LITHUANIAN UNIVERSITY OF HEALTH SCIENCES
MEDICAL ACADEMY

Vėtra Markevičiūtė

CALCIUM SULPHATE/HYDROXYAPATITE AUGMENTATION AND BISPHOSPHONATE IMPROVES SCREW ANCHORAGE IN FRAGILE BONE

Doctoral Dissertation
Medical and Health Sciences,
Medicine (M 001)

Kaunas, 2024

Dissertation has been prepared at the Department of Orthopaedics and Traumatology of Lithuanian University of Health Sciences during the period of 2018–2024.

Dissertation is defended extramurally.

Scientific Consultant

Assoc. Prof. Dr. Deepak Bushan Raina (Lund University, Medical and Health Sciences, Medicine – M 001).

Dissertation is defended at the Medical Research Council of the Lithuanian University of Health Sciences:

Chairperson

Prof. Dr. Žilvinas Dambrauskas (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001).

Members:

Prof. Dr. Valentinas Uvarovas (Vilnius University, Medical and Health Sciences, Medicine – M 001);

Prof. Dr. Algidas Basevičius (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001);

Prof. Dr. Justinas Stučinskas (Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine – M 001);

Assoc. Prof. Dr. Annette W-Dahl (Lund University, Medical and Health Sciences, Medicine – M 001).

Dissertation will be defended at the open session of the Medical Research Council of the Lithuanian University of Health Sciences on 23rd of August 2024 at 1 p.m. in the auditorium A-202 of the Center for Advanced Pharmaceutical and Health Technologies of the Lithuanian University of Health Sciences.
Address: Sukilėlių ave. 13, LT-50166 Kaunas, Lithuania.

LIETUVOS SVEIKATOS MOKSLŲ UNIVERSITETAS
MEDICINOS AKADEMIJA

Vėtra Markevičiūtė

BISFOSFONATAI IR AUGMENTACIJA KALCIO SULFATO/HIDROKSIAPATITU PAGERINA SRAIGTO TVIRTINIMĄSI TRAPIAME KAULE

Daktaro disertacija
Medicinos ir sveikatos mokslai,
medicina (M 001)

Kaunas, 2024

Disertacija rengta 2018– 2024 metais Lietuvos sveikatos mokslų universiteto Ortopedijos traumatologijos klinikoje.

Disertacija ginama eksternu.

Mokslinis konsultantas

doc. dr. Deepak Bushan Raina (Lundo universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Disertacija ginama Lietuvos sveikatos mokslų universiteto Medicinos mokslo krypties taryboje:

Pirmininkas

prof. dr. Žilvinas Dambrauskas (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Nariai:

prof. dr. Valentinas Uvarovas (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina – M 001);

prof. dr. Algidas Basevičius (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001);

prof. dr. Justinas Stučinskas (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina – M 001);

doc. dr. Annette W-Dahl (Lundo universitetas, medicinos ir sveikatos mokslai, medicina – M 001).

Disertacija bus ginama viešajame Lietuvos sveikatos mokslų universiteto Medicinos akademijos Medicinos mokslo krypties tarybos posėdyje 2024 m. rugpjūčio 23 d. 13 val. Lietuvos sveikatos mokslų universiteto Naujausių farmacijos ir sveikatos technologijų centro A-202 auditorijoje.
Disertacijos gynimo vietos adresas: Sukilėlių pr. 13, LT-50166 Kaunas, Lietuva.

