clinical short-term precision root mean square standard deviation ranged from 0.031 to 0.047 g/cm^2 and root mean square coefficient of variation ranged from 3.12% to 6.57% depending on ROI.

Conclusion: Simulated hip flexion or rotation of the femur affected periprosthetic BMD measurements, which stresses the importance of a reproducible set-up during DXA scans in order to reduce measurement errors caused by positioning variation and to achieve a high precision. The high clinical, short-term precision indicated that adherence to the scan protocol reduced measurement error.

Key words

Bone mineral density, Osseointegrated implant, Precision, Transfemoral amputation

Abbreviations

Bone mineral density (BMD); Bone mineral content (BMC); Dual energy X-ray absorptiometry (DXA); Least significant change (LSC); Osseointegration (OI); Precision error (PE); Region of interest (ROI); Root mean square standard deviation (RMS-SD)**;** Transfemoral amputees (TFA); coefficient of variation (CV).

Manuscript

The gold standard method for measuring bone mineral density (BMD) is dual energy X-ray absorptiometry (DXA). This method has proven useful for diagnosing osteoporosis and fracture risk assessment, and for examining BMD changes in serial measurements (1). DXA studies of transfemoral amputees (TFA) describe a substantial BMD loss in the hip, femoral neck, and trochanteric zone in the residual bone over time (2-4). A contributing factor is decreasing activity and loading on the femoral bone, which leads to disuse atrophy, BMD loss, and eventually osteopenia/osteoporosis (5). Some TFAs experience problems such as sores, pain, sweating, or unreliability of the prosthesis being securely suspended while wearing a traditional prosthetic socket, causing them to abandon or limit their prosthetic use (6). However, these problems may be alleviated with an osseointegration (OI) implant (7, 8). The OI implant system does not use a socket-suspended system; instead, it comprises a bone-anchored implant (fixture) and a percutaneous device (abutment) to mount the external prosthetic leg. This system eliminates socket-related problems and increases prosthetic use, joint movement, and patient mobility (8-10). The operation is a two-stage surgical procedure. During stage 1, the fixture is inserted into the bone marrow cavity of the residual femur and left for 6months to osseointegrate. During stage 2, the abutment is inserted into the fixture and the patient commences a 4-6 month rehabilitation program with gradual load on the OI implant system until full weight bearing is allowed (8). A fundamental aspect for successful osseointegration and implant survival is an intimately coupled bone-implant interface.

A radiostereometric study reports that the OI implant achieves stable fixation in the bone (11). Yet, at the same time, plain radiographic evaluation reports periprosthetic distal endosteal bone resorption with thinning and increased porosity of the cortical bone (11, 12). Similar

studies from generic CT-based finite element models report periprosthetic bone resorption in the distal residual femur (13). Periprosthetic BMD measurements have been described around hip and knee implants in studies evaluating strain-adaptive remodeling changes and investigating BMD relations to implant migration (14-17). To improve OI implant survival, we need more knowledge about the association between periprosthetic BMD, osseointegration, and implant fixation. Analysis of clinical serial BMD measurements, requires that the precision error (PE) should be calculated in order to judge if observed changes in periprosthetic BMD are true or merely reflect lack of precision. The PE refers to the ability to reproduce the same result in an identical setting, since the PE is influenced by the short- and long-term variability of the scanner, patient positioning, and scan analysis (1). The BMD around OI implants has not previously been investigated. The aim of our study was to evaluate the precision and feasibility of a protocol for periprosthetic BMD measurement around OI implants by simulating clinically relevant variations in leg position in an ex vivo study, and investigate the short-term precision by repeated patient examinations in a clinical setting.

Methodology

Ex vivo study.

Two cadaveric femoral bones were cut through the diaphysis and the proximal parts were kept for this study. After medullary reaming and thread cutting with surgical equipment for OI implant surgery, steel fixtures (OI implant, Integrum AB, Göteborg) with diameters of 16 mm and 16.5 mm, respectively, were inserted into the femora with intimate cortical contacts, and afterwards they were wrapped in plastic for hygienic purposes.

In specimen A (male, 43 years), the OI implant was inserted slightly lateral to the femoral midline resulting in a thin lateral cortical bone. In specimen B (female, 98 years), the OI implant was inserted close to the midline (figure 1).

The femoral bones were securely mounted on a positioning jig (figure 2) (18) that permitted adjustment of flexion and rotation within 1°, and the outline of the jig was marked on the densitometer table for similar positioning throughout the experiment. The mounted femoral bones were visually positioned parallel to the densitometer table and perpendicular to the X-ray beam (neutral position) . Pilot scans with a soft tissue equivalent placed below the femoral bones were conducted, and optimal thickness was confirmed (5 cm nylon plate and a 3 cm acrylic plate) for the software to detect the different tissue edges (bone, soft tissue, and artefact) correctly. Scans were repeated 5 times in increments of 5° from neutral anteroposterior position (0°) to 20° flexion and from neutral (0°) to 20° external rotation, thereby resembling the possible movement pattern of the residual femur during clinical positioning.

Clinical Study

The PE was tested in the clinical setting by performing double scans in 20 TFAs with OI implants. Scans were obtained 2 years (SD 1.4) after second stage surgery at Aarhus University Hospital between 2014 and 2015. The patients were scanned according to a specified protocol, where each patient was positioned in supine position and instructed to keep the residual limb stationary in relaxed position. Rice bags were then placed around the residual limb to imitate more soft tissue (since soft tissue was often quite atrophic) to support the leg and to avoid air in the scan field, since air in the scan field would adversely affect the automatic tissue recognition by the software and cause incorrect tissue segmentation. Between the repeated examinations, the patients rose from the scanner bed and were repositioned before the second scan. According to Danish law, a formal approval from the Regional Ethical Committee was not required, as the study was a part of a quality assurance investigation (inquiry number 135/2016). The study was approved by the Danish Data Protection Agency (2012-58-0005).

Scan Acquisition

Scans were acquired with the GE Lunar Prodigy Advance 2005 densitometer and analyzed with the enCORE 14.10.022 software. Scan mode standard “ortho hip” was used, and scans were performed in AP-plane, scan-window 25.2 cm long and 15 cm wide, beginning from the distal part of the osseous fixed prosthesis and were aborted 3 sweeps proximal of the prosthesis. One technician performed all clinical scans according to the defined protocol.

Scan Analysis

The enCORE software uses a dynamic tissue detection algorithm and all areas in the scan were segmented as either bone, tissue, air, artefact (metal), or neutral. The edge of the bone area

was automatically outlined and the areas marked as artefact were automatically removed from the densitometry measurement. Based on the bone remodeling around the OI implant described on plain radiographs by Nebergall et al. (11), a template of 7 ROI was made for periprosthetic measurements of BMD (figure 1). The ROI template was applied and positioned on the first scan, then locked on the outline of bone-edges and saved, and copied to subsequent scan on the same patient and cadaveric femoral bones allowing for easier template fitting. Measures of BMD (g/cm^2) were obtained for each ROI and analyses were completed by one observer.

Scan Quality

It was not a straight forward procedure to perform DXA around the OI implants in the transfemoral amputees. It was not possible to use a general device for maintaining an amputated leg in the same position during DXA examinations, because some patients had a very short residual femur and most had pain after surgery, and thus the clinical approach was to place the femur in a relaxed, comfortable position. If special steps were needed in patient positioning, it was documented with a photo and noted in the scan protocol. The software did in some cases detect the bone and artefact edges incorrectly and demanded manual correction. Out of 40 scans (repeated examinations), 27 cases needed minor manual adjustment of the implant and 21 cases needed minor (n = 9), intermediate (n = 10), or major (n = 2) adjustment of the bone (figure 3). All edge detection errors were corrected before analysis.

Statistical Analysis

Ex vivo study

BMD during flexion and rotation was analyzed with a log linear mixed model with random effects for femoral bones and positions (the random effects were assumed to be independent). BMD changes and interactions between the femoral bones at different positions were analysed in each ROI. The model assumptions were evaluated by visual diagnosis of the residuals and best linear unbiased predictors (BLUPS) or fitted values of the model (qq plot and plot of residuals versus BLUPS). Average BMD was presented as percent change from neutral position and relative BMD (rBMD) as the difference between specimens A and B. Precision was expressed as a percentage of coefficient of variation (CV) and calculated by dividing the standard variation by the mean BMD of repeated measurements and multiplying by 100. The data handling was supervised by a biostatistician.

Clinical Study

Normal distribution was evaluated on qq plots. Any changes in BMD between serial scans were assumed to be caused by measurement error, as both scans for each patient were acquired the same day. Short-term BMD precision was defined as root mean square standard deviation (RMS SD) and root mean square coefficient of variation (RMS CV) in percent for each ROI and calculated according to the algorithms described by Bonnick et al. (19). The least significant change (LSC) that could be measured was determined and LSC percent of CV was calculated by multiplying RMS percent of CV by 2.77 (19, 20). All statistical analyses were carried out using Stata (v 13.1, StataCorp LP, College Station, TX) and statistical significance was assumed at $p < 0.05$.

Results

Ex Vivo Study

In all ROI, the BMDs between the cadaveric femoral bones during flexion or rotation were significant different (rBMD) ($p < 0.001$). We found an interaction between the femoral bones and an effect of changing the femoral bone position on BMD ($p < 0.001$). The average BMD (figure 4) changed significantly in most positions compared to neutral position ($p < 0.04$).

In ROI 1, the average BMD changed up to 3.8% ($p < 0.023$) and the difference between specimens A and B increased up to 3.4% during flexion and 1.1% during rotation ($p < 0.001$). In the lateral ROI (2, 3, and 4), the average BMD decreased by 3.9%, 9.2%, and 9.9%, respectively, during flexion ($p < 0.001$). The rBMD differences between the femoral bones seemed to diverge with increasing flexion or rotation. The differences were especially evident when comparing the thin cortical bone in specimen A to the thick cortical bone in specimen B. The largest differences between the femoral bones during flexion were 27.8% (ROI 2), 27.7% (ROI 3) and 25.9% (ROI 4). During rotation the largest differences were 10.5% (ROI 2), 11% (ROI 3), and 10% (ROI 4) ($p < 0.001$).

Small changes in average BMD of the medial ROIs were seen: 5 changed up to 6.3%, 6 up to 2.3% and 7 up to 2.7%, during flexion and/or rotation ($p < 0.041$). The largest difference between specimens A and B in the medial ROI during flexion was 5% (ROI 5), 5.1% (ROI 6), and 5.3% (ROI 7). During rotation, the corresponding changes were 4.8% (ROI 5), 5.4% (ROI 6), and 10% (ROI 7) ($p < 0.036$). The precision of the repeated measurements in neutral and outermost positions ranged from 0.31% to 5.93% CV depending on ROI.

Clinical study

Twenty TFAs (14 males), mean age 52.3 years (range 35 to 70 years), had DXA examinations performed twice after second stage OI surgery. The short-term precision RMS SD ranged from 0.031 to 0.047 g/cm^2 and RMS as a percent of CV ranged from 3.12% to 6.57% (table 1).

Discussion

The aim of the study was to describe the effect of leg position on BMD changes around OI implants in femoral amputees, and to determine the precision error of BMD measurements in proximity of the OI implant. The precision of repeated measurements in the ex vivo study was < 6%, but changing the leg position changed BMD adjacent to the OI implant up to 9.9%. The average BMD resulted in an inaccurate estimate of the BMD change, due to the interaction between the femoral bones and changing positions causing up to 28% difference in the effect of changing positions between specimens A and B in the lateral ROI compared to 10% in the medial ROI. A contributing factor to this interaction was the lateral implantation of the fixture in specimen A occurring due to an anatomical variation of the femoral marrow channel, resulting in a small bone areal for the bone mineral content (BMC) measurement. Engelke et al. determined that bone areal variation was a strong predictor for BMC imprecision (21), which may explain why the largest variation in BMD was observed in the three lateral ROI with a thin cortical bone (small bone areal).

These findings show that care must be taken to position patients alike at every follow-up in serial examination. The clinical protocol for leg stump positioning was rather simple and easy for the patient as well as the technician to follow, and proved to have acceptable clinical short-term precision (< 6.6%). However, in retrospect, it could have been improved with a snap-lock device to keep the abutment of the OI implant locked during the examination—similar to the locking mechanism for the prosthetic leg.

Measurement of individual changes in periprosthetic BMD in clinical studies necessitates the use of a method with high precision. The precision error in our study was comparable to other studies assessing precision of BMD measurements in relation to acetabular, hip, and knee implants (< 8.4% CV) (18, 22-25). Depending on ROI, the changes in BMD measured in proximity of OI implants must exceed the LSC; 9% to 18%, in order to identify a true (above the detection limit) biological BMD change in a longitudinal study.

The limitations of this study are few but important. First, the method only examined the femur in the anterior-posterior position and was dependent on meticulous patient positioning by only one technician for precise measurements as described. Second, the analysis was conducted on software developed for a different anatomical region and implant type (ortho-hip), requiring the software to be tricked into running in automatic mode and avoiding air gaps by use of tissue-equivalent material. Nonetheless, edge detection errors were present in more than half of the cases and had to be manually corrected. Third, the number of patients and scans did not provide the 30° of freedom in the statistical analysis as recommended by the International Society for Clinical Densitometry to ensure that the precision error and LSC were accurate and unbiased (1), which may underestimate the calculated precision error (26). Finally, due to disuse osteopenia/osteoporosis after amputation and before OI surgery, the population represented a very heterogeneous group with large between-patient BMD values. A larger sample size would be preferable, however, all transfemoral amputees with an OI implant in Denmark were included.

At present, the OI implant surgical procedure is performed in 10 countries (27-29) and the validation of our DXA protocol for TFA may be an important step in patient risk assessment.

In longitudinal studies, detailed information about periprosthetic BMD changes may help locate regions around the implant which are susceptible to stress shielding (30) and investigate associations between BMD changes and implant survival in TFAs (31). Using a larger ROI enclosing a larger bone area may improve precision, but will lack detail (24). Based on the experiences by Nebergall et al. (11), the use of a 7-ROI model is ideal to examine periprosthetic cortical thinning and endosteal resorption affects BMD.

In conclusion, the ex vivo study demonstrates that changing the leg stump position even 5° has a significant impact on BMD measurements. Average BMD changed up to 9.9%, and the relative difference between specimens A and B varied up to 27.8% on the lateral side and up to 10% on the medial side. The clinical study confirms that the precision of BMD measurements in proximity of the OI implant using the Lunar Prodigy scanner is acceptable (< 6.6%) and comparable with other implant precision studies. Adhering to the scan protocol secures minimal change in leg positioning and ensures that follow-up BMD measurements are comparable in a clinical setting with a high precision.

Acknowledgement:

We would like to thank project coordinator/DXA operator Inger Krog-Mikkelsen from the Orthopaedic Research Unit, University Hospital of Aarhus for guidance and assistance with DXA scans, and biostatistician Aparna Udupi from the Biostatical Advisory Service (BIAS), University Hospital of Aarhus for statistical advice.

References

1. Baim S, Wilson CR, Lewiecki EM, Luckey MM, Downs RW,Jr, Lentle BC. Precision assessment and radiation safety for dual-energy X-ray absorptiometry: position paper of the International Society for Clinical Densitometry. J Clin Densitom. 2005 Winter;8(4):371-8.

2. Sherk VD, Bemben MG, Bemben DA. BMD and bone geometry in transtibial and transfemoral amputees. J Bone Miner Res. 2008 Sep;23(9):1449-57.

3. Flint JH, Wade AM, Stocker DJ, Pasquina PF, Howard RS, Potter BK. Bone mineral density loss after combat-related lower extremity amputation. J Orthop Trauma. 2014 Apr;28(4):238-44.

4. Kulkarni J, Adams J, Thomas E, Silman A. Association between amputation, arthritis and osteopenia in British male war veterans with major lower limb amputations. Clin Rehabil. 1998 Aug;12(4):348-53.

5. Gailey R, Allen K, Castles J, Kucharik J, Roeder M. Review of secondary physical conditions associated with lower-limb amputation and long-term prosthesis use. J Rehabil Res Dev. 2008;45(1):15-29.

6. Hagberg K, Branemark R. Consequences of non-vascular trans-femoral amputation: a survey of quality of life, prosthetic use and problems. Prosthet Orthot Int. 2001 Dec;25(3):186-94.

7. Sullivan J, Uden M, Robinson KP, Sooriakumaran S. Rehabilitation of the trans-femoral amputee with an osseointegrated prosthesis: the United Kingdom experience. Prosthet Orthot Int. 2003 Aug;27(2):114-20.

8. Hagberg K, Branemark R. One hundred patients treated with osseointegrated transfemoral amputation prostheses--rehabilitation perspective. J Rehabil Res Dev. 2009;46(3):331-44.

9. Hagberg K, Branemark R, Gunterberg B, Rydevik B. Osseointegrated trans-femoral amputation prostheses: prospective results of general and condition-specific quality of life in 18 patients at 2-year follow-up. Prosthet Orthot Int. 2008 Mar;32(1):29-41.

10. Hagberg K, Haggstrom E, Uden M, Branemark R. Socket versus bone-anchored trans-femoral prostheses: hip range of motion and sitting comfort. Prosthet Orthot Int. 2005 Aug;29(2):153-63.

11. Nebergall A, Bragdon C, Antonellis A, Karrholm J, Branemark R, Malchau H. Stable fixation of an osseointegated implant system for above-the-knee amputees. Acta Orthop. 2012 Apr;83(2):121-8.

12. Tsikandylakis G, Berlin O, Branemark R. Implant survival, adverse events, and bone remodeling of osseointegrated percutaneous implants for transhumeral amputees. Clin Orthop Relat Res. 2014 Oct;472(10):2947-56.

13. Tomaszewski PK, van Diest M, Bulstra SK, Verdonschot N, Verkerke GJ. Numerical analysis of an osseointegrated prosthesis fixation with reduced bone failure risk and periprosthetic bone loss. J Biomech. 2012 Jul 26;45(11):1875-80.

14. Baad-Hansen T, Kold S, Nielsen PT, Laursen MB, Christensen PH, Soballe K. Comparison of trabecular metal cups and titanium fiber-mesh cups in primary hip arthroplasty: a randomized RSA and bone mineral densitometry study of 50 hips. Acta Orthop. 2011 Apr;82(2):155-60.

15. Boden H, Adolphson P, Oberg M. Unstable versus stable uncemented femoral stems: a radiological study of periprosthetic bone changes in two types of uncemented stems with different concepts of fixation. Arch Orthop Trauma Surg. 2004 Jul;124(6):382-92.

16. Tjornild M, Soballe K, Hansen PM, Holm C, Stilling M. Mobile- vs. fixed-bearing total knee replacement. Acta Orthop. 2015 Apr;86(2):208-14.

17. Wilkinson JM, Eagleton AC, Stockley I, Peel NF, Hamer AJ, Eastell R. Effect of pamidronate on bone turnover and implant migration after total hip arthroplasty: a randomized trial. J Orthop Res. 2005 Jan;23(1):1-8.

18. Stilling M, Soballe K, Larsen K, Andersen NT, Rahbek O. Knee flexion influences periprosthetic BMD measurement in the tibia. Suggestions for a reproducible clinical scan protocol. Acta Orthop. 2010 Aug;81(4):463-70.

19. Bonnick SL, Johnston CC,Jr, Kleerekoper M, Lindsay R, Miller P, Sherwood L, et al. Importance of precision in bone density measurements. J Clin Densitom. 2001 Summer;4(2):105-10.

20. Kiebzak GM, Morgan SL, Peace F. Which to use to evaluate change in BMD at follow-up: RMS-SD or RMS-%CV? J Clin Densitom. 2012 Jan-Mar;15(1):26-31.

21. Engelke K, Gluer CC, Genant HK. Factors influencing short-term precision of dual X-ray bone absorptiometry (DXA) of spine and femur. Calcif Tissue Int. 1995 Jan;56(1):19-25.

22. Jayasuriya RL, Wilkinson JM. Pelvic periprosthetic bone mineral density measurement around cemented vs cementless acetabular prostheses. J Clin Densitom. 2014 Jan-Mar;17(1):116-20.

23. Tjornild M, Soballe K, Bender T, Stilling M. Reproducibility of BMD measurements in the prosthetic knee comparing knee-specific software to traditional DXA software: a clinical validation. J Clin Densitom. 2011 Apr-Jun;14(2):138-48.

24. Penny JO, Ovesen O, Brixen K, Varmarken JE, Overgaard S. Bone mineral density of the femoral neck in resurfacing hip arthroplasty. Acta Orthop. 2010 Jun;81(3):318-23.

25. Abu-Rajab RB, Watson WS, Walker B, Roberts J, Gallacher SJ, Meek RM. Peri-prosthetic bone mineral density after total knee arthroplasty. Cemented versus cementless fixation. J Bone Joint Surg Br. 2006 May;88(5):606-13.

26. Gluer CC, Blake G, Lu Y, Blunt BA, Jergas M, Genant HK. Accurate assessment of precision errors: how to measure the reproducibility of bone densitometry techniques. Osteoporos Int. 1995;5(4):262-70.

27. Aschoff HH, Juhnke DL. Evaluation of 10 years experience with endo-exo femur prostheses - background, data and results. Z Orthop Unfall. 2012 Dec;150(6):607-14.

28. Aschoff HH, Kennon RE, Keggi JM, Rubin LE. Transcutaneous, distal femoral, intramedullary attachment for above-the-knee prostheses: an endo-exo device. J Bone Joint Surg Am. 2010 Dec;92 Suppl 2:180-6.

29. The OPRA treatment in your country [Internet].; 2014 []. Available from: http://www.integrum.se/index.php/patient-info.

30. Huiskes R. The various stress patterns of press-fit, ingrown, and cemented femoral stems. Clin Orthop Relat Res. 1990 Dec;(261)(261):27-38.

31. Fini M, Giavaresi G, Torricelli P, Krajewski A, Ravaglioli A, Belmonte MM, et al. Biocompatibility and osseointegration in osteoporotic bone. J Bone Joint Surg Br. 2001 Jan;83(1):139-43.

Figure 1. The OI implant is marked as metal artefact (blue) due to the density of the metal, and the yellow line marks the bone edges. **(A-B)** Anteroposterior scan view of specimen A with a thin lateral cortical bone and specimen B with thick cortical bone. ROI 1 was placed proximal to OI implant, ROI 2-4 lateral, and ROI 5-7 medial to the OI implant. **(C-D)** Scan views from the clinical setting showing patients with thin and thick cortical bones.

Abbr: ROI, Regions of interest

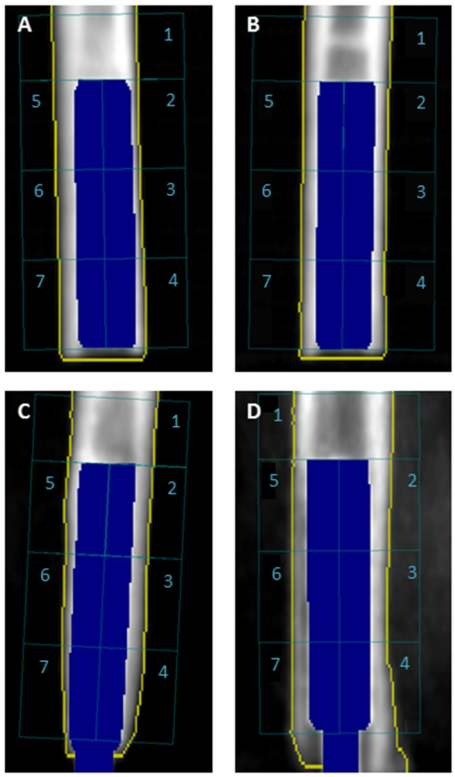
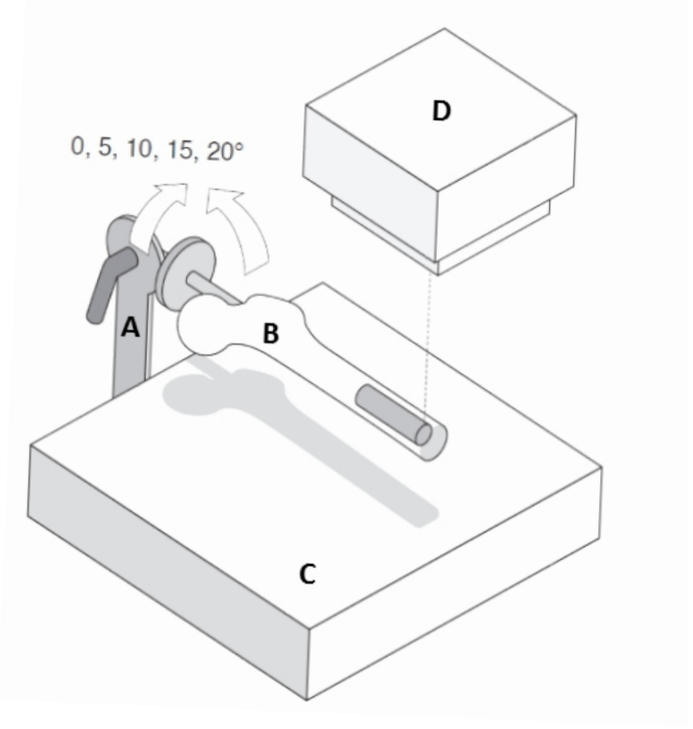


Figure 2. Ex vivo DXA setup



(A) Positioning jig (**B)** Cadaveric femoral bone (**C)** Soft tissue equivalent (**D)** DXA X-ray arm. The dotted line marks the start of DXA scan. The arrows show the degrees of flexion and external rotation from neutral position.