CONTENTS

To My Family and My Teachers…

ABSTRACT

Osteoporosis affects more than 200 million people worldwide, and by 2050, that figure is anticipated to double. A noticeable decline in BMD and an increase in trabecular bone porosity are typical symptoms of the condition. This makes it difficult to secure fracture fixation hardware in fragile cancellous bone. The aim of this work is to develop methods to improve immediate mechanical anchorage and enhance bone formation around orthopaedic hardware used for the fixation of hip fractures. Within the scope of this doctoral thesis, a calcium sulphate/hydroxyapatite (CaS/HA) biomaterial and systemic bisphosphonate zoledronic acid was used to modulate peri-implant bone formation. Our main research questions were: can a CaS/HA material implanted around a metallic implant increase the immediate mechanical bone anchorage and can a CaS/HA material be biologically activated by systemically administered bisphosphonate and consequently improve bone quality. This thesis comprises of 5 different studies involving both pre-clinical and clinical research. Our initial studies demonstrated that implants augmented with CaS/HA material in osteoporotic bone and activation of HA particles by systemically administered ZA (7–14 days after surgery) leads to new bone formation. Pre-clinical data demonstrate that biomechanical advantages can be achieved from CaS/HA augmentation of lag-screws in osteoporotic bone. It has also been demonstrated that bisphosphonate ZA seeks the HA particles and promote bone formation in cancellous bone. The following questions pertained to how the pre-clinical studies could be translated into a clinical study? For this reason, the two following studies were aimed to test the feasibility of a new stratification index (FAME), that could help to identify individuals who would benefit most from augmentation treatments and had a high risk of fracture and a low risk of death before the procedure. The feasibility of using the FAME index was validated in two separate studies following, after which a proof-of-concept clinical study was initiated. In a clinical setting, looking into the clinical efficacy of the developed methods in the fragile and elderly population undergoing treatment of pertrochanteric fracture stabilization with a dynamic hip screw system, this combination could potentially be superior to present techniques for augmenting osteoporotic trochanteric fractures thereby preventing reoperations.

ABBREVIATIONS

AP	–	Anteroposterior
APFN	–	Aimstrong proximal femoral nail
ASA	–	American Society of Anaesthesiologist
AO-OTA	–	AO Foundation/Orthopaedic Trauma Association
BCIS	–	Bone cement implantation syndrome
BMD	–	Bone Mineral Density
BMI	–	Body mass index
BMP2	–	Bone morphogenic protein 2
BV	–	Bone volume
BV/TV	–	Bone volume fraction
BVF	–	Bone void filler
CaP	–	Calcium phosphate
CaS	–	Calcium sulfate
CCD	–	Caput-collum-diaphyseal
CaS/HA	–	Calcium sulphate/hydroxyapatite
CI	–	Confidence interval
CM	–	Collagen membrane
CMN	–	Cephalomedullary nail
CT	–	Computer tomography
DHS	–	Dynamic Hip Screw
FAME	–	Fracture and Mortality Risk Evaluation
FAME-HL	–	High fracture-low mortality risk category
FRAX	–	Fracture Risk Assessment Tool
FRAX-H	–	High fracture risk category
FZA	–	Fluorescent ZA
GMD	–	Gentamicin-containing composite calciu sulfate/ hydroxyapatite
HA	–	Hydroxyapatite
HL	–	High-Low
HRQoL	–	Health-related quality of life
HHS	–	Harris hip score
mHA	–	HA microparticles
IM	–	Intramedullary nail
IMN	–	Intramedullary nailing
ISO	–	International organization for Standardization

LT – Lithuania
MCL – Medial collateral ligament
MDR – Medical device regulation (EU) 2017/745
MMA – Methylmethacrylate
MPD – Maximum penetration depth
MV – Mineralized tissue volume
MSCs – Mesenchymal stem cells
nHA – HA nanoparticles
OR – Odds ratio
PFN – Proximal femoral nail
PFNA – Proximal femoral nail antirotation
PMMA – Polymethyl Methacrylate
PRC – Packed red cell
QALY – Quality-adjusted life year
QCT – Quantitative computed tomography
QoL – Quality of life
OVX – ovariectomized
RCT – Randomized clinical trial
rhBMP-2 – Recombinant human bone morphogenic protein 2
RH – Relative humidity
ROI – Regions of interest
ROM – Passive range of motion
SD – Standard derivation
Sernbo-L – Low mortality risk category
SHS – Sliding hip screw
TAD – Tip-to-apex distance
TFNA – Trochanteric fixation nail advanced
TH – Pertrochanteric fractures
THA – Total hip arthroplasty
THR – Total hip replacement
TR – Turkey
TV – Total tissue volume
VAS – Visual analogue scale
WHO – World Health Organisation
XR – X-ray
ZA – Zoledronic Acid

INTRODUCTION

Osteoporosis refers to a skeletal disease characterized by low bone density and microarchitectural deterioration of bone tissue leading to increased bone fragility and susceptibility to fracture as defined by the WHO [1]. Fragility fractures are increasing with age and only partly halted by primary and secondary national prevention programs. Osteoporosis already affects more than 200 million people globally, and by 2050, that number is anticipated to double [2]. It is the most common metabolic bone disease in the world [3]. According to recent statistics, one in three women over the age of 50 and one in five men over the age of 65 will experience a bone fragility fracture, and this is a major burden for healthcare systems and society around the world [4]. Due to the aging population, fragility fractures are predicted to increase further in the next decade.