Figure 3. Uncorrected and manually corrected edge detection errors. **(A-B)** The typical site for minor artefact corrections was the proximal part of the implant. **(C-D)** A correction of a major bone and a minor artefact edge detection error.

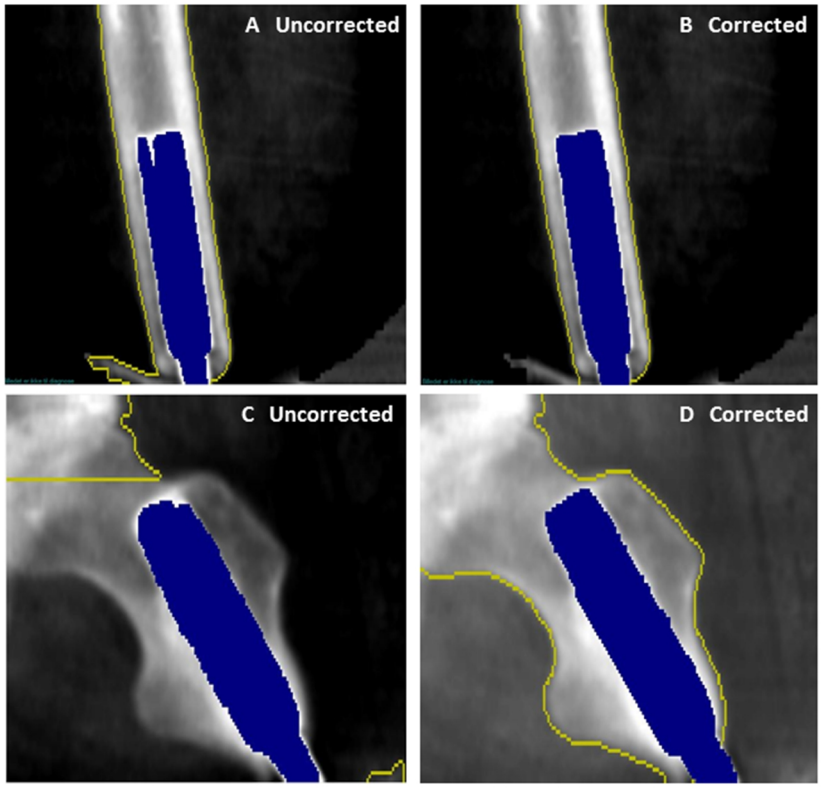


Figure 4: Variation in relative BMD in ROI 1 to 7 as a function of flexion (F5 to F20) or external rotation (R5 to R20). *Significant change of average BMD compared to neutral (p < 0.04). Abbr: ROI, Regions of interest; BMD, bone mineral density; F, flexion; R, rotation

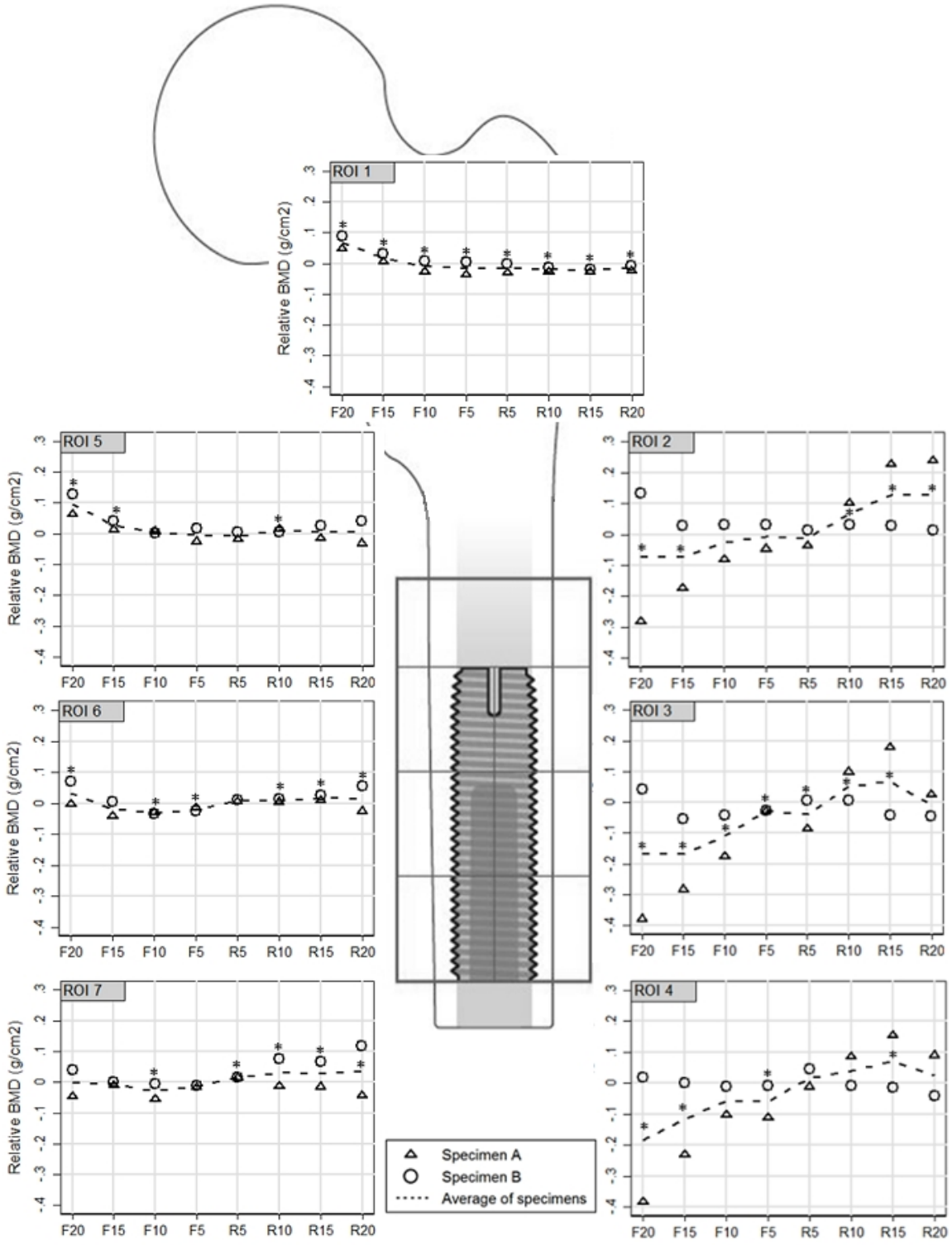


Table 1: Precision for clinical double examination n = 20

ROI	Mean BMD[a] (g/cm^2)	± SD (g/cm^2)	Range (g/cm^2)		RMS SD[b] (g/cm^2)	RMS CV[c]	LSC-SD (g/cm^2)	LSC-CV
1	1.145	0.570	0.456	2.227	0.031	4.66	0.085	12.91
2	1.181	0.469	0.505	2.027	0.034	3.12	0.093	8.65
3	1.081	0.490	0.210	1.913	0.041	4.58	0.115	12.68
4	0.870	0.608	0.465	1.777	0.040	6.57	0.111	18.21
5	1.302	0.457	0.587	2.041	0.047	4.33	0.129	12.00
6	1.201	0.385	0.412	1.885	0.037	3.88	0.103	10.76
7	1.095	0.356	0.453	1.781	0.043	4.61	0.118	12.77

[a]Mean bone mineral density from double examinations (± standard deviation, range)
[b]Root mean square standard deviation
[c]Root mean square coefficient of variation in percent
Abbr: ROI, region of interest; LSC, least significant change

Paper II

Loss of periprosthetic bone mineral density and higher PTH is associated with removal of osseointegrated implants in femoral amputees

List of authors and affiliations

1) Hansen, Rehne Lessmann[1,4], MD
2) Bente Lomholt Langdahl[2,4], Professor, MD, PhD, DMSc
3) Jørgensen, Peter Holmberg[3], MD, DMSc
4) Petersen, Klaus Kjær[3], MD
5) Søballe, Kjeld[1,3,4], Professor, MD, DMSc
6) Stilling, Maiken[1,4], MD, PhD

1: Orthopaedic Research Unit, University Hospital of Aarhus, Aarhus, Denmark

2: Department of Endocrinology and Internal Medicine, University Hospital of Aarhus, Aarhus, Denmark

3: Department of Orthopaedic Surgery, University Hospital of Aarhus, Aarhus, Denmark

4. Department of Clinical Medicine, Aarhus University, Denmark

Abstract

Background: The osseointegrated (OI) implant system comprises a bone anchored implant connected to an external prosthetic leg. The OI implant system is considered a treatment option for transfemoral amputees (TFAs) with a short residual femur and/or complications with the prosthetic socket.

Purpose: To evaluate changes in bone mineral density (BMD) and bone turnover markers (BTM) in TFAs treated with an OI implant.

Methods: An observational cohort of 20 consecutive patients (13 males), mean age 48 years (range 30-66), were treated with an OI implant during a two-stage surgical procedure. Examinations were conducted preoperatively, and 1, 3, 6, 7, 9, 12, 18, 24 and 30 months after the first stage surgery. Dual-energy X-ray absorptiometry (DXA) scans of seven periprosthetic regions of interests (ROIs), the proximal hip, and lumbar spine. Total serum calcium (Ca), 25-hydroxyvitamin D2+D3 (vitamin D), parathyroid hormone (PTH), N-terminal propeptide of type I procollagen (PINP), C-telopeptide of type I collagen (CTX-1), serum bone-specific alkaline phosphatase (BASP), osteocalcin (OC) were measured in the serum. DXA precision was examined by double examinations and presented as root-mean-square coefficient of variation (RMS-CV). A logistic regression analysis was used to determine potential predictors (change in periprosthetic BMD and preoperative BTMs) for implant loosening.

Results: Eight out of 20 implants (five fixtures and three abutments) were removed (removed implant (RI) group) and one patient was equipped with a modified socket prosthesis due to loosening. In the cohort, a decrease in mean periprosthetic BMD (95%CI) between 16% (9; 23) and 34% (24; 43) was measured in all ROIs six months after first stage surgery (p <0.001). In the RI group, a decrease in periprosthetic BMD (95%CI) between 26% (3;44) and 40% (23;54) was measured in ROI 1,2,3,5,6 and 7 (p < 0.03) at 30-month follow-up. In the non-removed implant (NRI) group, BMD was regained in all ROIs at 30-month follow-up and was not different from baseline (p >0.083). Precision in periprosthetic ROI 1-7 ranged from 3.1% to 6.6%, precision of spine BMD was 1.2%, precision of BMD in the hip of the amputated leg was 2.1% and 1.1% in the intact hip. The mean CTX concentration (95%CI) was 0.39 µg/L (0.25; 0.52) in the RI group and 0.28 µg/L (0.19; 0.37) in the NRI group (p = 0.15). However, in the time-series analysis a significant difference was measured between the groups at 18- and 24-months follow-up (p < 0.049). Preoperatively, three patients had elevated PTH (>6.9 pmol/l) and six patients had vitamin D insufficiency (<50 nmol/l). Median PTH (inter quartile range (IQR)) in the RI group was 5.8 pmol/L (4.6; 7.3) and 4.0 pmol/L (3.7; 4.5) in the NRI group (p = 0.01). At last follow-up examination, three patients had an elevated PTH concentration and six patients had vitamin D insufficiency. The odds of implant removal was 2.4 95%CI (0.92; 6.29, p =0.07) by each unit increase in PTH concentration.

Interpretation: Negative bone remodelling, in terms of lost periprosthetic BMD in the years following OI surgery, and change in bone metabolism, in terms of elevated PTH concentration, was associated with failure / implant loosening and removal of OI implants. Therefore we recommend to follow periprosthetic BMD and bone turnover markers, and to correct deficiencies, in the years after OI surgery.

Introduction

The osseointegration (OI) implant system for transfemoral amputees (TFAs) provides a direct bone-anchored attachment to the prosthetic leg alleviating problems associated with the prosthetic socket (1, 2). The system consists of an intraosseous titanium fixture (Integrum AB; Göteborg, Sweden) osseointegrated with the residual femoral bone and an abutment protruding the skin, making it possible to attach the external prosthetic leg with a snap-lock. The treatment involves a two-stage surgical procedure separated by six months and is followed by rehabilitation (3).

The Swedish Osseointegration Team treated 100 patients with an OI implant (Integrum AB; Göteborg, Sweden) from 1990 to 2008, and 20 implants were removed primarily due to failures occurring during the early phase (3). Later, 51 patients were enrolled in the Swedish prospective "Osseointegrated Prostheses for the Rehabilitation of Amputees" (OPRA) study with a two-year follow-up. In this cohort, four patients had a deep periprosthetic infection of which one led to implant removal, three were treated with antibiotics, and three implants were removed due to aseptic loosening (4). The United Kingdom Osseointegration Team removed two out of eleven OI implants (Integrum AB; Göteborg, Sweden) due to infection (5). A prospective cohort study found that 7 out of 39 patients had a periprosthetic OI implant infection after three years. One implant was removed, one resolved after antibiotic treatment and five ongoing deep infections did not affect prosthetic use (6). There are no available studies on the specific reasons for septic and aseptic OI implant removal. In general, the aetiology of aseptic implant loosening is thought to be multifactorial but is associated with local osteoclastic bone resorption (7). Generic CT-based finite element models find that periprosthetic bone loss is greater in the distal regions than the proximal regions adjacent to the OI implant and that these changes are consistent with stress shielding (8, 9). This is supported in the OPRA study by a radiographic increase in distal bone resorption and porosity adjacent to the OI implant. Additionally, the radiostereometric analysis indicates a stable OI implant fixation (10).

The gold standard for examining bone mineral density (BMD) changes in serial measurements and diagnosing osteoporosis is dual energy X-ray absorptiometry (DXA) (11). Periprosthetic BMD measurements have been described in numerous studies in joint replacement to assess strain-adaptive remodelling changes (12-15). DXA scans provide information on BMD and bone turnover markers (BTMs) indicate the rate of bone remodelling (16). BTMs such as N-terminal propeptide of type I procollagen (PINP), C-telopeptide of type I collagen (CTX-1), N-telepeptide of type-I collagen (NTX), serum bone-specific alkaline phosphatase (BASP), osteocalcin (OC) are among the markers investigated in patients with loose implants (17-19). BTMs may also be useful in evaluating periprosthetic osteolysis (20), although no markers have been validated and recommended for routine clinical use. Vitamin D (25-hydroxyvitamin D2+D3) is a hormone essential for bone mineralization, and low vitamin D levels may result in increased parathyroid hormone (PTH) concentrations and bone loss (21). Thus, identifying OI patients with an increase in PTH, low vitamin D and/or high BTMs at follow-up examination may help early identification of patients at risk of implant loosening or increased bone remodelling.

The primary aim of this prospective observational cohort study was to investigate the BMD and BTM changes in TFAs treated with an OI implant and followed for 30-month postoperative. The secondary aim was to investigate these changes in patients undergoing implant removal (RI) compared to patients with non-removed implants (NRI).

Method

Population

Patients referred to the Department of Orthopaedic Surgery for OI surgery between 2010 and 2013 were evaluated by a team of two senior consultants. Eligibility criteria: Age between 18-70 years, BMI <30 and a bone structure suitable for OI implant surgery. Exclusion criteria: Diabetes, arteriosclerosis, smoking, treatment with bisphosphonates, NSAID or cytostatic medicine, active cancer, kidney or hepatic insufficiency, dementia, pregnancy, and weight <100kg.

According to Danish law, formal ethical approval was not required since all examinations were performed according to an established quality assurance protocol (Inquiry number: 135/2016). Data was handled in accordance with the regulations by the Danish Data Protection Agency (Approval number 2012-28-005). In total, 20 patients were scheduled for primary OI implant surgery; 19 patients had a unilateral transfemoral amputation and 1 patient was scheduled for transfemoral amputation during OI surgery

Follow-up examinations and rehabilitation

The entire population was followed as an observational prospective cohort for 30 months. Examinations included DXA scans of the amputated femur, hips and spine and laboratory tests for evaluation of bone metabolism. The clinical outcome was OI implant removal as documented in patient records. Femur length was measured on preoperative CT images.

Examinations were conducted preoperatively (0) and 1, 3, 6, 7, 9, 12, 18, 24, 30 months after first stage (S1) surgery. Weight bearing on the OI implant was not possible during the six months between S1 and second stage (S2) surgery as the abutment was not inserted. Prophylactic IV dicloxacillin 2 g was administered 3 times/day; 1 day preoperatively, 1 day after S1 surgery and 10 days after S2 surgery. After S2 surgery, the initial rehabilitation included training using a short prosthesis for 12 weeks and a later training period using a long external prosthesis. The first 6 weeks, gentle exercise was allowed. In the following 6 weeks weight bearing started at 20 kg and increased 10 kg/week. After 12 weeks, the patient gradually increased weight bearing and time of prosthetic use until full mobility was achieved 12 months after S1 surgery.

Dual energy X-ray absorptiometry

Nineteen patients were DXA scanned on a GE Lunar Prodigy Advance scanner (General Healthcare, Madison WI) and one patient was scanned on a GE Lunar iDXA due to a temporary hardware upgrade. BMD around the OI implant was evaluated using a seven-template custom-designed ROI previously validated by our research group (22). BMD was measured in proximal femur (total hip) AP lumbar spine (L1-L4), using standard regions of interests (ROIs) (23). Precision error was determined by double examinations obtained as mean (SD) 2 years (1.4) after S2 surgery from 2014 to 2015. All DXA scans were performed by the same technician and all BMD analyses were completed by one observer (RLH) with enCORE 14.10.022 software (General Healthcare, Madison WI). The preoperative total hip and spine BMD was compared with a healthy Northern European age and gender matched reference population described in the Encore manual (24).

Biochemical measurements

Venous blood samples were obtained between 10AM and 15PM in non-fasting patients, and DXA scans were completed on the same day. After centrifugation, serum was stored at −80 °C until analysis.

The following methods were used to analyse the serum samples: Calcium (Ca) was determined by absorption spectrophotometry; vitamin D by high-performance liquid chromatography (API 5500, AB Sciex); bone specific alkaline phosphatase by ELISA; PTH, P1NP, CTX, OC were determined by electrochemiluminescence analysis (Cobas 6000 modul e601, Roche Diagnostic A/S).

Statistical analysis

The longitudinal dataset was analysed with a log linear mixed model with random effects for patients, thus between patient variations were allowed (with exponential variance between time points). The model assumptions were evaluated by visual assessment of the residuals and best linear unbiased predictors (BLUPS) or fitted values of the model (qq-plot and plot of residuals versus BLUPS). The primary outcome was measurements compared to baseline values calculated for all time points and presented as percent change with 95% confidence intervals (CI). Baselines were defined as: Preoperative BMD measurements in total hip and spine, preoperative blood test values, first postoperative (after 1 month) periprosthetic BMD measurement. A logistic regression analysis was used to determine the predictors of implant removal using the preoperative BTMs and the change in periprosthetic BMD. The odds ratio (OR) estimated the odds of implant removal during 3 years of follow-up.

Normality was evaluated by visual inspection of qq-plots. Parametric data were reported as mean with standard deviation (SD) or range, and non-parametric were reported as medians with 25%-75% inter quartile range (IQR) or range. Correlation between variables was examined with a Spearman's rank test.

A subgroup analysis of removed implants and non-removed implants was conducted. The groups were investigated in a time-series analysis using the log linear model and in a last follow-up analysis using a two-sample *t* test/ranksum test as appropriate. Precision of BMD measurements was presented as root mean square standard deviation (RMS-SD) and root mean square coefficient of variation in percent (RMS-CV)(25). Statistical analyses were carried out using Stata (v 13.1, StataCorp LP, College Station, TX) and statistical significance was assumed at $p<0.05$.

Results

Thirteen males and 7 females, mean age (range) 48 years (30 to 66) were scheduled for OI implant surgery. The patients were transfemorally amputated at a median (range) 4 years (0 to 39) prior to OI surgery, and the residual femur length (range) was 26 cm (12 to 36). Two patients had the abutment removed one month after the study period ended (was planned for implant removal at the last 30-month follow-up) and were counted as implant removals in the analysis. In total, nine implants were considered RI during the study period. Five patients had total implant removals (fixture and abutment), 3 had partial removals (abutment), and 1 patient could not use the OI implant (Appendix 1).

Periprosthetic BMD changes

At cohort level, a mean periprosthetic BMD loss between 16% (CI 9; 23) and 34% (CI 24; 43) was observed in all ROIs from S1 surgery to 6-month follow-up (p <0.001). From S1 surgery to 30-month follow-up BMD decreased between 17% (CI 3; 29) and 23% (CI 6; 63) in ROI 2,4,6 and 7 (p <0.01). There was no difference in periprosthetic BMD between the RI and NRI group in the time series analysis (Figure 1, p >0.12) or in the last follow-up analysis (Table2, p > 0.07). The periprosthetic BMD in both groups was lower at several time points compared to the postoperative baseline (Figure 1, p < 0.05). The OR of implant removal due to periprosthetic BMD change is presented in Table 3. The precision (RMS-CV) of BMD measurements: ROI 1 to 7 ranged from 3.1% to 6.6%, spine (L1-L4) was 1.2%, the proximal hip (total) of the amputated leg was 2.1% and the intact hip was 1.1%.

Changes in hip and lumbar spine

Preoperatively (Table 1), BMD was significantly lower in the hip of the amputated leg than the contralateral intact hip (p =0.0003) and the hip of an age and gender matched reference population (p =0.0005). The hip BMD of the amputated leg correlated with the length of the residual femoral bone (rho = 0.46, p = 0.047) and inversely with time since amputation (rho= -0.73, p = 0.0003). There was no difference between the intact hip (p =0.88) or spine BMD (p =0.2) compared to the reference population. The difference in total hip BMD between the amputated and intact side ranged from 37% (CI 25; 48) to 45% (CI 35; 54) depending on time point (p < 0.001) (Figure 2). There was no difference in total hip BMD between the RI and NRI groups at any follow-up examinations (p > 0.15). Lumbar spine BMD was unchanged during the 30-month follow-up (p > 0.60) and there was no difference between the RI and NRI groups at any time points (p > 0.30).

Bone turnover markers and bone metabolism

At cohort level, P1NP, CTX and BASP levels peaked 1 month after S1 surgery by 119% (CI 83; 162), 48% (CI 20, 83) and 147% (CI 95;213), respectively (p = 0.001). The time series analysis (Figure 3) showed a significant difference in the OC levels at baseline (p = 0.001) and in the CTX levels at 18 and 24 months (p < 0.049) between the RI and the NRI groups, whereas no difference was found in the last follow-up analysis (Table 3, p > 0.15). In the NRI group, BASP and OC levels were elevated at several time points compared to preoperative baseline values, thus, at 30-month follow-up, BASP levels had increased by 50% (CI 9; 106, p = 0.012) and OC levels by 28% (CI 4; 58, p = 0.019).

Preoperatively, 8 patients had PTH or vitamin D concentrations outside the reference interval: 2 patients had elevated PTH (>6.9 pmol/l), 5 patients had low vitamin D (<50 nmol/l), and 1 patient had elevated PTH and

low vitamin D. The time series analysis showed higher PTH concentrations in the RI group during the first year compared to the NRI group (Figure 4, p <0.031) and a similar group difference was found in the last follow-up analysis (Table 4, p = 0.01). The ORs are presented in table 3. The mean total calcium concentration during follow-up was 2.43 mmol/l (range 2.1; 2.67).

Discussion

This is the first study to evaluate the periprosthetic changes in BMD, and the changes in BTM after osseointegrated implant surgery.

Key findings:

- Eight out of 20 implants are removed during the trial
- BMD decreases in later removed implants and is regained in implants remaining in situ
- CTX increases in the RI group compared to the NI group at 18- and 24-month follow-up
- Low vitamin D was observed at several timepoints
- Elevated PTH may predict later implant removal

The TFAs in the cohort have osteoporotic bone in the hip of the amputated leg and normal bone in the intact hip compared to the age and gender matched reference population. The low BMD in the amputated leg is related to disuse atrophy and supports the results from previous studies (26-28).

Up to 34% periprosthetic BMD loss is observed in all ROIs during the first 6 months after S1 surgery. An interesting observation is that the periprosthetic BMD in the NRI group is unchanged at 30-month follow-up compared to the baseline values, as opposed to a periprosthetic BMD decrease in the RI group. Periprosthetic bone remodelling occurs due to the new loading conditions imposed by the OI implant, which is referred to as stress shielding (29).

Non-use atrophy affects all periprosthetic ROIs negatively because the OI implant is not subjected to loading in the six months between S1 and S2 surgery. In hip stem arthroplasty, a similar decrease in periprosthetic BMD due to stress shielding is observed in the calcar region after six months, whereas an increase in BMD is observed in the distal regions after one year (30, 31). CT finite element analysis suggests that the effect of stress shielding around the OI implant is greatest in the distal region and decreases proximally (8, 9). A similar effect is not found in the NRI group, as the smallest periprosthetic BMD changes are observed in the distal regions (Table 2). In contrast, the periprosthetic BMD changes around the removed implants are greatest in the proximal regions. Implant loosening may be associated with excessive periprosthetic bone loss (19); however, no significant difference in periprosthetic changes is found between the two groups. A decrease in periprosthetic BMD at the last follow-up resulted in 0.57 to 1.64 OR of implant removal.

One patient (ID = 8) had severe periprosthetic bone loss ranging from 36.8% to 52.8% (Appendix 1) and had the OI implant removed after 18 months. The patient was amputated and had the fixture implanted on the same day, but after falling directly on the external prosthetic knee he experienced pain during weight bearing indicating traumatic implant loosening.

Most likely, the decrease in BMD in the RI group is related to the osteolytic changes after infection and disuse osteoporosis, as patients with pain during weight-bearing typically have lower physical activity levels than those with well-functioning implants (32). This is also reflected by the decrease in hip BMD measured on the amputated side but not the intact side during follow-up.

Bone turnover markers

An increase in BTM is expected as a response to bone remodelling after OI implant surgery. In comparison, BASP and CTX concentrations increase rapidly three weeks after tibia fractures or hip replacement surgery, whereas OC concentrations increase at a slower pace (30, 33). Several months after hip replacement or fracture healing, the bone formation markers (BASP and OC) are still elevated indicating that the osteoblasts are active and bone is mineralizing (16, 30, 33). The increase in bone formation (BASP) and mineralization (OC) in the NRI group after S1 surgery most likely contribute to the recovery of periprosthetic BMD. At the last follow-up examination, the CTX-concentration was 0.39µg/L in the RI group and 0.28 µg/L in the NRI group. In comparison, the mean CTX-concentration in loose hip implants ranges from 0.32 to 0.43µg/L (19, 34). Osteolysis leads to an increase in bone resorption and during type-I collagen degradation CTX and NTX is released (16). Elevated serum CTX concentrations are measured in rat models with particle-induced osteolysis (35, 36) and in patients with a potentially unstable fixation of a tibial implant component (17). Serum CTX shows a trend toward increased concentration in patients with aseptic loosening after THA (19, 34). Six out of nine implant removals are performed after the 18-month follow-up and significant changes in CTX-concentration are measured at 18- and 24-month follow-up after S1 surgery ($p<0.049$)., However, the elevation in CTX is not only caused by aseptic loosening as four implants are removed due to infection (septic loosening).

Vitamin D and PTH

Occult vitamin D deficiency or osteopenia/osteoporosis is common in orthopaedic patients (37) and in lower limb amputees (38). Thus, all patients in this study are referred to an endocrinologist upon discovery of biomarkers or a T-score outside the normal reference interval. The patients are screened for metabolic diseases and recommended to take calcium and vitamin D supplements. The median PTH concentration of 5.84pmol/L in the RI group is higher compared to the 4.02pmol/L in NRI group at last follow-up examination. The OR indicated that elevated PTH concentrations increased the odds of implant removal by 2.4. Three patients with elevated PTH concentrations and four patients with low vitamin D had their OI implant removed, which indicates a potential for prediction of implant removal by these parameters. Elevated PTH concentrations over several days indirectly stimulate osteoclast activity, thereby causing an increase in bone turnover, which is usually associated with bone loss (39). In contrast intermittent treatment with recombinant PTH stimulates osteoblasts activity and increases BMD in humans (40) and improves osseointegration in animal studies (41). In rat models, vitamin D deficiency impairs osseointegration, whereas vitamin D supplementation improves implant osseointegration (42-44). The role of elevated PTH and low vitamin D in human OI implant surgery is unclear, but PTH may affect fixation of the OI implant adversely.

OI implant failure

The number of implant removals (5 fixtures and 3 abutments) during the 30-month follow-up is high. The Swedish Osseointegration Team removed 10 out of 15 OI implants (Integrum AB, Göteborg) during the first

four years and explained it by learning curve; however, the causes of implant removals were not described in the paper (3).

Limitations

The study population is small, but includes all patients treated with an Integrum OI implant in Denmark. The precision of DXA scans are validated in a previous study and found to be similar to double examinations of acetabular, hip, and knee implants (< 8.4% CV) (45-49). The circadian rhythm and food intake affects bone marker concentrations, and further vitamin D concentrations display seasonal variance (16); thus, blood samples were collected during daytime and analysed as batches to minimize analytical variability.

Conclusion

In total 40% of the implants are removed (five fixtures and three abutments) during the study period. A large periprosthetic BMD loss is observed between S1 and S2 surgery due to non-use atrophy. Periprosthetic BMD around the implants that were later removed decreases due to septic/aseptic loosening at 30-month follow-up, whereas the BMD around surviving implants recovered to baseline values. Severe periprosthetic bone loss may be associated with implant removal.

CTX concentrations are higher in the RI group at 18- and 24-month follow-up and may possibly be related to implant loosening. PTH concentrations are higher among patients with removed implants compared to patients with non-removed implants.

Correction of low vitamin D and elevated PTH concentrations should be considered before OI surgery. Focus on antiresorptive therapy to minimize the bone loss related to stress-shielding is interesting as a treatment solution to avoid implant removal but needs further investigation.

References

1. Hagberg K, Hansson E, Branemark R. Outcome of percutaneous osseointegrated prostheses for patients with unilateral transfemoral amputation at two-year follow-up. Arch Phys Med Rehabil. 2014 Nov;95(11):2120-7.

2. Hagberg K, Branemark R, Gunterberg B, Rydevik B. Osseointegrated trans-femoral amputation prostheses: prospective results of general and condition-specific quality of life in 18 patients at 2-year follow-up. Prosthet Orthot Int. 2008 Mar;32(1):29-41.

3. Hagberg K, Branemark R. One hundred patients treated with osseointegrated transfemoral amputation prostheses--rehabilitation perspective. J Rehabil Res Dev. 2009;46(3):331-44.

4. Branemark R, Berlin O, Hagberg K, Bergh P, Gunterberg B, Rydevik B. A novel osseointegrated percutaneous prosthetic system for the treatment of patients with transfemoral amputation: A prospective study of 51 patients. Bone Joint J. 2014 Jan;96-B(1):106-13.

5. Sullivan J, Uden M, Robinson KP, Sooriakumaran S. Rehabilitation of the trans-femoral amputee with an osseointegrated prosthesis: the United Kingdom experience. Prosthet Orthot Int. 2003 Aug;27(2):114-20.

6. Tillander J, Hagberg K, Hagberg L, Branemark R. Osseointegrated titanium implants for limb prostheses attachments: infectious complications. Clin Orthop Relat Res. 2010 Oct;468(10):2781-8.

7. Sundfeldt M, Carlsson LV, Johansson CB, Thomsen P, Gretzer C. Aseptic loosening, not only a question of wear: a review of different theories. Acta Orthop. 2006 Apr;77(2):177-97.

8. Tomaszewski PK, van Diest M, Bulstra SK, Verdonschot N, Verkerke GJ. Numerical analysis of an osseointegrated prosthesis fixation with reduced bone failure risk and periprosthetic bone loss. J Biomech. 2012 Jul 26;45(11):1875-80.

9. Tomaszewski PK, Verdonschot N, Bulstra SK, Rietman JS, Verkerke GJ. Simulated bone remodeling around two types of osseointegrated implants for direct fixation of upper-leg prostheses. J Mech Behav Biomed Mater. 2012 Nov;15:167-75.

10. Nebergall A, Bragdon C, Antonellis A, Karrholm J, Branemark R, Malchau H. Stable fixation of an osseointegated implant system for above-the-knee amputees. Acta Orthop. 2012 Apr;83(2):121-8.

11. Baim S, Wilson CR, Lewiecki EM, Luckey MM, Downs RW,Jr, Lentle BC. Precision assessment and radiation safety for dual-energy X-ray absorptiometry: position paper of the International Society for Clinical Densitometry. J Clin Densitom. 2005 Winter;8(4):371-8.

12. Baad-Hansen T, Kold S, Nielsen PT, Laursen MB, Christensen PH, Soballe K. Comparison of trabecular metal cups and titanium fiber-mesh cups in primary hip arthroplasty: a randomized RSA and bone mineral densitometry study of 50 hips. Acta Orthop. 2011 Apr;82(2):155-60.

13. Boden H, Adolphson P, Oberg M. Unstable versus stable uncemented femoral stems: a radiological study of periprosthetic bone changes in two types of uncemented stems with different concepts of fixation. Arch Orthop Trauma Surg. 2004 Jul;124(6):382-92.

14. Tjornild M, Soballe K, Hansen PM, Holm C, Stilling M. Mobile- vs. fixed-bearing total knee replacement. Acta Orthop. 2015 Apr;86(2):208-14.

15. Wilkinson JM, Eagleton AC, Stockley I, Peel NF, Hamer AJ, Eastell R. Effect of pamidronate on bone turnover and implant migration after total hip arthroplasty: a randomized trial. J Orthop Res. 2005 Jan;23(1):1-8.

16. Hlaing TT, Compston JE. Biochemical markers of bone turnover - uses and limitations. Ann Clin Biochem. 2014 Mar;51(Pt 2):189-202.

17. Li MG, Thorsen K, Nilsson KG. Increased bone turnover as reflected by biochemical markers in patients with potentially unstable fixation of the tibial component. Arch Orthop Trauma Surg. 2004 Jul;124(6):404-9.

18. von Schewelov T, Carlsson A, Dahlberg L. Cross-linked N-telopeptide of type I collagen (NTx) in urine as a predictor of periprosthetic osteolysis. J Orthop Res. 2006 Jul;24(7):1342-8.

19. Wilkinson JM, Hamer AJ, Rogers A, Stockley I, Eastell R. Bone mineral density and biochemical markers of bone turnover in aseptic loosening after total hip arthroplasty. J Orthop Res. 2003 Jul;21(4):691-6.

20. Sumner DR, Ross R, Purdue E. Are there biological markers for wear or corrosion? A systematic review. Clin Orthop Relat Res. 2014 Dec;472(12):3728-39.

21. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. N Engl J Med. 1997 Sep 4;337(10):670-6.

22. Hansen RL, Langdahl BL, Jørgensen PH, Petersen KK, Søballe K, Stilling M. (Submitted) Bone Mineral Density Measurements around Osseointegrated Implants: A Precision Study and Validation of Scan-Protocol for Transfemoral Amputees. Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry.

23. Lewiecki EM, Watts NB, McClung MR, Petak SM, Bachrach LK, Shepherd JA, et al. Official positions of the international society for clinical densitometry. J Clin Endocrinol Metab. 2004 Aug;89(8):3651-5.

24. GE Healthcare Lunar. enCORE-baseret røntgen-knogledensitometer Brugermanual. In: ; 2015. p. 315.

25. Bonnick SL, Johnston CC,Jr, Kleerekoper M, Lindsay R, Miller P, Sherwood L, et al. Importance of precision in bone density measurements. J Clin Densitom. 2001 Summer;4(2):105-10.

26. Sherk VD, Bemben MG, Bemben DA. BMD and bone geometry in transtibial and transfemoral amputees. J Bone Miner Res. 2008 Sep;23(9):1449-57.

27. Flint JH, Wade AM, Stocker DJ, Pasquina PF, Howard RS, Potter BK. Bone mineral density loss after combat-related lower extremity amputation. J Orthop Trauma. 2014 Apr;28(4):238-44.

28. Kulkarni J, Adams J, Thomas E, Silman A. Association between amputation, arthritis and osteopenia in British male war veterans with major lower limb amputations. Clin Rehabil. 1998 Aug;12(4):348-53.

29. Huiskes R. The various stress patterns of press-fit, ingrown, and cemented femoral stems. Clin Orthop Relat Res. 1990 Dec;(261)(261):27-38.

30. Arabmotlagh M, Sabljic R, Rittmeister M. Changes of the biochemical markers of bone turnover and periprosthetic bone remodeling after cemented hip arthroplasty. J Arthroplasty. 2006 Jan;21(1):129-34.

31. Nysted M, Benum P, Klaksvik J, Foss O, Aamodt A. Periprosthetic bone loss after insertion of an uncemented, customized femoral stem and an uncemented anatomical stem. A randomized DXA study with 5-year follow-up. Acta Orthop. 2011 Aug;82(4):410-6.

32. Rosenbaum TG, Bloebaum RD, Ashrafi S, Lester DK. Ambulatory activities maintain cortical bone after total hip arthroplasty. Clin Orthop Relat Res. 2006 Sep;450:129-37.

33. Veitch SW, Findlay SC, Hamer AJ, Blumsohn A, Eastell R, Ingle BM. Changes in bone mass and bone turnover following tibial shaft fracture. Osteoporos Int. 2006;17(3):364-72.

34. Landgraeber S, Loer F, Heep H, Classen T, Grabellus F, Totsch M, et al. Tartrate-resistant acid phosphatase 5b and C-terminal telopeptides of type I collagen as markers for diagnosis of aseptic loosening after total hip replacement. Arch Orthop Trauma Surg. 2010 Apr;130(4):441-5.

35. Liu S, Virdi AS, Sena K, Hughes WF, Sumner DR. Bone turnover markers correlate with implant fixation in a rat model using LPS-doped particles to induced implant loosening. J Biomed Mater Res A. 2012 Apr;100(4):918-28.

36. Ross RD, Virdi AS, Liu S, Sena K, Sumner DR. Particle-induced osteolysis is not accompanied by systemic remodeling but is reflected by systemic bone biomarkers. J Orthop Res. 2014 Jul;32(7):967-73.

37. Bogunovic L, Kim AD, Beamer BS, Nguyen J, Lane JM. Hypovitaminosis D in patients scheduled to undergo orthopaedic surgery: a single-center analysis. J Bone Joint Surg Am. 2010 Oct 6;92(13):2300-4.

38. Smith E, Comiskey C, Carroll A, Ryall N. A study of bone mineral density in lower limb amputees at a national prosthetics center. Journal of Prosthetics and Orthotics. 2011;23(1):14-20.

39. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev. 2001 Aug;22(4):477-501.

40. Lindsay R, Nieves J, Formica C, Henneman E, Woelfert L, Shen V, et al. Randomised controlled study of effect of parathyroid hormone on vertebral-bone mass and fracture incidence among postmenopausal women on oestrogen with osteoporosis. Lancet. 1997 Aug 23;350(9077):550-5.

41. Daugaard H, Elmengaard B, Andreassen TT, Lamberg A, Bechtold JE, Soballe K. Systemic intermittent parathyroid hormone treatment improves osseointegration of press-fit inserted implants in cancellous bone. Acta Orthop. 2012 Aug;83(4):411-9.

42. Kelly J, Lin A, Wang CJ, Park S, Nishimura I. Vitamin D and bone physiology: demonstration of vitamin D deficiency in an implant osseointegration rat model. J Prosthodont. 2009 Aug;18(6):473-8.

43. Dvorak G, Fugl A, Watzek G, Tangl S, Pokorny P, Gruber R. Impact of dietary vitamin D on osseointegration in the ovariectomized rat. Clin Oral Implants Res. 2012 Nov;23(11):1308-13.

44. Zhou C, Li Y, Wang X, Shui X, Hu J. 1,25Dihydroxy vitamin D(3) improves titanium implant osseointegration in osteoporotic rats. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012 Nov;114(5 Suppl):S174-8.

45. Jayasuriya RL, Wilkinson JM. Pelvic periprosthetic bone mineral density measurement around cemented vs cementless acetabular prostheses. J Clin Densitom. 2014 Jan-Mar;17(1):116-20.

46. Stilling M, Soballe K, Larsen K, Andersen NT, Rahbek O. Knee flexion influences periprosthetic BMD measurement in the tibia. Suggestions for a reproducible clinical scan protocol. Acta Orthop. 2010 Aug;81(4):463-70.

47. Tjornild M, Soballe K, Bender T, Stilling M. Reproducibility of BMD measurements in the prosthetic knee comparing knee-specific software to traditional DXA software: a clinical validation. J Clin Densitom. 2011 Apr-Jun;14(2):138-48.

48. Penny JO, Ovesen O, Brixen K, Varmarken JE, Overgaard S. Bone mineral density of the femoral neck in resurfacing hip arthroplasty. Acta Orthop. 2010 Jun;81(3):318-23.

49. Abu-Rajab RB, Watson WS, Walker B, Roberts J, Gallacher SJ, Meek RM. Peri-prosthetic bone mineral density after total knee arthroplasty. Cemented versus cementless fixation. J Bone Joint Surg Br. 2006 May;88(5):606-13.

Table 1: The preoperative BMD in the hip (total) and spine (L1-L4) compared with an age and gender matched reference population

	Data Mean	Standard deviation	Range (min max)	Reference Normal*	Reference Osteopenia†	Reference Osteoporosis‡	P-value§
Total hip BMD (g/cm^2) n=20							
Intact side	1.03	0.16	0.78 to 1.44	1.02	0.97	0.88	0.88
Amputated side	0.69	0.29	0.34 to 1.33				0.0005
Spine BMD (g/cm^2) n=20							
L1-L4	1.13	0.22	0.89 to 1.69	1.19	1.15	1.09	0.2

* Reference Normal: Age and gender matched reference value

† Reference Ostepenia: Upper BMD limit for reference osteopenia (Reference mean -(1*SD))

‡ Reference Osteoporosis: Upper BMD limit for reference osteoporosis (Reference mean -(2.5*SD))

§ P-value: Significance level for the difference between Data mean and reference normal.

Table 2: Periprosthetic BMD changes around the OI implant at last follow-up examination. Periprosthetic BMD is presented as mean difference from baseline measurements.

Measurements at last follow-up	Removed implants				Non-removed implants			
Periprosthetic ROIs (g/cm^2)	Mean diff	95%CI			Mean diff	95%CI		p-value
ROI 1	0.41	0.17	; 0.65		0.25	-0.02	; 0.52	0.30
ROI 2	0.28	-0.03	; 0.59		0.14	-0.10	; 0.38	0.42
ROI 3	0.38	0.07	; 0.69		0.17	-0.06	; 0.40	0.22
ROI 4	0.25	-0.10	; 0.61		-0.05	-0.35	; 0.25	0.15
ROI 5	0.30	0.04	; 0.56		0.13	-0.22	; 0.48	0.36
ROI 6	0.40	0.14	; 0.65		0.14	-0.10	; 0.39	0.11
ROI 7	0.32	0.09	; 0.55		0.03	-0.23	; 0.29	0.07

Table 3: Odds ratio of implant removal during 3-years of follow up

Predictor	Odds Ratio	95%CI Lower	95%CI Upper	Unadjusted p-value*
BMD change† (g/cm^2)				
ROI 1	0.86	0.18	4.17	0.85
ROI 2	1.32	0.16	11.06	0.80
ROI 3	0.63	0.08	4.87	0.66
ROI 4	0.66	0.08	5.33	0.69
ROI 5	0.93	0.14	6.12	0.94
ROI 6	0.57	0.07	4.60	0.60
ROI 7	1.64	0.20	13.23	0.64
Preoperative blood tests				
PTH (pmol/L)	2.40	0.92	6.29	0.07
Vitamin D (nmol/L)	0.99	0.95	1.03	0.63
BASP (µg/L)	1.09	0.90	1.31	0.39
OC (µg/L)	1.09	0.98	1.21	0.13

**The p-values are not adjusted for multiple comparison; thus, the interpretation should focus on the 95% CI.*

† BMD (ROI 1-7) change, in terms of the change in BMD from S1 to the last follow-up examination

Table 4: Bone turnover markers and bone metabolism at last follow-up examination. Bold font represents data presented as median value with IQR and (*) analysis is performed with a ranksum test.

Measurements at last follow-up	Removed				Non-removed			p-value
Bone turnover markers	Mean	95%CI			Mean	95%CI		
P1NP (µg/L)	60.55	38.98	82.12		60.39	44.51	76.27	0.99
CTX (µg/L)	0.39	0.25	0.52		0.28	0.19	0.37	0.15
OC (µg/L)	25.78	21.84	29.72		22.73	16.55	28.91	0.39
BASP (U/L)	29.28	15.59	42.96		22.65	12.62	32.67	0.38
Bone metabolism	Mean	95%CI			Mean	95%CI		
Vitamin D (nmol/L)	58.00	36.27	79.73		58.66	45.83	71.50	0.95
PTH (pmol/L)*	5.84	4.64	7.33		4.02	3.67	4.46	**0.01**

Figure 1: Time-series graph of the mean BMD (95%CI) in ROI 1-7 in the RI and NRI group during 30 months of follow-up

Statistically significant BMD changes from the postoperative baseline are labelled as (†) in the RI group and (‡) in the NRI group.

Compared to baseline, BMD is reduced between 26% CI (3;44) to 40% CI (23;54) in the removed group (*p <0.03)* and returns to baseline value in the non-removed group (*p* >0.083) at 30-month follow-up.