The dynamic balance between bone formation and resorption is necessary for normal bone metabolism [1]. Aging, estrogen shortage, and prolonged immobility are just a few factors that alter normal apoptosis, autophagy, and inflammation. This results in aberrant activation of osteoclasts, which eventually overwhelm bone creation by bone resorption [1]. The two types of risk factors for osteoporosis are classified as modifiable (smoking, alcohol consumption, physical inactivity, dietary calcium deficiency, and long-term glucocorticoid use) and non-modifiable (gender, age, race, and genetic characteristics) [5].

Osteoporosis can be further divided into two types: primary (occur in both sexes at all ages, but often follows menopause in women and occurs later in life in men) and secondary (a result of medications (eg, glucocorticoids), or other medical conditions [6]. Various methods are used to measure osteoporosis [7] and the diagnosis is based on patient medical history, careful physical examination, FRAX index, conventional X-rays of the thoracic and lumbar spine, BMD measurements, and laboratory investigations [4, 8]. In clinical practice, drug therapy such as calcium, vitamin D, hormone replacement therapy, bisphosphonates, etc., are commonly used to mitigate osteoporosis [1].

The pronounced reduction in the BMD and an increase in porosity of trabecular bone [6] makes anchorage of fracture fixation devices challenging in fragile cancellous bone.

Like how hypertension and high cholesterol are risk factors for stroke and heart disease, osteoporosis raises the likelihood of sustaining a fracture [3]. The “silent” aspect of the illness, which doesn’t express itself clearly until a fracture occurs, which presents additional difficulties for the healthcare system [9]. Typical osteoporotic fractures include vertebral compression

fractures, hip fractures, proximal humerus, and distal radius fractures – considered "indicator fractures" for osteoporosis [10]. The life expectancy in hip fracture patients decreases by nearly 25 % when compared to age and gender matched populations.

Approximately 20 % of hip fracture patients require long-term nursing home care, and up to 60 % do not fully regain pre-fracture independence [11]. Patients dealing with pain, physical limitations, and loss of freedom frequently have psychosocial problems after fractures, most notably sadness and low self-esteem [3].

Most fractures in older persons are caused, at least in part, by decreased bone mass [3]. It is estimated that nearly 2.5 million individuals sustain a proximal femur fracture yearly and the number is expected to reach 5 million in the year 2050 [2, 12]. Hip fractures are associated with 8.4–36 % excess mortality at 1 year, with higher mortality in men than in women [13].

The novelty of the study

Osteoporosis is a common disorder that is characterized by low bone mass with microarchitectural disruption and skeletal fragility. Failures due to loss of fixation is thus a prominent risk caused by inferior quality of the cancellous bone around the implant material or surgery related such as insufficient repositioning or sub-optimal placement of the fixation system. An additional reoperation in an already fragile patient, reduces quality of life and most importantly, increases the risk of premature death. Rate of failure that requires revision surgery is about 3–7 % depending on the fracture type [14, 15]. In addition, around 2 % of the cases are reported to have a postoperative infection [16, 17].

As failure of internal fixation of fractures in osteoporotic bone typically occurs through breakage of the bone that surrounds the implant, rather than the implant itself, and augmentation of the bone could be considered in patients with trochanteric fractures.

Neither the volume of the augmentation material to be used is standardized nor is the surgical technique. Our idea is creating a novel method for augmenting a lag screw by delivering a CaS/HA biomaterial at the implant interface in osteoporotic bone.

In addition, activating a local biomaterial to generate new surrounding bone, – by systemic administration of a bisphosphonate is a concept that has not been clinically evaluated.

The main study questions:

1. Can a CaS/HA material implanted around a metallic implant increase the immediate mechanical anchorage of the implant to the bone?
2. Can a CaS/HA material be biologically activated by systemically administered bisphosphonate?
3. To translate the pre-clinical findings into a clinical study, can the patients be stratified to minimize patient risk?
4. What is the clinical efficacy of the developed methods in a fragile and elderly population undergoing treatment of pertrochanteric fracture?

1. THE AIM AND OBJECTIVES OF THE STUDY

1.1. The hypothesis of the study

By injecting an in-situ setting CaS/HA biomaterial around an implant using a novel surgical technique, an improved mechanical bone anchorage will be achieved.

The CaS/HA biomaterial reinforcing a fragile cancellous bone will create a biomechanically stronger composite.

Postoperative biomodulation i.e. giving a systemic bisphosphonate Zoledronic acid that seeks and biologically activates the calcium enriched HA will increase bone formation around the implant.