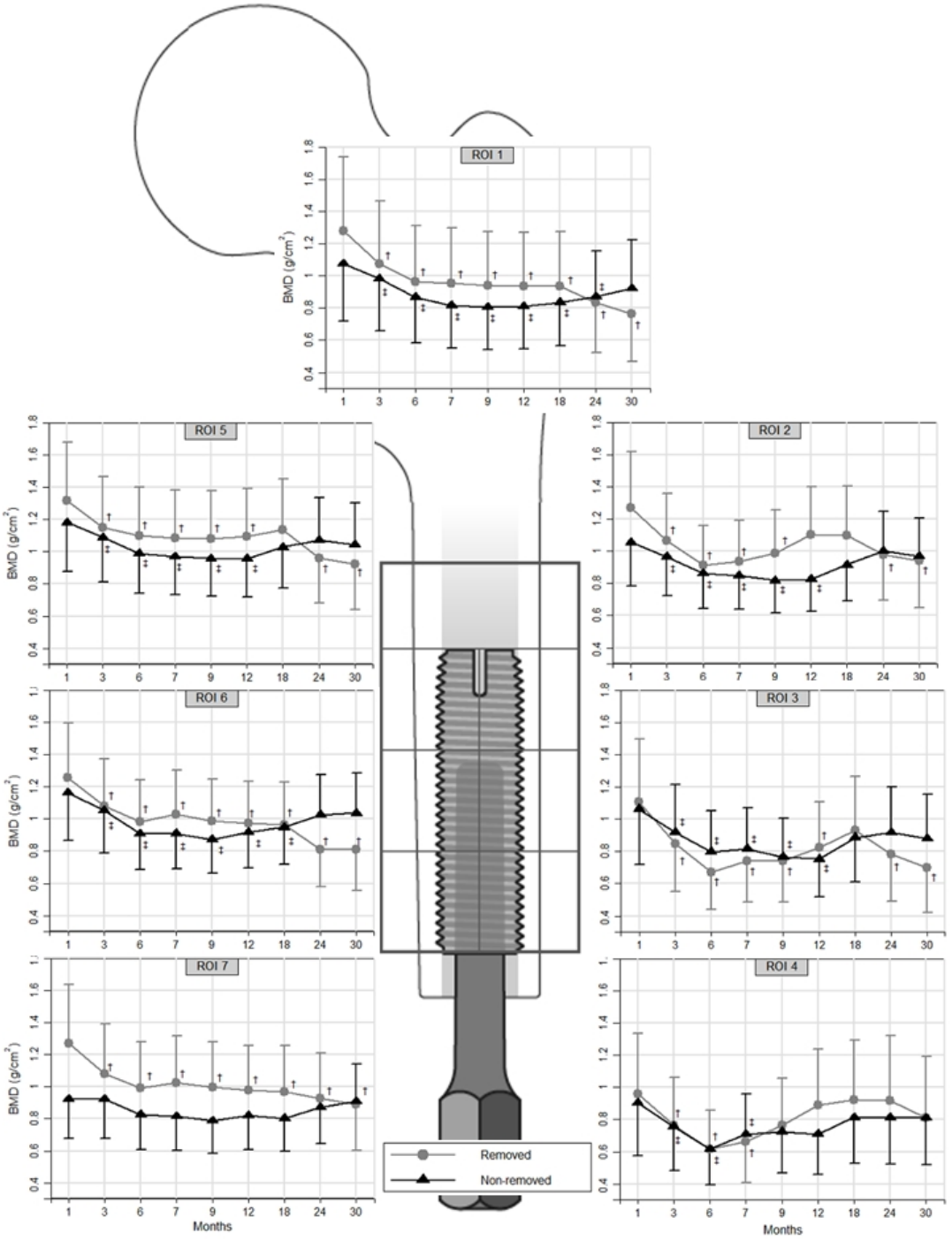


Figure 2: Time series graph of mean total hip BMD (95%CI) in the amputated and intact side during 30 months of follow-up.
(*) BMD on the amputated side was reduced to 13% 95%CI (8; 19) compared to preoperative baseline values ($p < 0.028$).
BMD on the intact side remained unchanged ($p > 0.23$) at all time points.

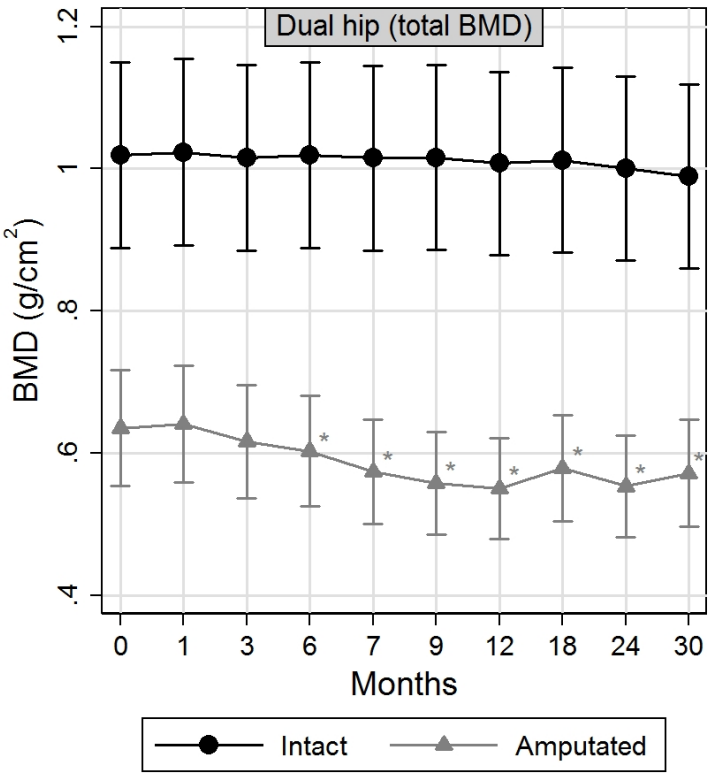


Figure 3: Mean bone turnover marker concentration (95%CI) at each time point in the removed and non-removed group.
Significant group difference (*); Significant changes from the preoperative baseline in the removed group (†) and the non-removed group (‡)

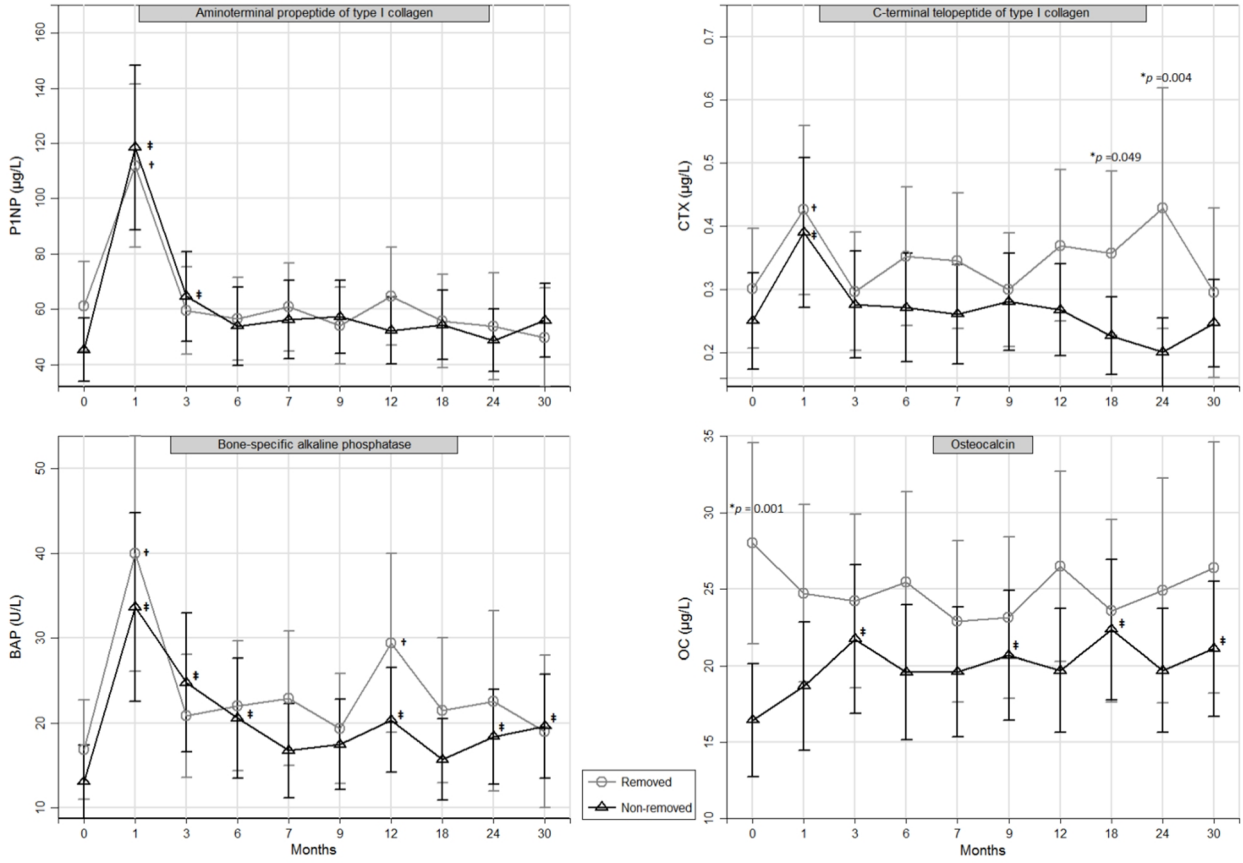


Figure 4: Mean PTH and vitamin D (95%CI) at each time point in the removed and non-removed group. (*) Statistically significant group differences; Significant changes from the preoperative baseline in the removed group (†) and the non-removed group (‡)

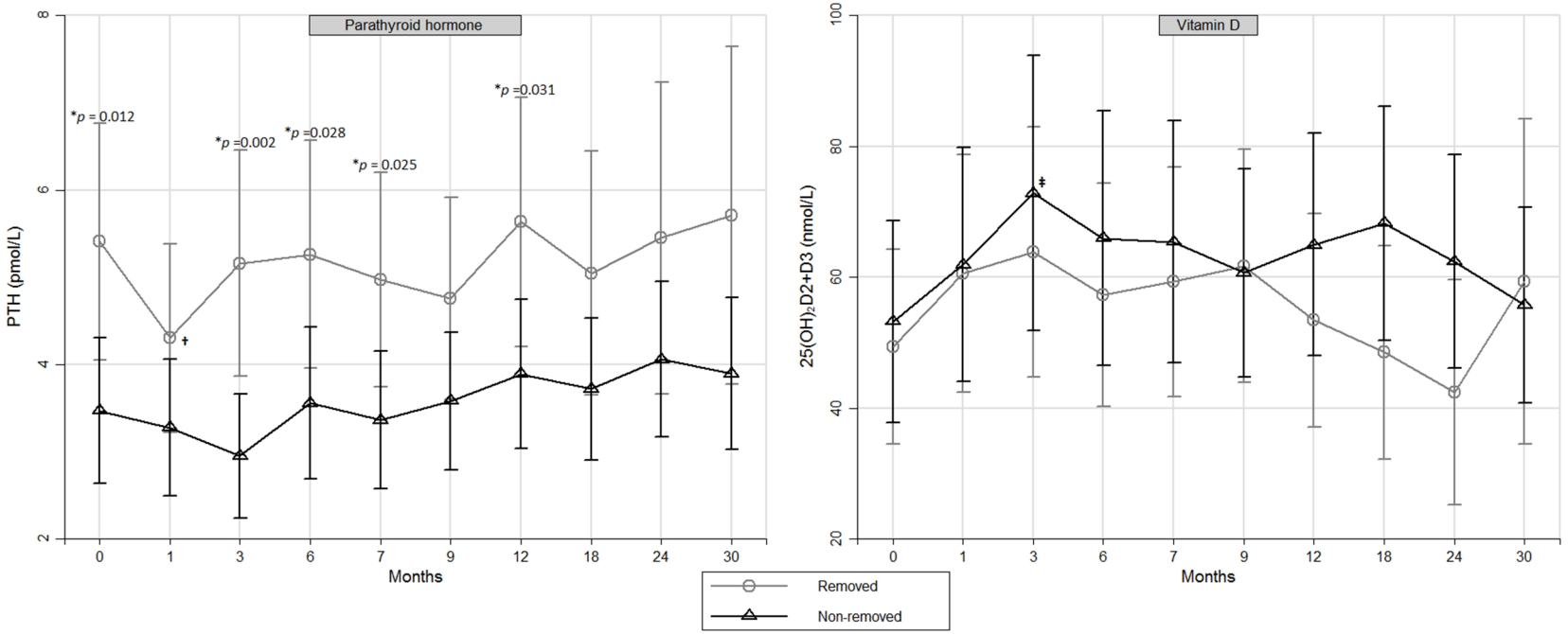
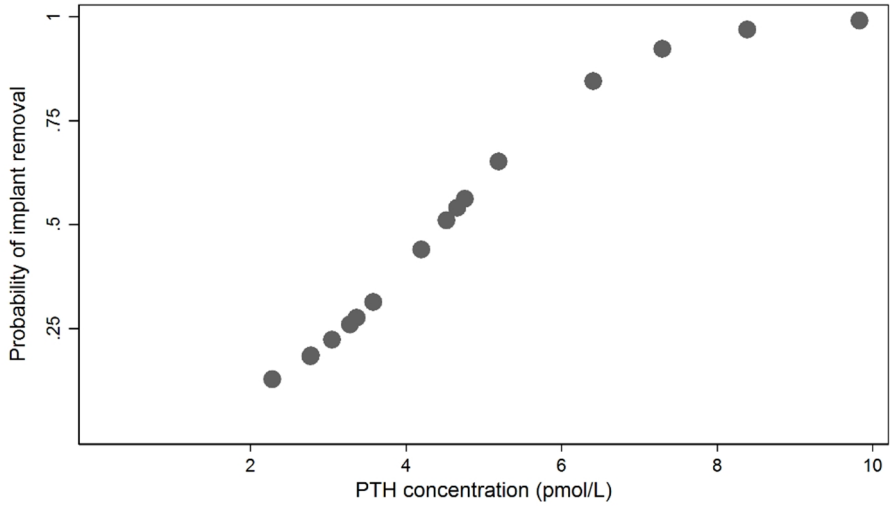


Figure 5: Logistic regression curve showing the probability of implant removal by increasing preoperative PTH concentration



Appendix 1:

	Percent change in BMD from baseline to last follow-up							**BTM, PTH, vit. D at last follow-up exam.**						**Clinical outcome**			
ID	ROI 1	ROI 2	ROI 3	ROI 4	ROI 5	ROI 6	ROI 7	P1NP	CTX	BASP	OC	PTH	Vit D	Last examination	Removals	Cause of implant removal	OI implant in situ
1	21.9	-6.0	12.8	-19.1	9.9	36.7	41.8	90.56	**0.67**	46	36.15	**9.27**	**30**	12	+	Pain	17
2	.	.	.	.	.	.	.	115.1	0.65	**54**	25.4	5.26	79	12	++	Infection	18
3	61.2	57.4	50.8	22.0	43.7	53.8	47.9	61.25	0.50	36.5	24.17	5.84	**19**	18	++	Infection	27
4	50.8	44.9	56.7	52.4	53.9	59.7	49.5	58.05	0.35	**50.4**	27.84	**7.33**	81	30	+	Pain	31
5	12.0	7.4	10.0	10.8	18.9	27.8	19.5	36.89	0.32	15.2	25.13	2.03	**42**	18	++	Infection	19
6	32.9	16.3	30.2	22.6	2.6	7.4	9.4	34.07	0.30	**8.9**	21.21	6.64	**39**	30	+	Pain	31
7	-13.1	-35.1	-17.3	-91.9	-16.5	-12.3	-22.3	49.65	0.29	18.2	29.19	4.45	107	12	++	Infection	16
8	44.2	46.0	52.8	48.4	38.9	41.7	36.8	69.67	0.27	24.8	25.04	4.64	57	12	++	Trauma	18
9	15.9	7.8	15.9	11.2	7.1	14.7	12.8	29.7	0.15	**9.5**	17.89	**8.59**	68	30	-*	Aseptic loosening	
10	7.6	1.4	3.0	1.5	-27.9	2.4	13.9	92.25	0.52	16	32.99	3.89	**28.3**	24			
11	43.4	49.5	48.8	-6.3	44.9	-18.6	2.0	108.7	0.46	20.6	45.78	5.07	50	30			
12	9.4	35.3	32.1	-67.1	37.5	38.0	-58.1	80.51	0.38	**62.6**	21.11	5.03	71	30			
13	.	.	.	.	.	.	.	56.78	0.35	18.4	22.28	3.67	**21**	30			
14	-21.0	-9.9	7.9	12.2	-6.4	0.8	-7.4	36.2	0.29	18.3	15.96	2.92	65	30			
15	.	.	.	.	.	.	.	42.86	0.27	**3.4**	15.76	4.05	71	30			
16	5.9	7.1	0.0	12.7	-11.5	-0.6	-2.0	61	0.25	16.7	19.95	3.72	65	30			
17	46.0	20.0	33.5	.	39.1	44.4	39.4	55.77	0.18	33	13.6	2.89	59	30			
18	36.2	5.4	2.8	18.2	10.9	5.0	23.7	47.42	0.15	19.1	18.57	4.46	73	30			
19	-23.4	-37.4	-23.7	-14.1	-22.2	1.3	-8.6	38.7	0.13	22.9	23.38	4.07	84	30			
20	.	.	.	.	.	.	.	44.07	0.13	18.1	20.65	4.02	58	30			

Implant removals are presented in grayscale rows;

ROI 1 to 7 is presented as the percent change BMD from baseline to the last examination;

BTM, PTH, vitamin D are presented as the absolute value at the last examination;

Removals, total OI implant removal(++) or removal of abutment(+);

Last examination in terms of number of months after S1 surgery;

OI implant in situ in terms of the number of months after S1 surgery the fixture or abutment remained in the patient ;

Patient (ID=9) does not use the OI implant due to pain, but uses a modified prosthetic socket, thus considered an OI implant failure (*);

Four patients are missing baseline BMD measurements, thus percent change from baseline values cannot be calculated (.)

Paper III

Higher migration of removed compared with non-removed osseointegrated implants for transfemoral amputees. A prospective 2 year RSA study with 5-years clinical follow-up

List of authors and affiliations:

1) Hansen, Rehne Lessmann[1,4], MD
2) Bente Lomholt Langdahl[2,4], Professor, MD, PhD, DMSc,
3) Jørgensen, Peter Holmberg[3], MD, DMSc,
4) Petersen, Klaus Kjær[3], MD
5) Søballe, Kjeld[1,3,4], Professor, , MD, DMSc
6) Stilling, Maiken[1,4], MD, PhD

1: Orthopaedic Research Unit, University Hospital of Aarhus, Aarhus, Denmark

2: Department of Endocrinology and Internal Medicine, University Hospital of Aarhus, Aarhus, Denmark

3: Department of Orthopaedic Surgery, University Hospital of Aarhus, Aarhus, Denmark

4. Department of Clinical Medicine, Aarhus University, Denmark

Abstract

Background: The osseointegrated (OI) prosthesis is a treatment option for transfemoral amputees with a short residual femur and/or difficulties caused by the prosthetic socket. Implant removal due to aseptic and septic loosening is not uncommon, but implant migration patterns in relation to failure has not previously been described.

Purpose: The aim was to investigate the migration pattern of removed and non-removed OI implants and the correlation between OI implant migration and preoperative bone mineral density.

Methods: An observational cohort of 17 consecutive patients (11 males), mean age 50 (range 32-66) were treated with an OI implant. Preoperative DXA scans of the proximal hip (total) and lumbar spine (L1-L4) and postoperative stereoradiographs of the OI implant during 24-month follow-up were obtained. RSA precision was examined by double examinations. X, Y, and Z translations and total translations (TT) were evaluated using CAD-implant models. Implant survival was followed up to 60-month follow-up.

Results: Six total implant removals (fixture and abutment) and four partial removals (abutment) were conducted, and one patient did not use the OI-implant. At the last RSA follow-up, the removed implant (RI) group had migrated 0.55 mm 95%CI(-0.03; 1.14, p = 0.009) TT and -0.07 mm 95%CI(-0.23; 0.08, p = 0.19) distal more than the non-removed implant group (NRI). Odds ratio for implant removal was 22.5 95%CI(1.6; 314, p = 0.021) if the OI implants migrated distally. The OI implant migration did not correlate with the T-score of the hips or lumbar spine (p > 0.45)

Interpretation: The OI implants that were later removed migrated continuously and more than the non-removed OI implants at last follow-up. 65% of the OI implants were removed within 5 years of follow-up, and distal implant migration greatly increased the odds of implant removal.

Introduction

An osseointegration (OI) implant for transfemoral amputees (TFA) provides a bone-anchored attachment for the external prosthetic leg, thereby alleviating problems associated with the prosthetic socket (1, 2). The OI implant system comprises an intraosseous titanium fixture and an exosseous abutment implanted during a two-stage surgical procedure. At stage 1 (S1), the fixture is inserted into the residual femur and allowed to osseointegrate for 6 months. At stage 2 (S2), the fixture is connected to the percutaneous abutment making it possible to attach an external prosthesis. After S2 surgery, the patients follow a 6-months rehabilitation program and gradually increase the load on the OI implant until full weight-bearing is possible (1). A successful osseointegration is imperative for implant survival and requires limited micromotion, a stable fixation and intimate contact to the bone (3).

From 1990 to 2008, the Swedish Osseointegration team reported OI implant (Integrum AB, Sweden) removal in 20 out of 100 patients, and due to a learning curve the first 15 patients had 10 implants removed (1). From 1999 to 2007, they enrolled 51 patients (55 implants) in the prospective "Osseointegrated prostheses for the Rehabilitation of Amputees" (OPRA) study with 2-years of follow-up. Four implants were lost, 1 septic and 3 aseptic, and in this specific cohort they reported a cumulative implant survival rate at two years of 92% (4).

Radiostereometric analysis (RSA) is considered the gold standard for measuring implant migration with respect to surrounding bone (5). Detection of implant migration at an early stage has proven useful for predicting aseptic loosening in knee and hip arthroplasty (6, 7) and the high accuracy and precision of RSA (8, 9) makes investigation possible with a small study population.

A marker-based RSA study of the OPRA cohort with analysis of 40 out of 55 implants (including 2 out of 4 failures) at the 2-year follow-up indicated a stable OI implant fixation (10), however the migration pattern of the loose implants was not described. To improve OI implant survival, more knowledge about the association between osseointegration and OI implant migration is important.

The primary aim of this prospective observational cohort study was to examine the migration of the OI implant for TFA using model-based RSA with 24-month follow-up. A secondary aim was to investigate the

migration pattern of OI implants compared to those later removed based on the 5-year clinical outcome. The tertiary aim was to investigate the correlation between OI implant migration and preoperative bone mineral density.

Study settings and participants

Seventeen consecutive patients (11 males), mean age 50 (range 32-66) were operated between 2010 and 2013 (11). The surgical criteria were healthy and motivated TFAs, with an age between 18-70 years, BMI <30, and a bone structure suitable for OI implant surgery. Exclusion criteria were diabetes, arthrosclerosis, smoking, treatment with bisphosphonates, NSAID or cytostatic medicine, active cancer, kidney or hepatic insufficiency, dementia, pregnancy, and weight >100kg.

According to Danish law, formal approval from the Regional Ethical Committee was not required as all examinations were performed according to an established quality assurance protocol (inquiry number 135/2016). Data were handled according to the regulations of the Danish Data Protection Agency (approval of 2012-28-005).

Follow-up examinations

Sixteen patients had a transfemoral amputated leg at the time of OI surgery and one patient was scheduled for one-stage transfemoral amputation and OI surgery. Implant fixation was investigated with model-based RSA and preoperative bone mineral density (BMD) with dual x-ray absorptiometry (DXA) scans. Radiostereometric imaging was conducted 1, 3, 6, 12, 18 and 24 months after S2 surgery. The OI implant position at 1-month follow-up was used as a baseline (Table 1) for all migration analyses and implant survival was reported up to 60 months follow-up.

DXA analysis

Preoperative DXA scans were performed using a Lunar Prodigy Advance 2005 (General Healthcare, Madison WI) DXA scanner and analysis was conducted with enCORE 14.10.022 software. BMD on the both sides was measured in the proximal hip (total hip) and lumbar spine (l1-l4) using standard regions of interest (ROI). All DXA scans were performed by the same technician and all BMD analyses were completed by one observer.

The prevalence of osteoporosis and osteopenia was estimated according to the WHO classifications (normal, T-score T>-1; osteopenia T≤-1; osteoporosis T<-2.5)

RSA setup

Until 2014 all stereoradiographs were obtained using a standard RSA setup(12) consisting of two-ceiling fixed, synchronized roentgen tubes (Acro-Ceil/Medira; Santax Medico; Denmark) both positioned at a 20^{o} angle with the vertical plane, and an unfocussed uniplanar carbon calibration box (Box 24; Medis Specials, Leiden, the Netherlands) (Figure 1). All stereoradiographs were digitized images (Fuji CR (ST-VI IP), 200 µm pixels pitch). In 2014, the radiography equipment was replaced with an automated RSA system (Adora RSA; NRT, Denmark) also with ceiling fixed and synchronized roentgen tubes (Varian Medical Systems, USA). Stereoradiographs were direct digital (Canon CXDI-70C**,** 125 µm pixel pitch). We continued using the same RSA setup (tube position, patient position and calibration box).

Radiostereometric analysis

All operations were performed as a joint venture with two surgeons. During S1 surgery, 6-10 tantalum beads (Ø 1.0 mm) were inserted into the femoral cortical bone distal and proximal to the implant (Figure 1) using a bead gun (Wennbergs Finmek AB, Sweden).

CAD models of the inserted fixtures were provided in 11 different sizes (Ø 16mm to 23mm) by the manufacturer (Integrum AB, Sweden) and were implemented in the model-based RSA software by the software provider (RSAcore, Leiden, The Netherlands). The final implant-models were created with 10,000 triangles in order to maintain enough model detail to specify the edges of the threaded surface. X, Y, and Z translations and total translations (TT) of the OI implant with respect to the bone markers were calculated. The Y-axis was aligned with the longitudinal axis of the fixture (Figure 1), and implant migration along the y-axis in proximal direction (subsidence) was defined as positive motion and the distal direction as negative implant migration. The cut-off for stable markers was 0.35mm (rigid body error) and the same bone markers around the implant were selected for all follow-up analyses to ensure a similar rigid body reference. The mean rigid body error was 0.14 (range 0.024 to 0.35). Sixteen patients had ≥ 3 stable bone markers; one

patient only had one stable bone marker during follow-up examinations. Primarily due to a longitudinal alignment of the bone markers, six patients had a condition number (CN) >120, and the mean CN was 138.4 (range 37 to 406). Only translations were assessed, and high CNs were thus accepted. Precision of RSA was determined by obtaining two sets of stereoradiographs on the same patient (n=12) within an interval of a few minutes (double examinations). The patient was repositioned between the examinations by either sitting or standing before returning to the supine position. Model-based analysis of all radiographs was performed by one observer using model-based RSA 4.0 (RSAcore, Leiden, The Netherlands) software.

Statistics

Longitudinal implant migration was analysed with a linear mixed model with random effects for patients, thus allowing between patient variation (with exponential variance between time points). The model assumptions were evaluated by visual assessment of the residuals and BLUPS (best linear unbiased predictors) or fitted values of the model (qq-plot and plot of residuals vs BLUPS). Translations were assessed along the X-, Y-, and Z-axis. Translation (TT) was calculated using the Pythagoras theorem $T = \sqrt{X^2 + Y^2 + Z^2}$. The primary outcome was the mean TT and Y translation with 95% confidence interval (CI) after S2 surgery. A measure of continuous migration at a cohort level was defined as the difference in TT and Y-translations between 3 months and 24 months of follow-up.

A subgroup analysis of removed implants (RI) and non-removed implants (NRI) were undertaken. The RI group comprised of total implant removal (fixture and abutment) and partial removal (abutment). The patients were followed until partial/total implant removal or until 60 months of follow-up. A logistic regression analysis was used to determine the predictors (Y- and total translations) of implant removal at the last follow-up examinations. The odds ratio (OR) estimated the odds of implant removal during 5 years of follow-up.

Normal distribution was evaluated on qq-plots. Parametric data were analysed using *t* test and reported as means with 95% CI. Non-parametric data were analysed using rank sum and *U* test, and reported as medians with 25%-75% inter quartile range (IQR).

Precision was assessed by double examinations. Any difference between the first and second examination was assumed to be caused by measurement error. The difference in migration (mm) represented the bias of the system and the standard deviation (SD) the precision. The mean (SD) of TT was reported instead of the log-transformed values for interpretational reasons. The 95% CI of the measurement error/precision limit was expressed as ±1.96 x precision SD according to the RSA ISO standard (13). The correlation between preoperative T-score in the hip of the amputated leg, intact hip and lumbar spine with Y and total translation was examined with a Spearmans' rank test. A p-value <0.05 was considered statistically significant. Statistical analysis was performed using STATA 13.1 (Stata Corp LP, College Station, Texas).

Results

RSA cohort measurements

The mean clinical follow-up time was 31 months CI (22.7; 39.5). For the entire cohort (Figure 2), the total translation (TT) was 0.40mm CI (0.10; 0.70) at the 3-month follow-up and 0.90mm CI (0.54; 1.27) at the 24-months follow-up. The difference between 3 and 24 months of follow-up (continuous migration) was 0.50mm CI (0.10; 0.90, p = 0.014) in TT. The OI implants migrated 0.02mm CI (-0.10; 0.05) distal at 3 months, -0.13mm CI (-0.23; -0.04) at 24 months (Figure 3) and the difference (continuous migration) was -0.11mm CI (-0.22; -0.01, p = 0.037) in Y translations.

Clinical outcomes and prediction

Nine OI implants migrated above the y-axis precision limit (Table 2) as shown in Figure 3, and six fixtures plus four abutments were removed during the 60-month follow-up period (Table 3). One patient (N) was categorized as a removed in the subgroup analysis due to symptoms indicating aseptic loosening and RSA indicating continued implant migration. The OR of implant removal was 22.5 CI(1.6; 314) if the implants migrated distally and 0.04 CI(0.01; 0.62) if the implants migrated proximally (p = 0.021). In TT, the OR of implant removal was 1.13 CI(0.31; 4.08, p = 0.86)

Analysis of removed versus non-removed implants

A subgroup analysis of eleven RI and six NRI during the 60 months of follow-up was undertaken. The mean implant survival time was 22 months CI (14.3; 30.2) in the RI group which was less compared with 48 months CI (37.4; 57.2) in the NRI group (p = 0.0005). The time-series analysis in Figure 4 displays a similar TT between the groups at all time points (p > 0.49). At 24 months, the RI group migrated -0.28mm CI (-0.41; -0.16) distal compared with -0.01mm CI (-0.12; 0.11) distal migration in the NRI group (p = 0.002). In the time-series analysis, the difference (continuous migration) in distal migration was -0.23mm CI (-0.36; -0.09, p = 0.001) and 0.65mm CI (0.10; 1.21, p = 0.021) in TT.

At the last follow-up analysis (Table 4), there was no difference between the groups in Y translations (p = 0.16) or TT (p = 0.76), but significant difference from baseline to last follow-up (continuous migration) in TT of the removed implants (p = 0.009).

BMD effect on migration pattern

The preoperative BMD and T-score (Table 5) were similar between the subgroups (p>0.64). The OI implant migration at last follow-up examination did not correlate with the T-scores in the hips or lumbar spine (p>0.45) (Table 6).

Discussion

This is the first study using model-based RSA to evaluate the osseointegrated implant fixation for TFA and describe the migration pattern of loose and stable implants. The difference between early and late total translation indicated that the OI implants migrated continuously in the RI group (Table 4).

Implant migration pattern

Eleven implants (including patient N) were removed and seven of those were removed after the migration exceeded the y-axis precision limit of 0.11mm (Table 2). This is consistent with the established understanding that early implant migration above the precision limit of RSA is a prognostic marker for premature implant removal (14). Two implants migrated above the Y-axis precision (implant N and Q) but remained in situ during the 5-year clinical follow-up period. Implant N subsided 6 months after S2 surgery and the patient reported pain during weight bearing at the 12-month follow-up. At the 24-month follow-up, the OI implant had

migrated -0.32mm distally and weight-bearing on the OI implant had ceased. This patient had large areas with indurated skin grafts after necrotizing fasciitis which made it difficult to reconstruct the soft tissue cover if the implant was removed. Instead of removing the OI implant, we equipped the patient with a prosthetic socket including a silicon bottom leaving space to the abutment, thereby transferring the weight to soft tissue. Implant Q migration varied between -0.07 to -0.41mm during follow-up. This patient had a very short stump and the residual femur was pointing in an anterior direction during RSA examinations. Combined with a large CN (389), this may be a factor contributing to the large variation in distal migration. Patient Q was using the OI implant during the clinical follow-up.

The majority of OI implants in the cohort did not necessarily subside after implantation, but seemed to favour a slight -0.13mm CI (-0.23, -0.04, n=10) distal migration at 2 years, which was even greater for the removed implants, viz. Y= -0.28mm CI (-0.41, -0.16). Nebergall et al. found no OI implant migration pattern favouring distal or proximal direction and reported Y= 0.00mm CI (-0.53 to 0.13, n=40) migration at 24 months (10). Proximal migration (subsidence) is an important measure of stem fixation in the femoral bone (6) and it is an early prognostic indicator for loosening of stems in total hip arthroplasty. However, we found that distal migration at the last RSA examination markedly increased the odds (OR=22.5) of implant removal (Table 3). A possible explanation for the divergent proximal and distal migration pattern may be that the load and traction on the OI implant during the gait cycle was transferred directly to the periprosthetic bone and was not reduced by muscles and tendons during the swing phase as in a hip replacement. A study of the kinetics in TFA treated with OI implants illustrated that the maximum load on the abutment in the longitudinal axis was 780N during the stance phase, but decreased down to -85N during the swing phase due to the traction created by gravity on the external prosthesis (15). Even though the magnitude of the distal forces was small, the distal forces do play a role in the causal explanation for the migration pattern of the removed implants. The OR indicated that an increase in TT may add to the odds of implant removal. Ryd et al used the maximum total point of motion (MTPM) in order to identify patients with continuous knee implant migration. Defining continuous migration as MTPM >0.2mm at any follow-up at 1-2 years after surgery could identify implants at

risk of removal with a predictive power of 85% (7). Applying the same principle on OI implants with a TT >0.2mm between the last two follow-ups >1 years after surgery (Table 3) indicated that 8 out of 10 implants were at a high risk of removal.

Aseptic/septic loosening

Six fixtures and 4 abutments were removed within 5 years of follow-up. Similarly, 10 out of 15 OI implants (Integrum AB, Sweden) were removed by the Swedish osseointegration team during the first 4 years; however, the causes of implant removal were not described in the paper (1). The migration pattern of the removed OI implants in the OPRA study was unknown (10), but the implants were all removed within the first 2 years after S2 surgery. (10). The high number of implant removals in this study may be due to only partial osseointegration before S2 surgery which would seem to be a consequence the OI implants having a limited capacity to withstand weight-bearing. In an experimental bovine GAP-model, unstable titanium implants showed no bone ingrowth and the implant offered no resistance to push-out compared with stable titanium implants (16). The percutaneous abutment provided an open access to the bone and increased the risk of an ascending intramedullary infection which can be a reason for loosening. Several implant designs have been attempted to seal off the skin implant interface and minimize the infection risk (17, 18). To reduce the infection risk after S2 surgery, the tissue thickness around the abutment was surgically reduced. This allowed the skin to adhere onto the bone end and for soft tissue downgrowth facing the abutment (19). Even with this soft tissue seal, infections remain an unresolved problem (20).

Bone mineral density

Aro et al. found that hydroxyapatite-coated femoral stems subsided more in patients with osteopenia/osteoporosis (21), and same results were found with hydroxyapatite-coated titanium alloy cups (22). But even though the preoperative T-score was very low in the amputated hip it did not correlate with OI implant migration. Other unknown factors may have contributed to implant migration, e.g. infection or aseptic loosening.

RSA and precision

No other OI implant RSA studies have previously presented precision estimation. The precision along the X- and Z-axis in our study was lesser than the precision in hip stem studies using model-based RSA (SD <0.2mm) (23, 24). Nonetheless, the y-axis precision (SD 0.06mm) was higher than with hip stems, probably because the threads along the OI implant assisted the CAD-model fitting in proximal/distal direction.

The main strengths of the present study are prospective follow-up on the full cohort of transfemoral amputees with OI implants. Limitations include the small number of patients in the cohort, and data were missing at some time points (Table 1). However, implant stability can be evaluated acceptably with a small study population as a consequence of the high precision of RSA (12). Moreover, by using a linear mixed model, it was possible to handle missing data and keep all patients in the analysis. Seven patients had a high CN (>120) indicating the bone marker configuration approached a straight line, which decrease the precision around the rotational axis (25). This is difficult to avoid with a long slim bone model (femur), and therefore, we focussed solely on translations.

Conclusion

Continuous migration of removed implants was measured in TT. Implant removal was associated with higher distal implant migration compared to non-removed OI implants. The T-scores in hips or lumbar spine did not correlate with implant migration. Compared with other OI implant studies a high number of implant removals occurred and it is advisable to monitor OI patients closely and preferably with RSA for early prediction of implant loosening. Since only a limited number of transfemoral amputees are treated with OI implants worldwide and novel OI implant designs are emerging, osseointegration teams should join in multicentre studies to clarify complications and benefits of different OI implant designs, and model-based RSA would be an important tool to monitor implant fixation.

References

1. Hagberg K, Branemark R. One hundred patients treated with osseointegrated transfemoral amputation prostheses--rehabilitation perspective. J Rehabil Res Dev. 2009;46(3):331-44.

2. Sullivan J, Uden M, Robinson KP, Sooriakumaran S. Rehabilitation of the trans-femoral amputee with an osseointegrated prosthesis: the United Kingdom experience. Prosthet Orthot Int. 2003 Aug;27(2):114-20.

3. Albrektsson T, Branemark PI, Hansson HA, Lindstrom J. Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. Acta Orthop Scand. 1981;52(2):155-70.

4. Branemark R, Berlin O, Hagberg K, Bergh P, Gunterberg B, Rydevik B. A novel osseointegrated percutaneous prosthetic system for the treatment of patients with transfemoral amputation: A prospective study of 51 patients. Bone Joint J. 2014 Jan;96-B(1):106-13.

5. Karrholm J, Gill RH, Valstar ER. The history and future of radiostereometric analysis. Clin Orthop Relat Res. 2006 Jul;448:10-21.

6. Karrholm J, Borssen B, Lowenhielm G, Snorrason F. Does early micromotion of femoral stem prostheses matter? 4-7-year stereoradiographic follow-up of 84 cemented prostheses. J Bone Joint Surg Br. 1994 Nov;76(6):912-7.

7. Ryd L, Albrektsson BE, Carlsson L, Dansgard F, Herberts P, Lindstrand A, et al. Roentgen stereophotogrammetric analysis as a predictor of mechanical loosening of knee prostheses. J Bone Joint Surg Br. 1995 May;77(3):377-83.

8. Valstar ER, Vrooman HA, Toksvig-Larsen S, Ryd L, Nelissen RG. Digital automated RSA compared to manually operated RSA. J Biomech. 2000 Dec;33(12):1593-9.

9. Borlin N, Thien T, Karrholm J. The precision of radiostereometric measurements. Manual vs. digital measurements. J Biomech. 2002 Jan;35(1):69-79.

10. Nebergall A, Bragdon C, Antonellis A, Karrholm J, Branemark R, Malchau H. Stable fixation of an osseointegated implant system for above-the-knee amputees. Acta Orthop. 2012 Apr;83(2):121-8.

11. Hansen RL, Langdahl BL, Jørgensen PH, Petersen KK, Søballe K, Stilling M. Loss of periprosthetic bone mineral density and higher PTH is associated with removal of osseointegrated implants in femoral amputees. (Unpublished).

12. Valstar ER, Gill R, Ryd L, Flivik G, Borlin N, Karrholm J. Guidelines for standardization of radiostereometry (RSA) of implants. Acta Orthop. 2005 Aug;76(4):563-72.

13. RSA ISO standard. Implants for surgery — Roentgen stereophotogrammetric analysis for the assessment of migration of orthopaedic implants. 2013. Report No.: ISO 16087.

14. Karrholm J. Radiostereometric analysis of early implant migration - a valuable tool to ensure proper introduction of new implants. Acta Orthop. 2012 Dec;83(6):551-2.

15. Lee WC, Frossard LA, Hagberg K, Haggstrom E, Branemark R, Evans JH, et al. Kinetics of transfemoral amputees with osseointegrated fixation performing common activities of daily living. Clin Biomech (Bristol, Avon). 2007 Jul;22(6):665-73.

16. Bechtold JE, Mouzin O, Kidder L, Soballe K. A controlled experimental model of revision implants: Part II. Implementation with loaded titanium implants and bone graft. Acta Orthop Scand. 2001 Dec;72(6):650-6.

17. Aschoff HH, Juhnke DL. Evaluation of 10 years experience with endo-exo femur prostheses - background, data and results. Z Orthop Unfall. 2012 Dec;150(6):607-14.

18. Pitkin M. Design features of implants for direct skeletal attachment of limb prostheses. J Biomed Mater Res A. 2013 Nov;101(11):3339-48.

19. Stenlund P, Trobos M, Lausmaa J, Branemark R, Thomsen P, Palmquist A. Effect of load on the bone around bone-anchored amputation prostheses. J Orthop Res. 2016 Jun 24.

20. Tillander J, Hagberg K, Hagberg L, Branemark R. Osseointegrated titanium implants for limb prostheses attachments: infectious complications. Clin Orthop Relat Res. 2010 Oct;468(10):2781-8.

21. Aro HT, Alm JJ, Moritz N, Makinen TJ, Lankinen P. Low BMD affects initial stability and delays stem osseointegration in cementless total hip arthroplasty in women: a 2-year RSA study of 39 patients. Acta Orthop. 2012 Apr;83(2):107-14.

22. Finnila S, Moritz N, SvedstroM E, Alm JJ, Aro HT. Increased migration of uncemented acetabular cups in female total hip arthroplasty patients with low systemic bone mineral density. A 2-year RSA and 8-year radiographic follow-up study of 34 patients. Acta Orthop. 2016 Feb;87(1):48-54.

23. Kaptein BL, Valstar ER, Stoel BC, Reiber HC, Nelissen RG. Clinical validation of model-based RSA for a total knee prosthesis. Clin Orthop Relat Res. 2007 Nov;464:205-9.

24. Kaptein BL, Valstar ER, Spoor CW, Stoel BC, Rozing PM. Model-based RSA of a femoral hip stem using surface and geometrical shape models. Clin Orthop Relat Res. 2006 Jul;448:92-7.

25. Ryd L, Yuan X, Lofgren H. Methods for determining the accuracy of radiostereometric analysis (RSA). Acta Orthop Scand. 2000 Aug;71(4):403-8.

Table 1: Patients analysed at each follow-up examination

Patient count at each interval (months after S2 surgery)							
	1	3	9	12	18	24	60
Patients eligible for follow up	17	17	17	16	14	11	
Patient missing follow up	1	2	1	2	1	1	
Implants removed				3*	3		4**
Patients analysed	16	15	16	12	10	10	

*1 abutment and 2 fixtures removed

**3 abutments and 1 fixture removed

Table 2: Precision of RSA.

The mean value expresses the bias of the system, and the standard deviation the precision. The precision limit is a value below or equal to the difference between two examinations occurring with a probability of 95%. Migration can only be regarded as “true migration” and not a “measurement error” when it is above the precision limit.

	Double examination (n=12)			
Axis of translation	x	y	z	TT*
Mean (mm)	0.06	0.002	-0.06	0.51
SD (mm)	0.20	0.06	0.25	0.28
Precision limit**	0.39	0.11	0.49	0.56

*The total translation was calculated using the 3D Pythagorean Theorem: $T = \sqrt{X^2 + Y^2 + Z^2}$

**Precision limit was calculated by multiplying SDx1.96.

Table 3: Presentation of individual migration pattern, clinical data and outcome.

	RSA data						Clinical data		
	Migration		Last RSA exam.	Cont. migration		Condition number	Revision	Cause	OI implant in situ
ID	Y-axis	TT		Y-axis	TT				
A	**0.19**	**1.99**	**12**	**0.09**	**1.99**	**406**	**++**	**Infection**	**21**
B	0.12	0.28	24	0.08	0.69	169	-	-	36
C	0.10	0.25	6	0.03	0.25	106	-	-	37
D	**0.10**	**1.05**	**3**	**0.10**	**1.05**	**45**	**++**	**Infection**	**13**
E	0.10	0.32	24	-0.05	0.28	37	-	-	54
F	0.09	0.42	24	0.00	0.13	87	-	-	58
G	0.02	1.62	24	0.01	0.29	169	-	-	45
H	**-0.02**	**0.13**	**24**	**0.03**	**0.16**	**58**	**+**	**Pain**	**26**
I	**-0.05**	**0.89**	**6**	**0.02**	**0.89**	**107**	**+**	**Pain**	**11**
J	**-0.07**	**0.35**	**6**	**-0.06**	**0.35**	**85**	**++**	**Trauma**	**12**
K	**-0.15**	**0.40**	**24**	**-0.06**	**0.38**	**222**	**+**	**Pain**	**25**
L	**-0.16**	**0.24**	**12**	**-0.10**	**0.24**	**48**	**++**	**Infection**	**12**
M	**-0.18**	**0.42**	**24**	**0.01**	**0.21**	**125**	**+**	**Pain**	**40**
N	**-0.32**	**0.39**	**24**	**-0.66**	**1.57**	**69**	**-***	**Pain**	**34**
O	**-0.37**	**3.03**	**24**	**-0.82**	**3.36**	**NA**	**++**	**Infection**	**41**
P	**-0.39**	**0.42**	**12**	**-0.44**	**0.42**	**92**	**++**	**Infection**	**10**
Q	-0.39	1.78	24	-0.30	0.74	389	-	-	54

Implants migrating above the y-axis precision limit (excessive migrators) are presented in grayscale rows;
Implant removals are highlighted with bold font;
Migration (mm) measured from baseline to the last RSA examination (months after S2 surgery);
Continuous migration (mm) measured as implant migration between the last two follow-up examinations;
Condition number where >120 indicates poor distribution of stabile/useful bone markers;
Removals, total OI implant removal(++) or removal of abutment(+);
Cause, in terms of cause of removals
OI implant in situ in terms of the number of months after S2 surgery the fixture or abutment remained in the patient
Patient N does not use the OI implant due to pain, but uses instead a modified prosthetic socket, thus considered an OI implant failure (*).

Table 4: Migration of removed and non-removed OI implant at last RSA examination.

Difference between the removal and the non-removal group (*); Changes from early (3 month) to late (up to 24 months) migration in the removed group (†) and the non-removed group (‡).

Translations, mm	**Absolute migration**		p-value		**Continuous migration**		p-value
Y-axis	Median (IQR)	Mean (95%CI)			Median (IQR)	Mean (95%CI)	
Removed implants	-0.15 (-0.32; -0.02)	-0.13 (-0.25; -0.01)	* $p = 0.16$		-0.07 (-0.19; 0.00)	-0.07 (-0.23; 0.08)	†$p = 0.19$
Non-removed implants	0.09 (0.02; 0.10)	0.01 (-0.20; 0.21)			-0.01 (-0.11; 0.03)	-0.05 (-0.18; 0.09)	‡$p = 0.50$
TT							
Removed implants	0.42 (0.35; 1.05)	0.85 (0.25; 1.45)	*$p = 0.76$		0.23 (0.06; 0.68)	0.55 (-0.03; 1.14)	†$p = 0.009$
Non-removed implant	0.37 (0.28; 1.62)	0.76 (0.02; 1.53)			0.17 (0.04; 0.42)	0.31 (-0.33; 0.95)	‡$p = 0.22$

Abbreviations: CI; Confidence interval, TT; Total translations, IQR; Inter quartile range

Table 5: Preoperative BMD measurements and T-scores in the removed and non-removed group

Preoperative DXA variables	Removed n=11			Non-removed n=6		p-value
BMD, g/cm^2	mean	95%CI		mean	95%CI	
Lumbar spine	1.18	1.01 ; 1.35		1.13	0.98 ; 1.28	0.64
Total hip intact side	1.05	0.92 ; 1.18		1.05	0.94 ; 1.16	0.99
Total hip amputated side	0.73	0.52 ; 0.95		0.71	0.42 ; 1.01	0.89
T-score						
Lumbar spine	-0.22	-1.65 ; 1.21		-0.60	-2.02 ; 0.82	0.70
Total hip intact side	-0.16	-1.15 ; 0.83		0.00	-0.83 ; 0.83	0.81
Total hip amputated side	-2.45	-4.07 ; -0.84		-2.57	-4.69 ; -0.46	0.92

Abbreviations: DXA; Dual X-ray Absorptiometry, BMD; bone mineral density; CI; Confidence interval, TT; Total translations

Table 6: Spearmans rank correlation of preoperative T-score and implant migration at last RSA follow-up examination.

	Correlation with Y translations	**Spearmans rho**	**p-value**
T-score	Lumbar spine	-0.20	0.45
	Total hip intact side	0.17	0.50
	Total hip amputated side	-0.07	0.77
T-score	**Correlation with total translations**		
	Lumbar spine	0.18	0.48
	Total hip intact side	-0.06	0.81
	Total hip amputated side	0.01	0.95

Figure 1: The setup of radiostereometric analysis. (A) During RSA examination the roentgen tubes are aligned at a 20° angle with the vertical plane focusing on the fixture as the image is taken. **(B)** The model-based RSA 4.0 software displays the cage markers in yellow and green, bone markers in red, and the CAD-fixture model in green fitted to the contours of the actual implant on the stereoradiographs (red lines). The Y-axis is the yellow line aligned with the model.