1.2. The main study aim

The aim of this work is to develop methods to improve immediate mechanical anchorage and enhance bone formation around orthopaedic hardware used for the fixation of hip fractures using a calcium sulphate/hydroxyapatite (CaS/HA) biomaterial and systemic bisphosphonate zoledronic acid to modulate peri-implant bone formation.

1.3. The specific objectives of the study

1. To identify an optimal surgical technique to deliver an injectable biomaterial CaS/HA in anchoring a bone-implant without applying pressure, which is associated with risk of material leakage into the surrounding vasculature.
2. To study if injection of a CaS/HA material at the bone-implant interface enhance the immediate biomechanical anchorage of a lag-screw in an osteoporotic saw-bone model.
3. To study in vivo implant integration and bone regeneration and if a CaS/HA biomaterial can be biologically activated by systemic administration of ZA.
4. To identify the optimal timing of ZA administration and bone formation around an augmented implant.
5. To investigate if additional calcium sulphate and phosphates combinations can be bioactivated.
6. To translate the preclinical findings into useful clinical practice, a new index FAME was developed combining FRAX and SERNBO (high fracture and high mortality risk) and studied in a clinical emergency setting and at one year follow up.

7. To verify the preclinical findings in a proof-of-concept RCT. Study and compare the clinical outcome and bone remodelling around a hip screw in patients receiving systemically administered ZA with or without CaS/HA augmentation. Bone remodelling was evaluated using the contralateral hip as control with post-operative CT, at 3 months and a final investigation with DEXA and CT at 6 months.

2. REVIEW OF THE LITERATURE

2.1. FRAX index

Since it was developed in 2008, FRAX has taken its place in the routine management of osteoporosis [18]. FRAX (Fig. 2.1.1) is a computer-based algorithm (http://www.shef.ac.uk/FRAX) developed by the WHO Collaborating Centre for Metabolic Bone Diseases [19].

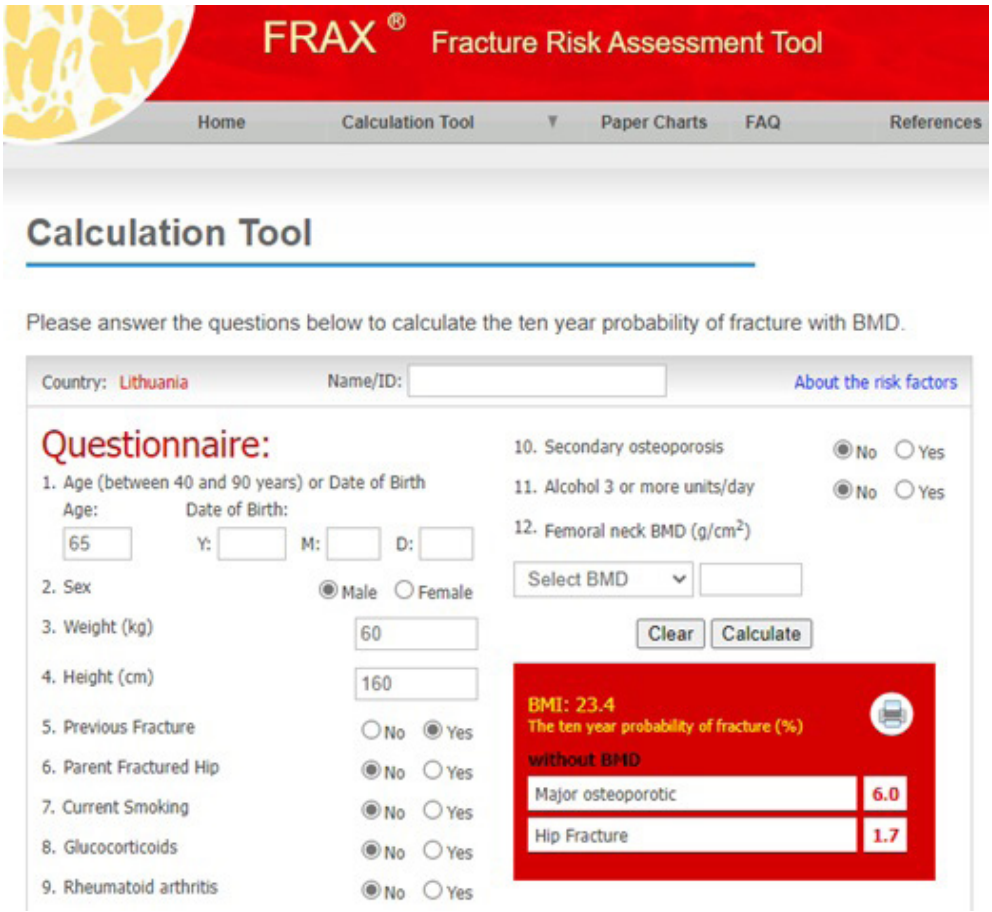

Fig. 2.1.1. *Screen page for the input of FRAX variables.*

(Lithuania mode,Version 4.3. http://www.shef.ac.uk/FRAX)