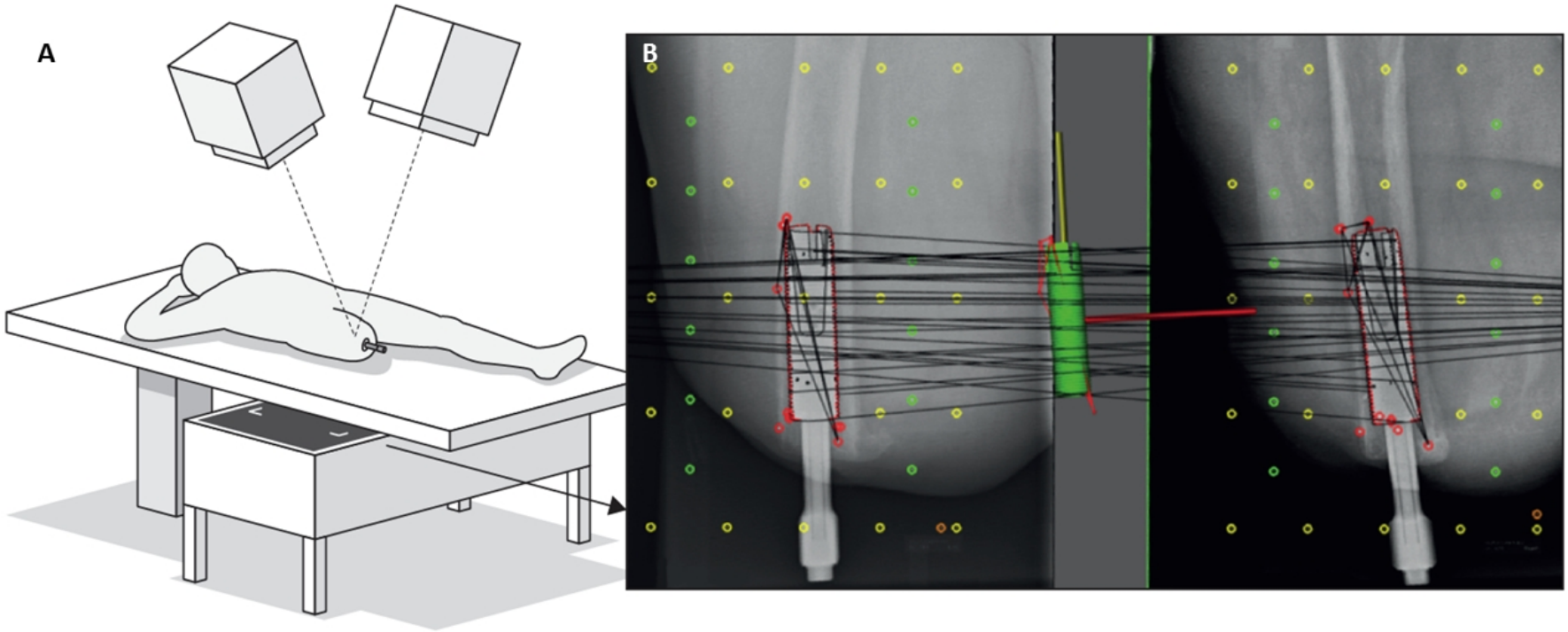


Figure 2: Implant migration pattern (total translation).

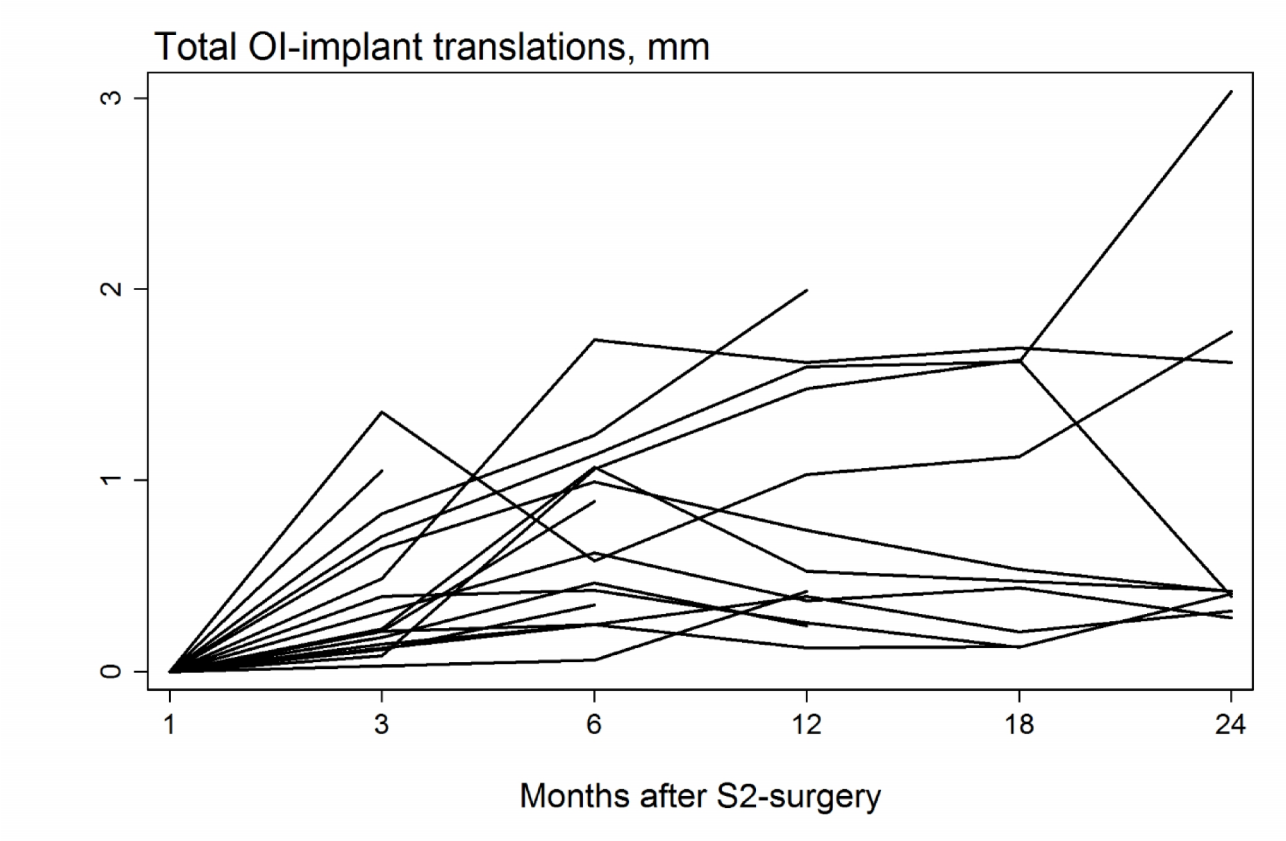


Figure 3: Implant migration pattern (Y-axis)
OI-implants migrating above the precision limit /dashed line) are labelled according to ID in Table 3.

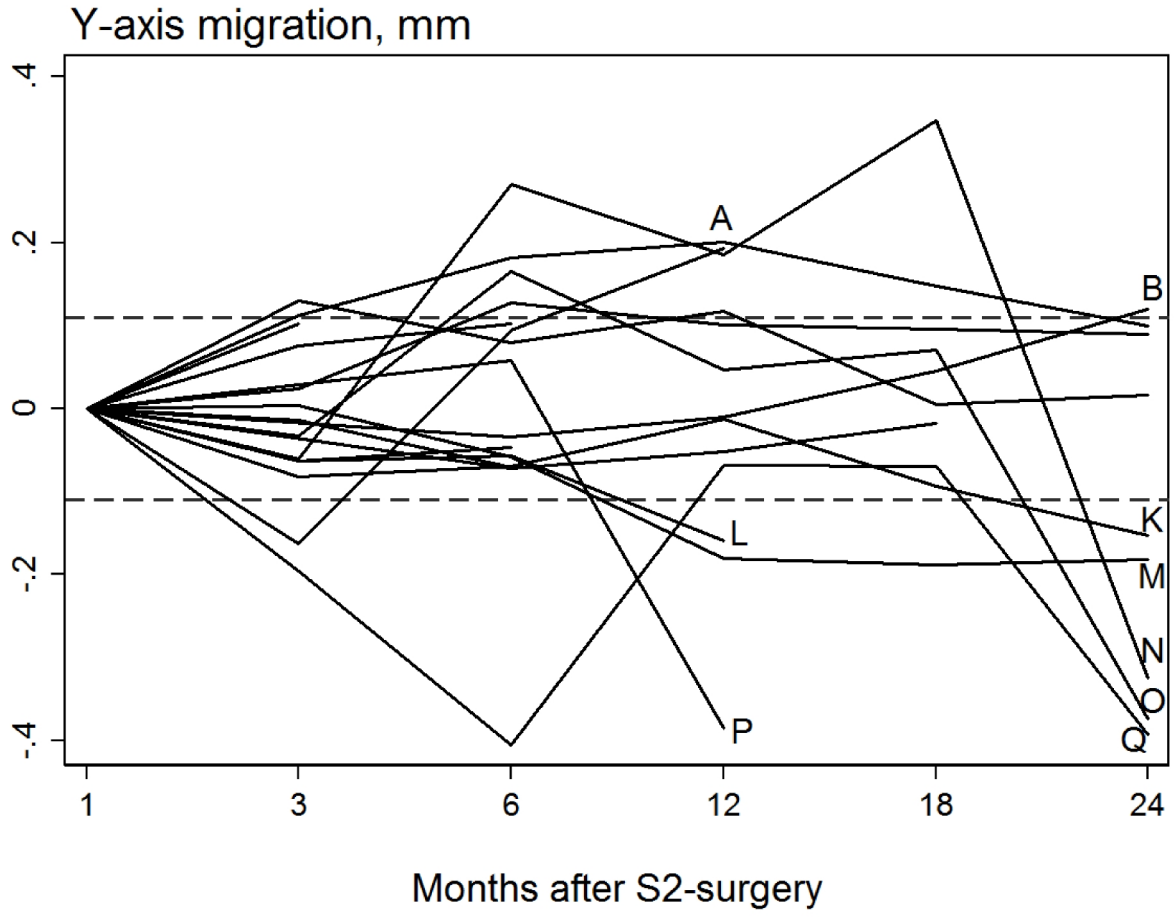
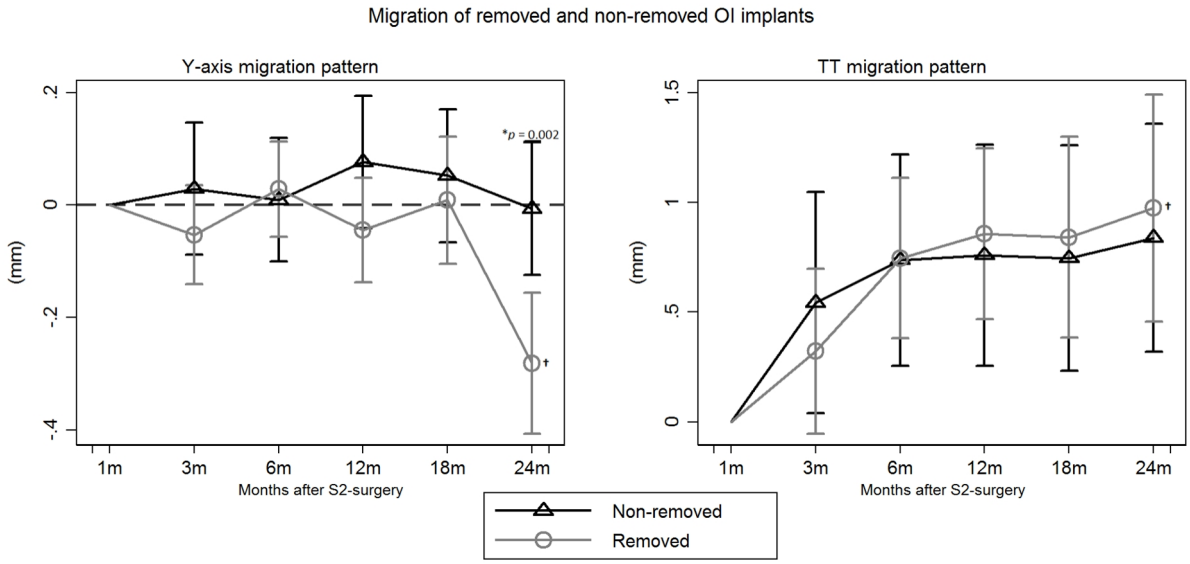


Figure 4: Time-series analysis of proximal/distal and TT migration pattern of removed versus non-removed OI implants.
(*) Significant difference between the removed and non-removed group.
(†) Significant changes compared with early migration (3 month) in the removal group.



Paper IV

The effect of denosumab on periprosthetic bone mineral density in six transfemoral amputees treated with osseointegrated implants: The observations from a terminated randomized controlled trial

List of authors and affiliations:

1. Hansen, Rehne Lessmann[1,4], MD
2. Bente Lomholt Langdahl[2,4], Professor, MD, PhD, DMSc
3. Jørgensen, Peter Holmberg[3], MD, DMSc
4. Petersen, Klaus Kjær[3], MD
5. Søballe, Kjeld[1,3,4], Professor, MD, DMSc
6. Stilling, Maiken[1,4], MD, PhD

1: Orthopaedic Research Unit, University Hospital of Aarhus, Aarhus, Denmark

2: Department of Endocrinology and Internal Medicine, University Hospital of Aarhus, Aarhus, Denmark

3: Department of Orthopaedic Surgery, University Hospital of Aarhus, Aarhus, Denmark

4. Department of Clinical Medicine, Aarhus University, Denmark

Abstract

Background: Transfemoral amputees have low bone mineral density (BMD) in the residual femur and osseointegration (OI) implant surgery impose stress shielding around the implant. The aim of this study was to investigate the effect of denosumab (dmab) on periprosthetic BMD and implant fixation in transfemoral amputees operated with OI implants.

Methods: This study was performed as a single-centre, double-blinded randomized controlled trial with 30-month follow-up. Six males scheduled for OI implant surgery were randomized to dmab (n=3) or placebo (n=3) treatment. Dual energy x-ray absorptiometry (DXA) examinations and drawing of blood samples were conducted preoperatively and at 1, 3, 6, 7, 9, 12, 18 and 30 months of follow-up. Radiostereometric images were obtained postoperatively after 7 months. The primary outcome was change in periprosthetic bone mineral density measured with DXA in 7 regions of interest (ROIs). Secondary outcome was migration using model-based radiostereometric analysis (RSA). Blood tests (C-telopeptide of type I collagen (CTX-1), N-terminal propeptide of type I procollagen (PINP), osteocalcin (OC), bone-specific alkaline phosphatase (BAP), 25-hydroxyvitamin D2+D3 (vitamin D) and parathyroid hormone (PTH) were performed to assess bone turnover. The clinical outcome was evaluated using the SF-36 and the questionnaire for persons with a transfemoral amputation

Results: The recruitment stopped after 3 years, since merely 6 out of planned 16 patients were enrolled in the study and no new patients were pending. Three patients completed the trial, 1 patient (placebo) completed the 18-month follow-up, 1 patient (denosumab) completed the 3-month follow-up and 1 patient (placebo) had the OI implant removed at 18-month follow-up.

DXA: Depending on ROI, the change in mean (SD) periprosthetic BMD ranged from -0.22 (0.1) to 0.155 (0.01) g/cm^2 in the dmab group, -0.578 (0.29) to -0.145 (0.29) g/cm^2 in the placebo group and the greatest difference between the groups was 0.59 g/cm^2 95%CI (-0.09; 1.28, p = 0.07). The proximal hip BMD of the amputated leg increased 0.03 g/cm^2 (0.01) in the dmab group and decreased -0.11 g/cm^2 (0.05) in the placebo group, with a 0.14 g/cm^2 95%CI (0.01; 0.26, p = 0.045).

RSA: In the dmab group, the mean (SD) distal implant migration was -0.32mm (0.29) and TT was 0.79mm (0.17), whereas the implants migrated 0.1mm (0.08) proximally and 0.54mm (0.47) TT in the placebo group. Three out of five patients could walk 500 meters, the prosthetic use had increased and all patients reported that their overall situation was good.

Conclusion: The trend in BMD indicates that dmab therapy preserves periprosthetic BMD at follow-up, but it does not prevent implant migration. A larger study to examine the periprosthetic BMD changes and OI implant stability after dmab treatment to confirm these trends with sufficient statistical power is warranted.

Introduction

The osseointegrated (OI) implant system for transfemoral amputees (TFAs) provides a bone-anchored attachment to the prosthetic leg. Two surgical procedures separated by six months are required. During stage 1 (S1) surgery the intramedullary titanium fixture (Integrum AB, Göteborg Sweden) is inserted into the amputated femur and during stage 2 (S2) surgery the percutaneous abutment is attached to the fixture. The abutment is connected the external prosthetic leg with a snap-lock (Figure 1) (1). TFAs have low bone mineral density (BMD) in the residual femur bone compared to the contralateral intact bone, due to disuse osteopenia/osteoporosis after amputation (2, 3). After OI implant surgery, a pronounced periprosthetic bone loss in the distal regions is observed on radiographs, however the implants remain stable (4). Generic CT-based finite element models find a similar strain-adaptive bone remodelling pattern around the OI implant consistent with bone stress shielding (5, 6).

Animal studies indicate that anti receptor activator of nuclear factor-κB ligand (anti-RANKL) treatment may reduce periprosthetic bone loss (7) and improve implant fixation (8). Papers describing the effect of anti-RANKL treatment on periprosthetic BMD in humans are pending (9). Dmab is a human monoclonal RANKL antibody, which inhibits the recruitment of osteoclasts and thereby it reduces bone resorption and increases BMD. Dmab is an antiresorptive treatment approved for osteoporotic treatment and proven to reduce fracture risk (10). The aim of this study is to compare the effect of a single dmab injection to saline injections administered 1 months before S1-surgery and 1 month before S2- surgery in transfemoral amputated patients scheduled for OI surgery with 30-months follow-up. We hypothesize that dmab is effective in reducing bone stress-shielding around the OI-implants and improves implant fixation compared with a placebo group.

Method

Trial design

A randomized single-center double-blinded placebo-controlled study was conducted at the orthopaedic department, Aarhus University hospital from 2013 to 2016. The patients, medical personnel and investigators were blinded to the treatment. Eligibility criteria are presented in table 1. The patients were enrolled in the project by two senior surgeons in collaboration with the co-investigator. The trial was approved from the Danish Data Protection Agency (1-16-02-32-13), the Danish Regional Ethics Committees (1-10-72-444-12) and the Danish Department of Health (2013081593). The trial was registered at EudraCT (2012-003574-66) and monitored by the Good Clinical Practice unit at Aarhus University hospital.

OI implant surgery

The OI implant system consisted of two parts: a titanium fixture and an abutment implanted during a two-stage surgical procedure. At stage 1 (S1) surgery, the fixture was implanted into the residual femur medulla using a retrograde approach and left to osseointegrate for 6 months. At stage 2 (S2) surgery, the percutaneous abutment was connected to fixture and the soft tissue around the abutment was surgically removed. After S2 surgery, the prosthetic leg was attached to the abutment, the patients followed a 6-month rehabilitation program and gradually increased weight-bearing on the OI implant.

Sample size, randomization and blinding

The calculation of sample size was performed on frequencies of patients reaching the primary outcome parameter assuming a two-sided alpha of 0.05, a power of 80% and a 1:1 ratio. We expected to find a 0.15g/cm^2 difference between the groups under the assumption that bone mineral density would increase 0.2 g/cm^2 (SD=0.1) in the dmab-group and 0.05 g/cm^2 (SD=0.1) in the control group. These estimated changes were based on previous studies examining the effect of bisphosphonate on periprosthetic BMD (11, 12) and the preliminary data from the first patients treated with OI implants at our department. With a 10% expected dropout, a total number of 16 patients would be needed. Patients were distributed in four blocks of four patients, randomizing a total of 16 patients in 1:1 ratio to placebo or dmab. The randomization procedure was done by the Hospital Pharmacy, Central Denmark Region in 2013, who also delivered the test drugs in sealed boxes. The test drug was administered by an independent nurse not associated to the project. To maintain blinding, all persons were instructed to leave the room before the seal was broken and the patient was instructed not to look at the syringe upon injection.

Intervention

All patients were examined for hypocalcaemia prior to treatment. The patients were injected subcutaneously in the shoulder region using an aseptic procedure with a syringe containing 1ml of dmab solution 60mg/ml (Prolia, Amgen) or 1ml of saline solution 9 mg/ml (Takeda pharma). The test drug was administered twice six months apart, one month before S1-surgery and one month before S2-surgery. All patients were provided with a daily oral supplement of 800mg calcium with 38µg vitamin D for one year.

Outcome parameters

The primary outcome was BMD (g/cm^2) measured with Dual-energy X-ray Absorptiometry (DXA). The secondary outcome was implant migration in terms of mean (SD) Y translation (mm) and total translations (TT) of the OI implant measured with model-based radiostereometric analysis (RSA) after S2 surgery. The tertiary outcome was changes in bone turnover markers in blood serum. Patient reported outcome was the questionnaire for persons with a transfemoral amputation (Q-TFA) and 36-Item Short-Form health survey (SF-36). In order to investigate the local bone resorption, samples of C-telopeptide of type I collagen (CTX) in the femoral bone proximal to the OI implant and in the iliac crest were collected the first 3 days after S1-surgery. CTX was tried sampled in the bone using microdialysis, but unfortunately all femoral probes were displaced into the subcutis a short period after the patient had awakened from surgery, thus, this method was terminated from the study.

DXA

Scans were performed preoperatively, 1, 3, 6, 7, 9, 12, 18 and 30 months after S1-surgery on the GE Lunar iDXA scanner (General healthcare, Madison WI). BMD was measured in the proximal femur (total hip) and the AP lumbar spine (L1-L4) using standard regions of interests (ROIs). One month after S1-surgery, BMD changes around the OI implant was examined with a custom-designed ROI validated in a previous study (13). The precision was determined by double examination performed six months after S2-surgery. One technician performed all scans according to a defined protocol (13) and the analyses were performed by one observer with the enCORE 14.10.022 software (General healthcare, Madison WI).

RSA

Six to ten tantalum beads (Ø 1.0 mm) were inserted into the cortical bone around the OI implant with a bead gun (Wennbergs Finmek AB, Sweden) at S1-surgery. Stereoradiographs were obtained 7 (1 month after S2-surgery), 9, 12, 18, 30 months after S1-surgery in a standard RSA setup (14), using the RSA system (Adora RSA; NRT, Denmark) with two-ceiling fixed, synchronized roentgen tubes (Varian Medical Systems, USA). Both roentgen tubes were auto-positioned at a 20^{o} angle with the vertical plane, and an unfocussed uniplanar carbon calibration box (Box 24; Medis Specials, Leiden, the Netherlands). Stereoradiographs were direct digital (Canon CXDI-70C**,** 125 µm pixel pitch). The cut-off for stable bone markers was 0.35mm, and the mean rigid body error was 0.14mm (range 0.03 to 0.32mm) and the mean condition number was 79.3 (range 39.3 to 113.9) (14). In the analysis, the same bone markers were selected at follow-up and the CAD-models of the OI-implant were used to calculate OI implant migration with respect to the bone markers. Signed X-, Y-, and Z- translations and total translations ($T \quad = \sqrt{X^2 + Y^2 + Z^2}$) was calculated. The Y-axis was aligned with the longitudinal axis of the fixture and proximal migration (subsidence) was defined as positive motion, whereas distal migration was defined as a negative motion. The precision (standard deviation) along the translations were determined by use of double examinations in a previous study conducted at our department (X=0.20mm, Y=0.06mm, Z=0.25mm) (15). Model-based analysis of all radiographs was performed by one observer with Model-based RSA 4.0 (RSAcore, Leiden, The Netherlands) software.

Blood tests

Blood samples were obtained during day-time in non-fasting patients. The samples were centrifuged at 4000 RPM at 4 °C for 10 minutes and the serum was stored into 2 ml tubes and kept at −80 °C. The concentration of bone turnover and bone metabolism markers were measured as a batch analysis.

C-telopeptide of type I collagen (CTX), parathyroid hormone (PTH), N-terminal propeptide of type I procollagen (P1NP) and osteocalcin (OC) concentrations were determined by electrochemiluminescence analysis (Cobas 6000 modul e601, Roche Diagnostic A/S). Calcium (Ca) was determined by absorption spectrophotometry. 25-hydroxyvitamin D2+D3 (Vitamin D) concentration was analysed by high-performance liquid chromatography (API 5500, AB Sciex) and bone specific alkaline phosphatase (BAP) by ELISA.