Based on clinical risk variables, with or without BMD, the FRAX algorithm has been created to assess the 10-year risk of hip and major osteoporotic fracture (hip, spine, humerus, or wrist fracture) [19, 20]. Fracture probability

varies markedly in different regions of the world [21]. Models are currently available for 78 nations or territories covering more than 80 % of the world population [22]. The risk factors included in FRAX are age, sex, BMI, personal history of fracture, parental history of hip fracture, current smoking, alcohol intake, glucocorticoid use, rheumatoid arthritis, other causes of secondary osteoporosis and with the option of including femoral neck BMD [20]. Patients can be divided into high, middle, and low risk categories using age-dependent intervention thresholds based on FRAX risk scores [8]. FRAX is based on analysis of data from 12 large prospective observational studies in about 60,000 untreated men and women in different world regions, having over 250,000 person-years of observation and more than 5,000 reported fractures reported [23]. FRAX is a substantial advancement in identifying men and women who are at high risk for osteoporosis-related fractures and enables the customization of pharmaceutical therapies for these individuals [19].

2.2. SERNBO index

After an intracapsular femoral neck fracture, the Sernbo score was initially described to determine which patients should have a hemiarthroplasty and which ones should have a total hip arthroplasty [24].

The Sernbo (Table 2.2.1) score uses four factors (age, social situation, mobility, and mental state) [25] to divide patients into a high-risk, intermediate and a low-risk group [26–29]. Information gathered during normal orthopaedic patient examination can be used to quickly calculate the Sernbo score [27]. The Sernbo score can only be 20, 17, 14, 11 or 8. The Sernbo score was graded according to the total number of points and divided into low risk (17–20 points), intermediate risk (14 points) and high risk (8–11 points), as described previously Mellner et al. [26].

Table 2.2.1. *The Sernbo scoring system*

Domain		**Score**	
Age	< 80 years	5	
	> = 80 years	2	
Social Situation	Independent (no carers)	5	
	Package of care / residential care/ nursing care	2	
Mobility	Walks unaided / one stick	5	
	Two sticks / frame / wheelchair / bedbound	2	
Mental state	Normal	5	
	History of dementia	2	
Total:			/20

The Sernbo score system is helpful for identifying hip fracture patients who are at high risk for mortality in the first year following surgery [26, 27].

2.3. Pertrochanteric fracture

There are two types of hip fractures: extracapsular and intracapsular [30]. The region from the extracapsular basilar neck region distally to the lesser trochanter until the medullary canal is defined as pertrochanteric (intertrochanteric) fractures, while the region from the lesser trochanter to 5 cm distally in the femoral diaphysis is subtrochanteric fractures.

The most widely used are the AO/OTA classification (Fig. 2.3.1) [31] and modified Evans classification system (Fig. 2.3.2) [31]. Therefore, if a fracture has two components, it is considered stable; if it has three or more, it is considered unstable [32]. The Jensen modification of the Evans' classification consists of five subtypes: type 1: undisplaced 2-part fracture; type 2: displaced 2-part fracture; type 3: 3-part fracture without posterolateral support due to dislocation of the greater trochanter fragment; type 4: due to a dislocated lesser trochanter fragment, a three-part fracture without medial support occurred and type 5: 4-part fracture without posterolateral and medial support [31]. The AO/OTA classifies trochanteric fractures into three groups: 31 A1 simple pertrochanteric, A2 multi-fragmentary pertrochanteric and A3 intertrochanteric patterns [33]. A2 type is further sub-divided into A 2.2 with one intermediate fragment and A2.3 with two or more intermediate fragments; they are both thought of as unstable pertrochanteric fracture patterns [33]. 42 % of all hip fractures [34] are pertrochanteric femur fractures, one of the most common types of fracture, especially among the elderly, which has a high risk of mortality and functional impairment [35].