Patient reported outcomes

Electronic questionnaires were sent directly to patient emails preoperatively, and 12, 18 and 30 months after first stage surgery. The SF-36 questionnaire was reported as a physical function (PF) and the physical component score (PCS). Each score ranges from 0 to 100 and a higher number represents better health. The Q-TFA described the prosthetic use, the walking distance and the patient estimated function level (range 1 to 5 and a high number represented a positive output) with prosthesis, problems with the prosthesis and the overall situation as an amputee (16).

Termination of trial and statistics

The trial was terminated before inclusion was completed; thus, the comparison was made on a descriptive level using the 95% confidence interval (CI) as only a limited number of patients were included. The outcome parameters were presented as Δ difference (Δ_{diff}) with 95%CI between the groups using unadjusted p-values; thus, the results focus on the 95%CI and not the significance level.

Normal distribution was assessed by qq-plots. Parametric data were analysed using *t* tests and reported as means with standard deviation or range (minimum to maximum value). The data was presented in graphs up to 30 months of follow-up; however, to include all patients in the analysis, the mean (SD) values and Δ difference (95% CI) between the groups were calculated at 6-months follow-up (primary outcome) and at 18-month follow-up (primary, secondary and tertiary outcome).

Only the allocation letter (A or B) of each group was known during the statistical analysis and writing the manuscript. All analyses were performed in Stata 13.1 (STATA corp., TX).

Results

The inclusion of new patients ceased in June 2016 for two reasons: Only a limited number of transfemoral amputees were enrolled in the study after three years and no new patients were pending. Due to the logistics and the setup of the study, it was not feasibel to continue the inclusion for another estimated six to eight years. A total of nine patients were assessed for eligibility and six patients were randomized and included in the analyses (Figure 2). Three patients completed the trial and the remaining were followed until 18-month follow-up (n=2, placebo) and 3-month follow-up (n=1, dmab). One patient (placebo) had the OI implant removed after 18 months due to traumatic loosening. Six males, mean age 55.5 years (range 36 to 66), were amputated due to trauma (n=2), tumour (n=2), infection (n=1) or a deep vein thrombosis (n=1) a median 15 years (range 2 to 40) ago.

Bone mineral density changes

The mean (SD) BMD change in ROI 1-7 (Figure 3) ranged from -0.152 (0.01) to 0.114 (0.17) g/cm^2 in the dmab group and -0.449 (0.27) to -0.175 (0.11) g/cm^2 in the placebo group 6 months after S1-surgery. The $\Delta group_{diff}$ for all BMD changes are presented in table 2. At 18-month follow-up, the BMD change in ROI 1-7 ranged from -0.22 (0.1) to 0.155 (0.01) g/cm^2 in the dmab group and -0.578 (0.29) to -0.145 (0.29) g/cm^2 in the placebo group. The mean proximal hip BMD on the amputated side increased 0.03 g/cm^2 (0.01) in the dmab group, decreased -0.11 g/cm^2 (0.05) in the placebo group. The precision of BMD measurements (n=5) was determined as root mean square % coefficient of variation (RMS %CV) on the iDXA scanner: ROI 1 to 7 ranged from 1.6 to 4.1%, spine (L1-L4) was 0.7%, amputated proximal hip (total) was 3.6% and intact hip was 1.1%.

Radiostereometric analysis

The mean (SD) total translation was 0.79mm (0.17) in the dmab group and 0.54mm (0.47) in the placebo group at 18-month follow-up (Figure 4). A distal OI implant migration of -0.32mm (0.29) was measured in the dmab group and 0.1mm (0.08) subsidence in the placebo-group at 18-months follow-up. One patient (id=6) had a total OI implant removal due to traumatic loosening with 1.63mm total translation between the last two follow-up examinations (the last radiograph image was taken a few days before implant removal). The $\Delta group_{diff}$ for migrations are presented in table 2.

Blood test

Preoperatively, 3 patients had low vitamin D (<50 nm/L) which was corrected within the first six months. At 6-month follow-up, the CTX in the dmab group was 0.1 µg/l (SD=0.07) and 0.33 µg/l (SD=0.09) in the placebo

group, whereas OC was 11.2 µg/l (SD=2.4) (dmab) and 23.3 µg/l (SD=5.8) (placebo) (Figure 5). The changes in BTMs at 18-months follow up are presented in table 2.

Patient reported outcomes

As presented in table 3 most patient reported outcome measures had improved or remained unchanged at the last follow-up examination. One patient (ID=6) had a loose implant and did not use the external prosthetic leg and one patient (ID=2) reported increased prosthetic use, but only walked shorter distances due to pain in the knee of the non-amputated leg. Both patients had a decreased PF and PCS at last follow-up examination. All patients found that their overall situation was unchanged or had improved.

Adverse events

In the dmab group, patient 1 (66 years) developed a rather large exostosis in the distal part of the residual femur (Figure 6) a few months after S1-surgery. The patient experienced pain in the distal femur during weight bearing and therefore he seldom used the socket-suspended prosthesis. After the second dmab injection, patient 1 experienced acute pain in the lower leg due to ischaemia. Two weeks earlier, the patient had experienced an ischaemic prodromal in the same leg, and was examined at another hospital, but was clinically normal at the time. The CT angiography showed a popliteal aneurysm with a thrombosis and excessive arteriosclerosis in the anterior and posterior tibial artery. The patient was treated with thrombolysis and vascular surgery. Today, the patient is well without any complications from the events and uses his OI implant.

In the placebo group, patient 5 sustained an iatrogenic fissure in the distal femur bone at S1-surgery. The lateral implant displacement from the femoral midline caused a thinning of the cortical bone, thus BMD in ROI 3 and 4 could not be estimated with DXA. The patient reported pain during partial weight bearing the first nine months after S2-surgery, but after achieving almost full weight bearing with the long prosthetic leg, the patient no longer experience pain during walking.

Patient 6 experienced a traumatic incident that caused the prosthetic leg to rotate externally without releasing the safety device (OPRA AXOR II, Integrum AB, Sweden), thus the torsion was transferred to the bone-anchored fixture. Afterwards, the abutment could rotate a few degrees from side to side, even though it was securely attached to the fixture. Six months after the accident the patient presented a normal radiograph of the amputated femur, however, RSA indicated aseptic loosening (Figure 4) and weight bearing on the implant was painful. Thus, the implant was removed after 18-month follow-up.

Discussion

The aim of this trial was to investigate the effect of dmab on periprosthetic BMD and OI implant fixation. The trial was terminated due to recruiting issues, and therefore the number of patients was insufficient for proper study power. In spite of that, there were indications that dmab therapy preserves the bone around the OI implant, however it did not seem to prevent implant migration.

DXA and RSA

There was no difference in periprosthetic BMD between the dmab and placebo groups at 6-months or at 18-month follow-up. However, the dmab group showed a trend towards less periprosthetic BMD loss. This trend was supported by the increase in the proximal hip BMD of the amputated leg at 18-month follow-up.

Several previous studies investigated the antiresorptive effect of bisphosphonates around hip stems and reported lesser BMD loss in several periprosthetic regions (11, 17, 18). These reports corresponded to the findings in our study, assuming that the antiresorptive effect of dmab was similar. We found no difference between the groups in Y migration or in TT. Similarly, a single dose pamidronate administered after total hip surgery did not affect the uncemented acetabular cup migration (19), nor did a weekly dose of risedronate affect the uncemented hip stem migration or outcome (11).

Additionally, OI implant migration seemed to increase between 12 month and 18 months (Figure 4). Earlier studies found that discontinuing dmab treatment caused the positive changes in BMD and BTMs to return to the original values within 12 months due to reactivation of osteoclasts (20). After reactivation, a rebound effect may have caused to osteoclasts to commence the bone remodelling around the OI implant and thereby caused additional micromotion. Micromotion in the early phases after hip arthroplasty is a strong risk factor for later implant revision (21). Maybe dmab treatment should have been continued.

Blood tests

A small peak in BTMs was measured in most patients as a response to OI implant surgery, which resembles observations after hip replacement surgery (22). However, this was different in the dmab group since CTX and OC decreased after S1-surgery. The decrease in CTX indicated that dmab administered 1 month prior to OI surgery reduced the osteoclastic response and less type-I collagen was degraded (23). The effect of dmab on the OC concentration was surprising, since a low concentration may indicate a reduced bone formation. However, a similar effect was measured in patients after a single infusion zoledronic acid after total hip arthroplasty without a negative effect on the implant migration (18).

Patient reported outcomes and mobility examinations

The patients reported that their overall situation as an amputee was good and the prosthetic use had increased. However, the walking distance did not seem to improve and three out of five patients could walk 500 meters or more at the last follow-up examination. This was lesser than the 2-year results reported from 39 OI implant users, reporting that 80% rated the overall situation as an amputee was good or extremely good, 50 % walked more than 500 meters at last follow up examination, and the PF and PCS improved (24).

Adverse events

Patient 1 experienced two adverse events, the exostosis formation was likely dmab-related, whereas the acute ischaemic incident was not, since the arteriosclerotic vessels observed on CT-angiography was a chronic disease develop years before the dmab injections. In patient 5 (placebo), the lateral cortical bone was almost resorbed and a considerable decrease in BMD was measured in all ROIs during the first 6 months after surgery, but this did not affect implant fixation at 18-months follow-up (Figure 5). Patient 6 had the OI implant removed after a traumatic incident. Implant removals were not an uncommon event, a recent study reported that the two-year survival of OI implants was 92% (25) and an earlier study reported up to 20 OI implant removals in 100 patients during 18 years of follow-up (1). Patients 6 also suffered from severe

periprosthetic bone loss during the first 6 months after surgery, but the implant did not migrate according to RSA until after the traumatic incident (Figure 4). Besides bone stress-shielding the changes in periprosthetic BMD could also be associated with aseptic loosening (26). If the implant was only partially osseointegrated the ability to withstand loading or traumatic incidents may be severely reduced (27).

Limitation

Due to a very small study population, the changes measured in most groups were non-significant. Even though this was a randomized controlled study the results and trends should be interpreted with caution.

Conclusion

There was no statistically significant difference in periprosthetic BMD change between dmab or placebo treatment in transfemoral amputees with an OI implant, however there was a trend that dmab treatment preserved periprosthetic BMD at follow-up.

The OI implant migration pattern was similar in both groups; except that one patient in the placebo group had the OI implant removed after a traumatic incident and for that reason had high last-follow-up implant migration. 5 out of 5 patients reported their overall situation as an amputee to be good after OI implant surgery. The results are interesting and we encourage a larger study to investigate the effects of dmab on periprosthetic BMD and OI implant fixation.

References

1. Hagberg K, Branemark R. One hundred patients treated with osseointegrated transfemoral amputation prostheses--rehabilitation perspective. J Rehabil Res Dev. 2009;46(3):331-44.

2. Sherk VD, Bemben MG, Bemben DA. BMD and bone geometry in transtibial and transfemoral amputees. J Bone Miner Res. 2008 Sep;23(9):1449-57.

3. Flint JH, Wade AM, Stocker DJ, Pasquina PF, Howard RS, Potter BK. Bone mineral density loss after combat-related lower extremity amputation. J Orthop Trauma. 2014 Apr;28(4):238-44.

4. Nebergall A, Bragdon C, Antonellis A, Karrholm J, Branemark R, Malchau H. Stable fixation of an osseointegated implant system for above-the-knee amputees. Acta Orthop. 2012 Apr;83(2):121-8.

5. Tomaszewski PK, van Diest M, Bulstra SK, Verdonschot N, Verkerke GJ. Numerical analysis of an osseointegrated prosthesis fixation with reduced bone failure risk and periprosthetic bone loss. J Biomech. 2012 Jul 26;45(11):1875-80.

6. Tomaszewski PK, Verdonschot N, Bulstra SK, Rietman JS, Verkerke GJ. Simulated bone remodeling around two types of osseointegrated implants for direct fixation of upper-leg prostheses. J Mech Behav Biomed Mater. 2012 Nov;15:167-75.

7. Aspenberg P, Agholme F, Magnusson P, Fahlgren A. Targeting RANKL for reduction of bone loss around unstable implants: OPG-Fc compared to alendronate in a model for mechanically induced loosening. Bone. 2011 Feb;48(2):225-30.

8. Bernhardsson M, Sandberg O, Aspenberg P. Anti-RANKL treatment improves screw fixation in cancellous bone in rats. Injury. 2015 Feb 19.

9. Skoldenberg O, Rysinska A, Eisler T, Salemyr M, Boden H, Muren O. Denosumab for treating periprosthetic osteolysis; study protocol for a randomized, double-blind, placebo-controlled trial. BMC Musculoskelet Disord. 2016 Apr 23;17:174,016-1036-5.

10. Cummings SR, San Martin J, McClung MR, Siris ES, Eastell R, Reid IR, et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N Engl J Med. 2009 Aug 20;361(8):756-65.

11. Skoldenberg OG, Salemyr MO, Boden HS, Ahl TE, Adolphson PY. The effect of weekly risedronate on periprosthetic bone resorption following total hip arthroplasty: a randomized, double-blind, placebo-controlled trial. J Bone Joint Surg Am. 2011 Oct 19;93(20):1857-64.

12. Yamasaki S, Masuhara K, Yamaguchi K, Nakai T, Fuji T, Seino Y. Risedronate reduces postoperative bone resorption after cementless total hip arthroplasty. Osteoporos Int. 2007 Jul;18(7):1009-15.

13. Hansen RL, Langdahl BL, Jørgensen PH, Petersen KK, Søballe K, Stilling M. (Submitted) Bone Mineral Density Measurements around Osseointegrated Implants: A Precision Study and Validation of Scan-Protocol for Transfemoral Amputees. Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry.

14. Valstar ER, Gill R, Ryd L, Flivik G, Borlin N, Karrholm J. Guidelines for standardization of radiostereometry (RSA) of implants. Acta Orthop. 2005 Aug;76(4):563-72.

15. Hansen RL, Langdahl BL, Jørgensen PH, Petersen KK, Søballe K, Stilling M. (Submitted) Higher migration of removed compared with non-removed osseointegrated implants for transfemoral amputees. A prospective 2 year RSA study with 5-years clinical follow-up. Acta Orthopaedica Scandinavica.

16. Hagberg K, Branemark R, Hagg O. Questionnaire for Persons with a Transfemoral Amputation (Q-TFA): initial validity and reliability of a new outcome measure. J Rehabil Res Dev. 2004 Sep;41(5):695-706.

17. Wilkinson JM, Eagleton AC, Stockley I, Peel NF, Hamer AJ, Eastell R. Effect of pamidronate on bone turnover and implant migration after total hip arthroplasty: a randomized trial. J Orthop Res. 2005 Jan;23(1):1-8.

18. Friedl G, Radl R, Stihsen C, Rehak P, Aigner R, Windhager R. The effect of a single infusion of zoledronic acid on early implant migration in total hip arthroplasty. A randomized, double-blind, controlled trial. J Bone Joint Surg Am. 2009 Feb;91(2):274-81.

19. Wilkinson JM, Eagleton AC, Stockley I, Peel NF, Hamer AJ, Eastell R. Effect of pamidronate on bone turnover and implant migration after total hip arthroplasty: a randomized trial. J Orthop Res. 2005 Jan;23(1):1-8.

20. Miller PD, Bolognese MA, Lewiecki EM, McClung MR, Ding B, Austin M, et al. Effect of denosumab on bone density and turnover in postmenopausal women with low bone mass after long-term continued, discontinued, and restarting of therapy: a randomized blinded phase 2 clinical trial. Bone. 2008 Aug;43(2):222-9.

21. Karrholm J, Borssen B, Lowenhielm G, Snorrason F. Does early micromotion of femoral stem prostheses matter? 4-7-year stereoradiographic follow-up of 84 cemented prostheses. J Bone Joint Surg Br. 1994 Nov;76(6):912-7.

22. Arabmotlagh M, Sabljic R, Rittmeister M. Changes of the biochemical markers of bone turnover and periprosthetic bone remodeling after cemented hip arthroplasty. J Arthroplasty. 2006 Jan;21(1):129-34.

23. Kendler DL, Roux C, Benhamou CL, Brown JP, Lillestol M, Siddhanti S, et al. Effects of denosumab on bone mineral density and bone turnover in postmenopausal women transitioning from alendronate therapy. J Bone Miner Res. 2010 Jan;25(1):72-81.

24. Hagberg K, Hansson E, Branemark R. Outcome of percutaneous osseointegrated prostheses for patients with unilateral transfemoral amputation at two-year follow-up. Arch Phys Med Rehabil. 2014 Nov;95(11):2120-7.

25. Branemark R, Berlin O, Hagberg K, Bergh P, Gunterberg B, Rydevik B. A novel osseointegrated percutaneous prosthetic system for the treatment of patients with transfemoral amputation: A prospective study of 51 patients. Bone Joint J. 2014 Jan;96-B(1):106-13.

26. Wilkinson JM, Hamer AJ, Rogers A, Stockley I, Eastell R. Bone mineral density and biochemical markers of bone turnover in aseptic loosening after total hip arthroplasty. J Orthop Res. 2003 Jul;21(4):691-6.

27. Albrektsson T, Branemark PI, Hansson HA, Lindstrom J. Osseointegrated titanium implants. Requirements for ensuring a long-lasting, direct bone-to-implant anchorage in man. Acta Orthop Scand. 1981;52(2):155-70.

Figure 1: The osseointegration implant

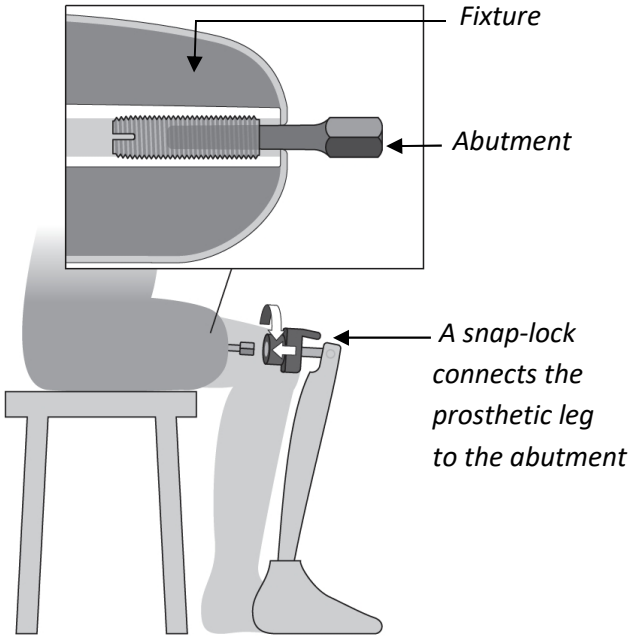


Figure 2: CONSORT 2010 Flow Diagram

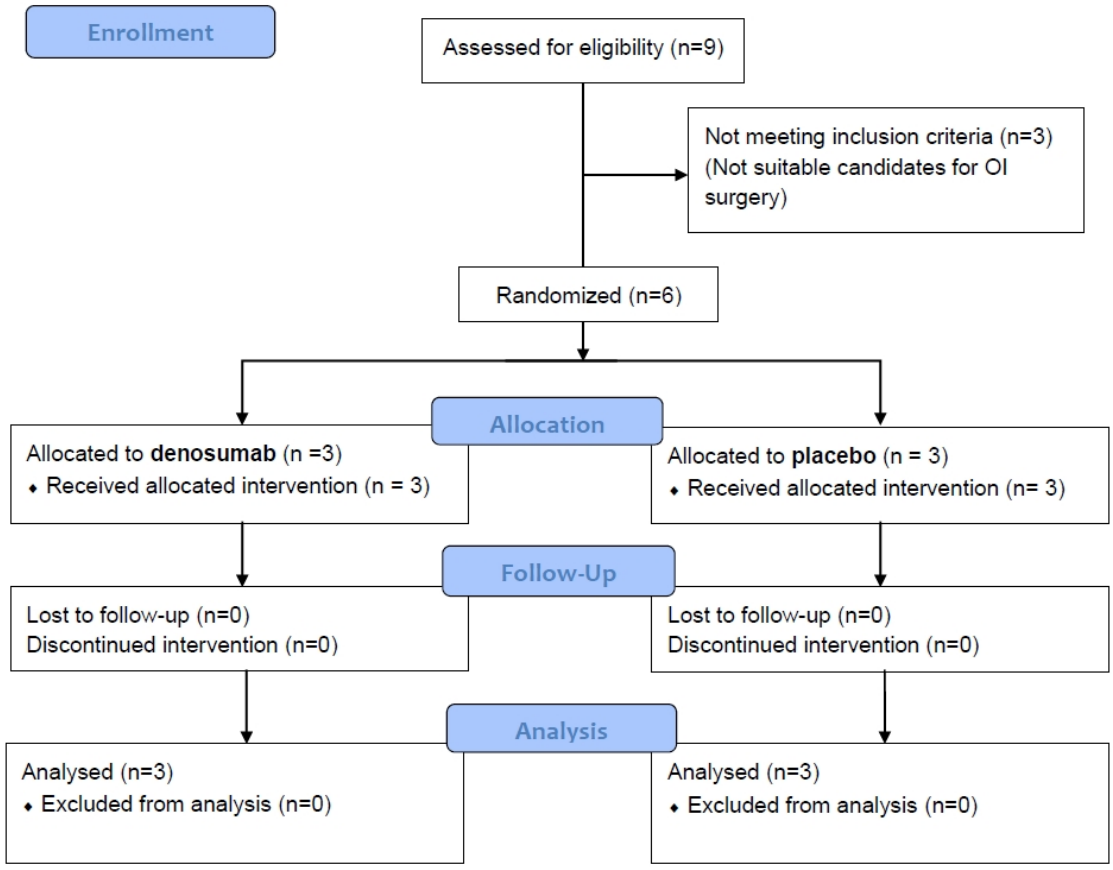
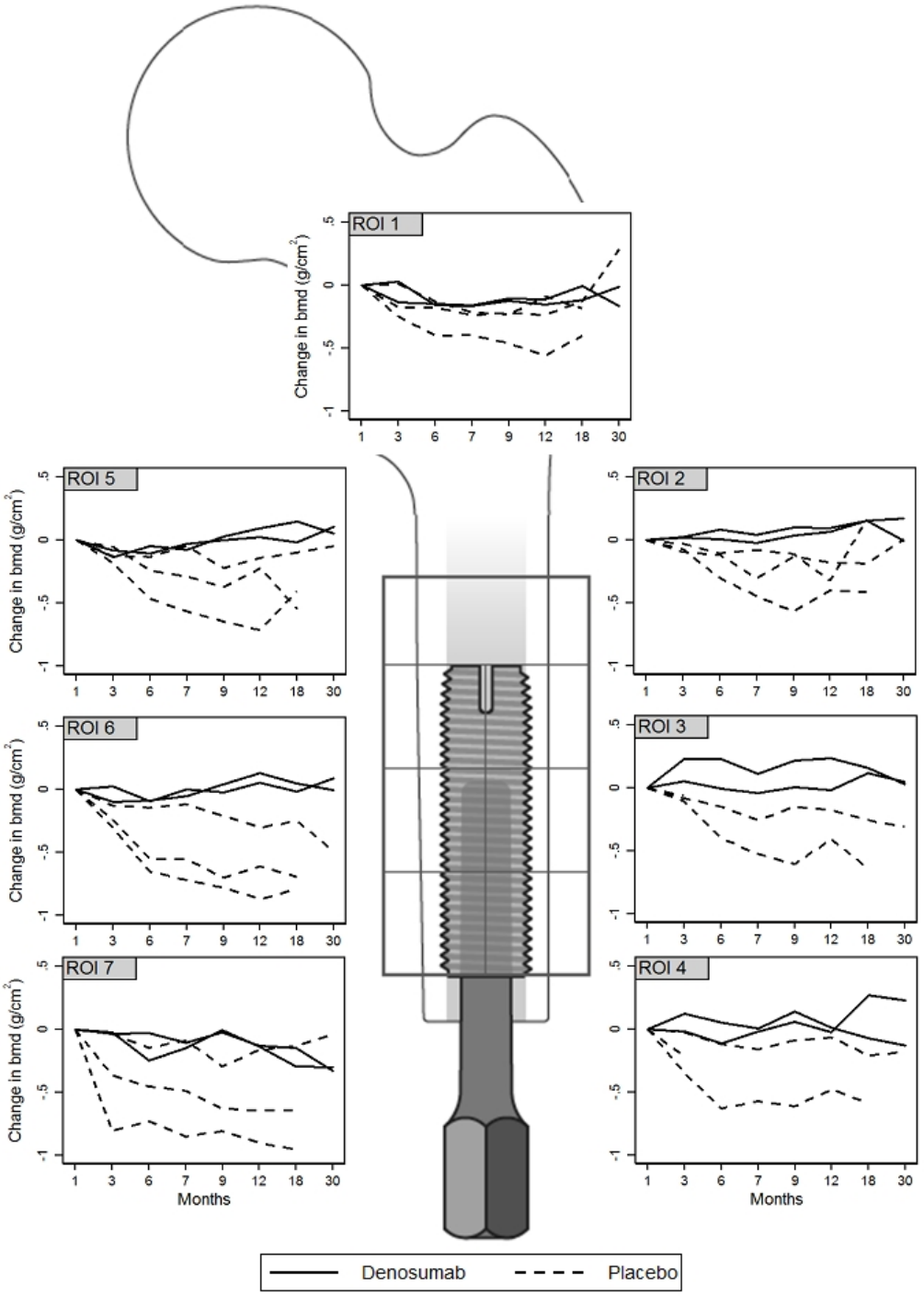
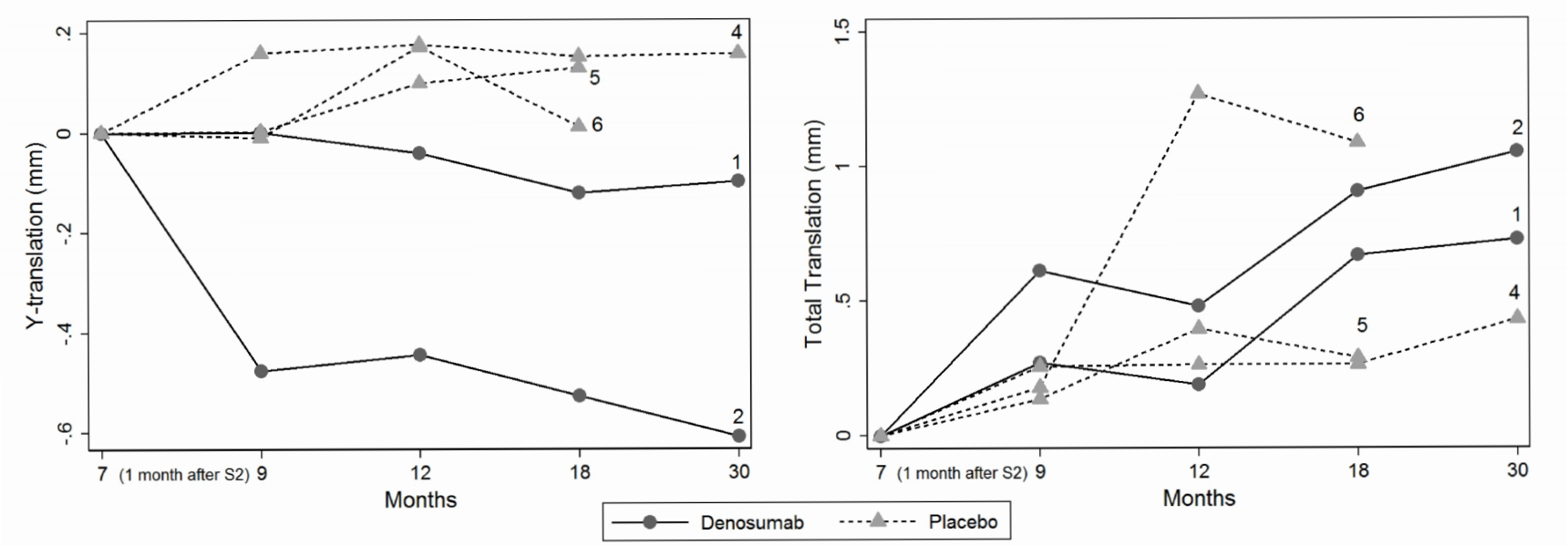


Figure 3: Change in periprosthetic BMD in ROI 1-7



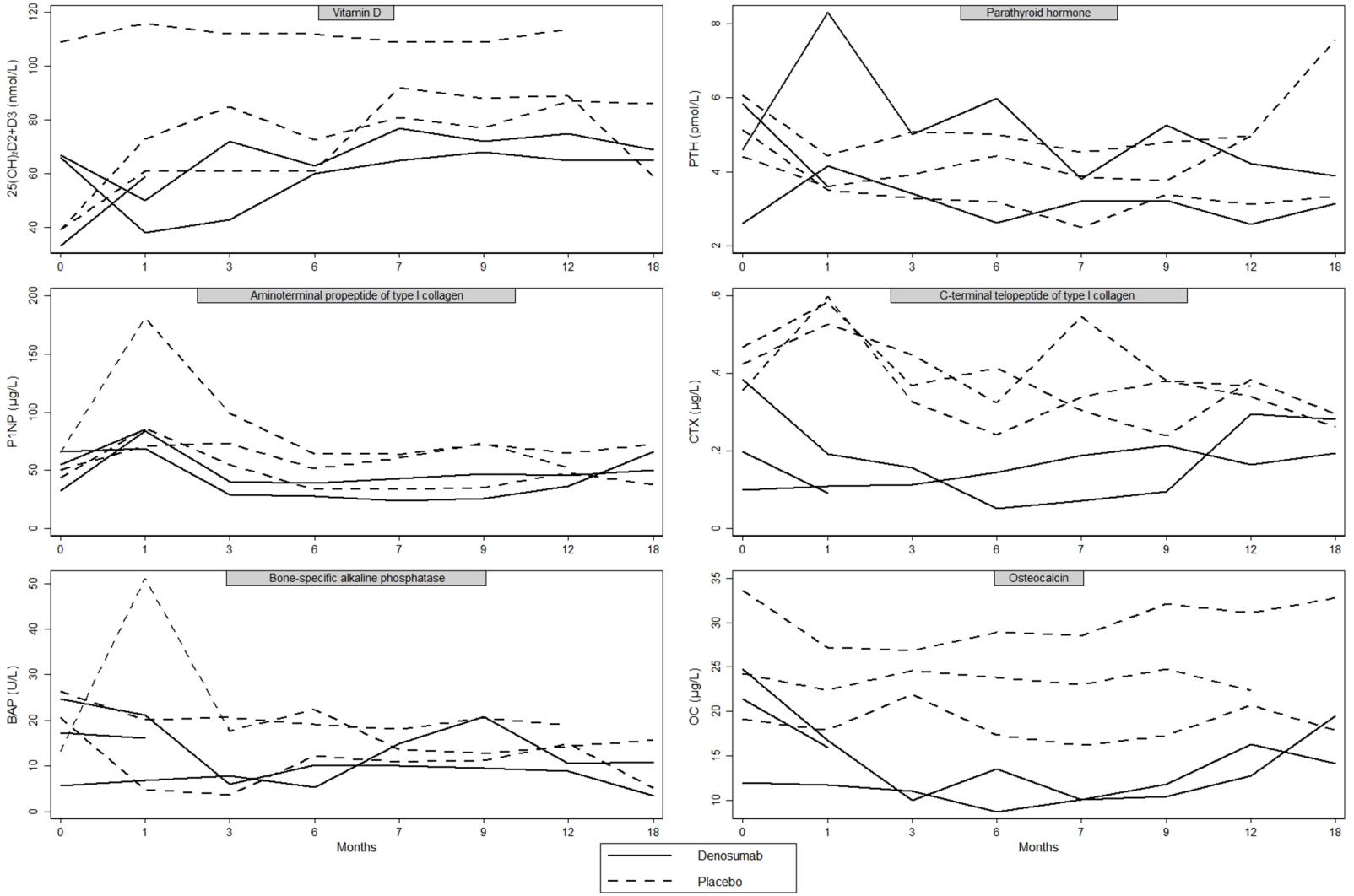
The change in BMD is presented as relative to the BMD value at 1-month follow-up. Individual graphs are presented for all ROIs. Changes in BMD after dmab treatment is presented as a solid line and as dashed line after placebo treatment.

Figure 4: OI implant Y translation and total translation



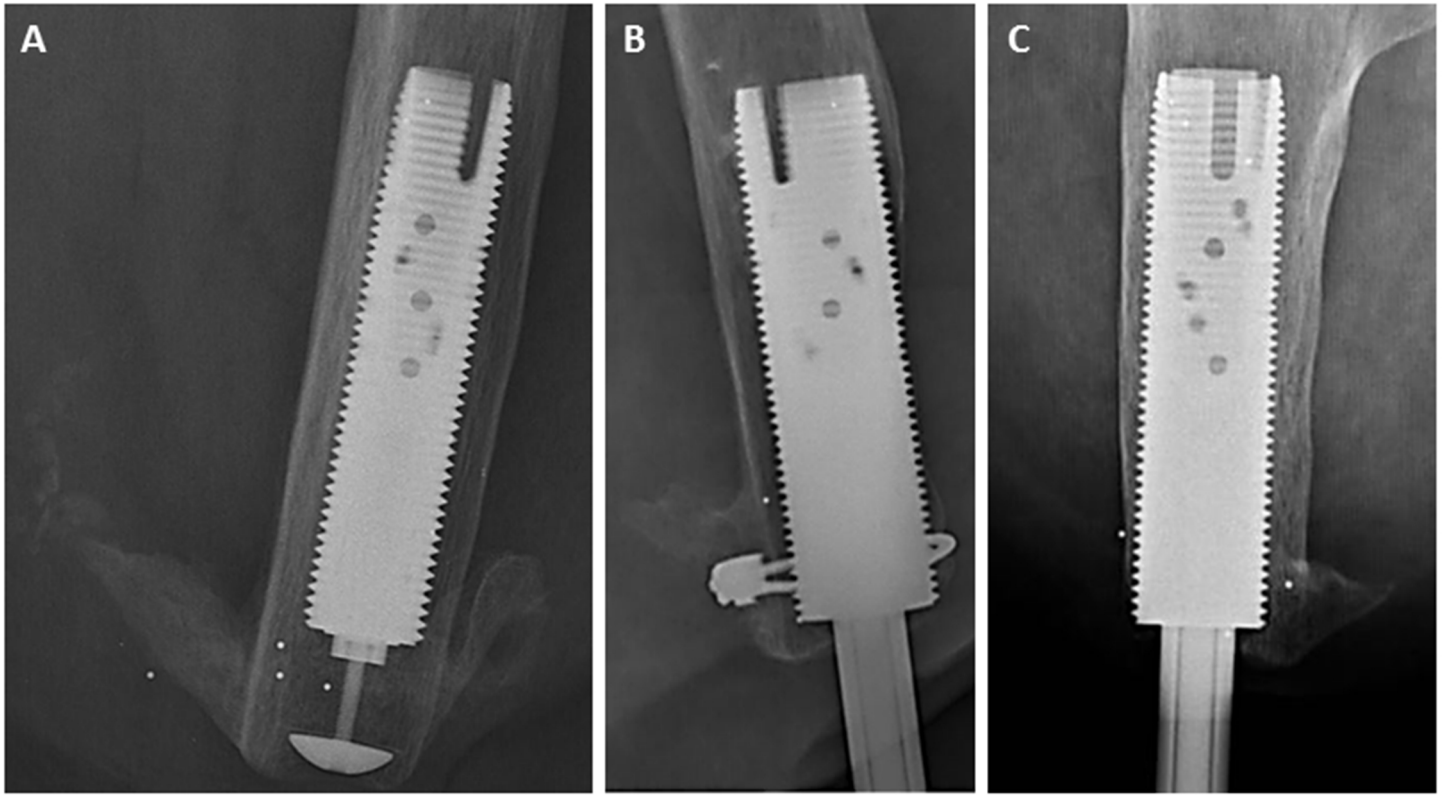
The individual graphs are labelled with patient id (1-6). Migration patterns after dmab treatment are presented as a solid line and as dashed line after placebo treatment.

Figure 5: Bone turnover marker and bone metabolism concentrations



Changes in BTMs after dmab treatment are presented as a solid line and as dashed line after placebo treatment.

Figure 6: Radiographs of OI implants after adverse events.



A: Radiograph of patient 1 showing the distal exostosis six months after S1-surgery. **B:** The lateral displacement of the fixture in patient 5 and the thinning of the lateral cortical bone after 18 months. **C:** No radiographic signs of implant loosening in patient 6 six months after an accident.

Table 1: Inclusion and exclusion criteria.

Inclusion criteria
Age between 18-70 years
Scheduled for OI-implant surgery
Body mass index <30
Female patients of childbearing age must produce a negative pregnancy test and use effective contraception
Informed consent
Exclusion criteria
Diabetes with complication
Atherosclerosis
Smoking
Drug abuse
Treatment with NSAID or cytostatic
Active cancer
Liver or kidney insufficiency
Dementia
Hip flexion contracture on the affected side >10 degrees
Body weight >100kg
Hypocalcaemia
Contraindications to denosumab

Table 2: Mean difference between the denosumab and placebo group.

		Mean (Δdiff)	Lower 95% CI	Upper 95% CI	Unadjusted p-value*
BMD range ROI 1-7 (g/cm^2)					
	Lower range (6m)	0.08	-0.26	0.42	0.5
	Upper range (6m)	0.22	-0.06	0.5	0.09
	Lower range (18m)	0.35	-0.64	1.35	0.34
	Upper range (18m)	0.59	-0.09	1.28	0.07
Hip BMD (g/cm^2) (18m)					
	Amputated side	0.14	0.01	0.26	0.045
	Intact side	-0.02	-0.17	0.12	0.65
Spine BMD (g/cm^2) (18m)					
	L1-L4	-0.03	-0.13	0.07	0.43
Model-based RSA (mm) (18m)					
	Y-translation	-0.42	-0.94	0.09	0.08
	Total translation	0.24	-0.9	1.39	0.55
Blood tests (18m)					
	P1NP (µg/L)	2.9	-78.3	84.1	0.9
	CTX (µg/L)	-0.04	-0.24	0.16	0.48
	BAP (U/L)	-3.3	-30.8	24.2	0.66
	OC (µg/L)	-8.5	-42.6	25.6	0.4
	Vitamin D (nmol/L)	-5.5	-64.2	53.2	0.73
	PTH (pmol/L)	-1.9	-11.1	7.3	0.46

The p-values were not adjusted for multiple comparison; thus, the interpretation should focus on 95%CI.

Table 3: Patient reported outcomes before OI surgery and at last follow-up examination.

Patient reported outcomes before S1-surgery (with prosthetic socket)												
	Prosthetic use		Walking distance with a prosthetic leg					Prosthetic function	Prosthetic problems	Overall situation	SF-36	
id	Days/week	Hours/day	10m	50m	200m	500m	2km				PF	PCS
1	7	0-3	+++	+++	+++	++	+	2	3	3	75	41.1
2	6	0-3	+++	+++	+++	-	-	1	4	3	50	44.1
3	5	7-9	+++	+++	+	-	-	2	4	4	35	40.4
4	7	10-12	+++	+++	+	-	-	1	5	2	90	38.3
5	7	>15	+++	+++	++	+	-	4	3	4	50	35.4
6	7	10-12	+++	+++	+++	+++	+	3	3	3	70	52.3

Patient reported outcomes at last follow-up examination (with OI implant)													
		Prosthetic use		Walking distance with a prosthetic leg					Prosthetic function	Prosthetic problems	Overall situation	SF-36	
id	Follow-up	Days/week	Hours/day	10m	50m	200m	500m	2km				PF	PCS
1	30	**7**	**13-15**	**+++**	**+++**	**++**	**+**	**+**	**4**	**2**	**4**	**90**	**43.7**
2	30	**6**	**4-6**	++	-	-	-	-	**2**	**2**	**4**	20	30.1
3	0	The patient has not completed follow-up examinations yet											
4	30	**7**	**>15**	**+++**	**+++**	**++**	**+**	**+**	**4**	**2**	**4**	**90**	**52.1**
5	18	**7**	**>15**	**+++**	**+++**	**++**	**+**	-	3	3	**4**	**55**	**42.5**
6	18*	1	0-3	-	-	-	-	-	1	5	**4**	60	41.9

Patients treated with denosumab are presented in grayscale rows. Improved or unchanged outcomes at the last follow-up examination are highlighted with bold font. Total OI implant removal (*).
Prosthetic use is reported as number of days a week and number of hours a day. Walking distance with a prosthetic leg during the last three months is reported as (+++) distance walked on a daily basis, (++) several times a week, (+) less than one time a week and (-) never.
Prosthetic function in terms of patient estimated function level with current prosthesis in terms of 1 = very low, 2 = low, 3 = medium, 4 = high 5 = very high. Prosthetic problems with current prosthesis in terms of 1 = very big, 2 = big, 3 = medium, 4 = low 5 = very low. Overall situation as amputated patient in terms of 1 = very bad, 2 = bad, 3 = medium, 4 = good 5 = very good. SF-36 physical function and physical component score

RCT

AARHUS UNIVERSITY

Declaration of co-authorship

Full name of the PhD student: Rehne Lessmann Hansen

This declaration concerns the following article/manuscript:

Title:	The effect of denosumab on periprosthetic bone mineral density in six transfemoral amputees, treated with osseointegrated implants: The observations from a terminated randomized controlled trial
Authors:	Hansen, Rehne Lessmann; Bente Lomholt Langdahl; Jørgensen, Peter Holmberg; Petersen, Klaus Kjær; Søballe, Kjeld; Stilling, Maiken

The article/manuscript is: Published ☐ Accepted ☐ Submitted ☐ In preparation ☒

If published, state full reference:

If accepted or submitted, state journal:

Has the article/manuscript previously been used in other PhD or doctoral dissertations?

No ☒ Yes ☐ If yes, give details:

The PhD student has contributed to the elements of this article/manuscript as follows:

A. No or little contribution
B. Has contributed (10-30 %)
C. Has contributed considerably (40-60 %)
D. Has done most of the work (70-90 %)
E. Has essentially done all the work

Element	Extent (A-E)
1. Formulation/identification of the scientific problem	D
2. Planning of the experiments and methodology design and development	E
3. Involvement in the experimental work/clinical studies/data collection	D
4. Interpretation of the results	E
5. Writing of the first draft of the manuscript	E
6. Finalization of the manuscript and submission	D

Signatures of the co-authors

Date	Name	Signature
~~12/12-16~~	~~Bente Lomholt~~	
12/12-16	BENTE LANGDAHL	Bente Lomholt
12/12-16	MAIKEN STILLING	Maiken Stilling

AARHUS
UNIVERSITY

13/12-16	KJÆR PETERSEN	
14/12-16	PETER HELTBERG JØRGENSEN	
14/12-16	KJELD SØBALLE	

In case of further co-authors please attach appendix

Date: 16/12-16

Signature of the PhD student

D+A

AARHUS UNIVERSITY

Declaration of co-authorship

Full name of the PhD student: Rehne Lessmann Hansen

This declaration concerns the following article/manuscript:

Title:	Loss of periprosthetic bone mineral density and higher PTH is associated with removal of osseointegrated implants in femoral amputees
Authors:	Hansen, Rehne Lessmann; Bente Lomholt Langdahl; Jørgensen, Peter Holmberg; Petersen, Klaus Kjær; Søballe, Kjeld; Stilling, Maiken

The article/manuscript is: Published ☐ Accepted ☐ Submitted ☐ In preparation ☒

If published, state full reference:

If accepted or submitted, state journal:

Has the article/manuscript previously been used in other PhD or doctoral dissertations?

No ☒ Yes ☐ If yes, give details:

The PhD student has contributed to the elements of this article/manuscript as follows:

A. No or little contribution
B. Has contributed (10-30 %)
C. Has contributed considerably (40-60 %)
D. Has done most of the work (70-90 %)
E. Has essentially done all the work

Element	Extent (A-E)
1. Formulation/identification of the scientific problem	C
2. Planning of the experiments and methodology design and development	C
3. Involvement in the experimental work/clinical studies/data collection	D
4. Interpretation of the results	C
5. Writing of the first draft of the manuscript	D
6. Finalization of the manuscript and submission	D

Signatures of the co-authors

Date	Name	Signature
12/12-2016	BENTE LANGDAHL	Bente Langdahl
12/12-2016	MAIKEN STILLING	Maiken Stilling
13/12-16	KJÆR PETERSEN	Kjær Petersen

AARHUS
UNIVERSITY

14-12-16	PETER HULMBERG JØRGENSEN	
14/12.16	KJELD SØBALLE	

In case of further co-authors please attach appendix

Date: 16/12-16

Signature of the PhD student

RSA

AARHUS UNIVERSITY

Declaration of co-authorship

Full name of the PhD student: Rehne Lessmann Hansen

This declaration concerns the following article/manuscript:

Title:	Higher migration of removed compared with non-removed osseointegrated implants for transfemoral amputees. A prospective 2 year RSA study with 5-years clinical follow-up.
Authors:	Hansen, Rehne Lessmann; Bente Lomholt Langdahl; Jørgensen, Peter Holmberg; Petersen, Klaus Kjær; Søballe, Kjeld; Stilling, Maiken

The article/manuscript is: Published ☐ Accepted ☐ Submitted ☒ In preparation ☐

If published, state full reference:

If accepted or submitted, state journal:

Has the article/manuscript previously been used in other PhD or doctoral dissertations?

No ☒ Yes ☐ If yes, give details:

The PhD student has contributed to the elements of this article/manuscript as follows:

A. No or little contribution
B. Has contributed (10-30 %)
C. Has contributed considerably (40-60 %)
D. Has done most of the work (70-90 %)
E. Has essentially done all the work

Element	Extent (A-E)
1. Formulation/identification of the scientific problem	C
2. Planning of the experiments and methodology design and development	C
3. Involvement in the experimental work/clinical studies/data collection	D
4. Interpretation of the results	C
5. Writing of the first draft of the manuscript	E
6. Finalization of the manuscript and submission	C

Signatures of the co-authors

Date	Name	Signature
12/12.2016	BENTE LANGDAHL	Bente Langdahl
12/12-2016	MAIKEN STILLING	Maiken Stilling
13/12-16	KLAUS KJÆR PETERSEN	Klaus Kjær Petersen

AARHUS
UNIVERSITY

14.12.16	PETER HOLMBERG JØRGENSEN	
14/12-16	KJELD SØBALLE	

In case of further co-authors please attach appendix

Date: 16/12-16

Signature of the PhD student

AARHUS UNIVERSITY

Declaration of co-authorship

Full name of the PhD student: Rehne Lessmann Hansen

This declaration concerns the following article/manuscript:

Title:	Bone Mineral Density Measurements around Osseointegrated Implants: A Precision Study and Validation of Scan-Protocol for Transfemoral Amputees.
Authors:	Hansen, Rehne Lessmann; Bente Lomholt Langdahl; Jørgensen, Peter Holmberg; Petersen, Klaus Kjær; Søballe, Kjeld; Stilling, Maiken

The article/manuscript is: Published ☐ Accepted ☐ Submitted ☑ In preparation ☐

If published, state full reference:

If accepted or submitted, state journal:

Has the article/manuscript previously been used in other PhD or doctoral dissertations?

No ☒ Yes ☐ If yes, give details:

The PhD student has contributed to the elements of this article/manuscript as follows:

A. No or little contribution
B. Has contributed (10-30 %)
C. Has contributed considerably (40-60 %)
D. Has done most of the work (70-90 %)
E. Has essentially done all the work

Element	Extent (A-E)
1. Formulation/identification of the scientific problem	D
2. Planning of the experiments and methodology design and development	D
3. Involvement in the experimental work/clinical studies/data collection	D
4. Interpretation of the results	C
5. Writing of the first draft of the manuscript	E
6. Finalization of the manuscript and submission	D

Signatures of the co-authors

Date	Name	Signature
12/12-2016	Bente Langdahl	
12/12-2016	BENTE LANGDAHL	Bente Langdahl
12/12-2016	Maiken Stilling	Maiken Stilling
13/12-16	KJÆR PETERSEN	

AARHUS
UNIVERSITY

14.12.16	PETER HOLMDORG JØRGENSEN	
14/12-16	KJELD SØBALLE	

In case of further co-authors please attach appendix

Date: 16/12 - 16

Signature of the PhD student