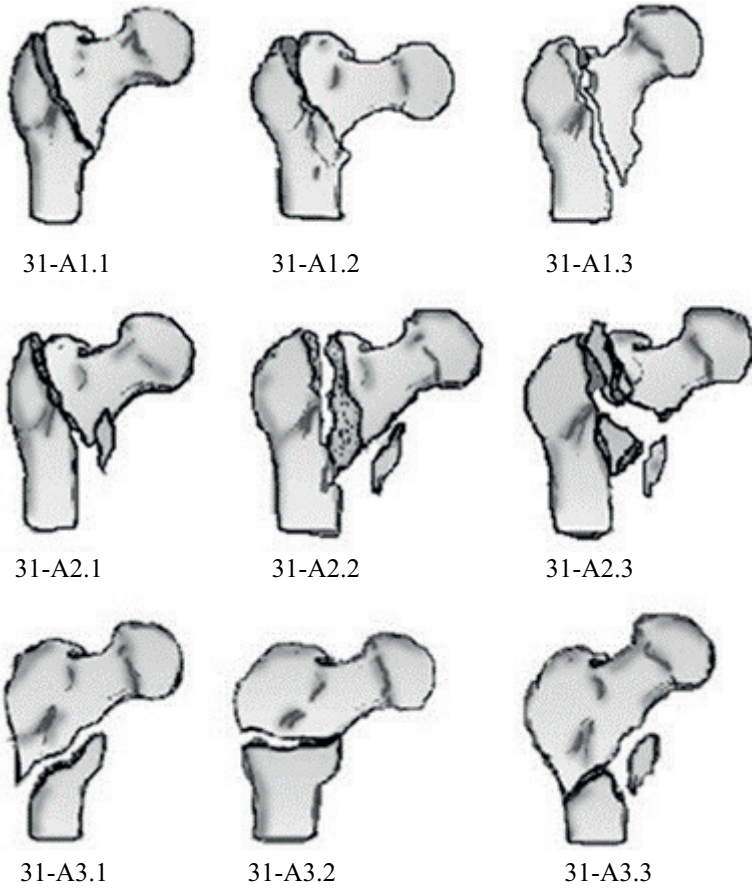

Fig. 2.3.1. *AO/OTA classification of pertrochanteric fractures of the proximal femur.*

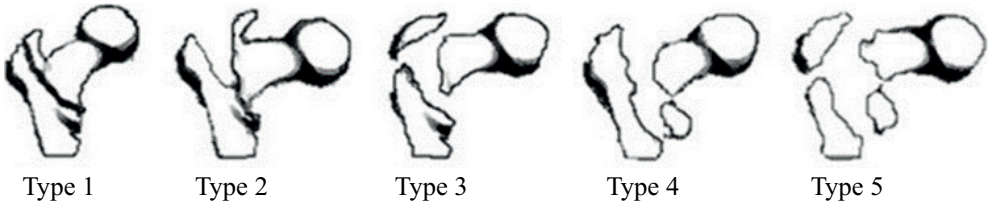

Fig. 2.3.2. *Jensen's modification of Evans classification of pertrochanteric fractures of the proximal femur.*

2.4. Treatment

Surgical fixation is typically used to treat proximal femoral fractures [36] which results in pain alleviation, early weight bearing, and in most cases restoration to pre-injury levels of function [35, 37].

During surgery, a surgeon can choose to use a DHS (the gold standard of trochanteric fracture treatment, for the stable fractures [38]) or gamma nail system or PFNA system (mainly for unstable fractures [38]). DHS requires a lateral femoral plate while the latter two systems have an intramedullary nail. A recent meta-analysis comparing the DHS to PFNA showed no differences between the two in-terms of reduction of failure rates, but PFNA led to shorter surgery time and lower blood loss [39]. In a large multicentre the INSITE RCT by Schemitsch et al. [40] they discovered no appreciable differences in patients treated with the IMN or a SHS for trochanteric fractures in terms of HRQOL, revision surgical procedures, fracture healing rate, or adverse effects at one year postoperative. Sharma et al. [38] in a study showed that PFN provides a shorter surgery with a smaller incision that leads to less wound complications. However recently studies have also shown an increased short-term mortality with PFN [41]. So, both surgical methods- DHS and IM have their own pros and cons.

2.5. Fixation outcomes and complications

DHS and intramedullary nails and screws are both associated with high failure rates, in unstable trochanteric fractures [42]. The literature reports a wide range of complications following hip fracture surgery, from 12.5 % to 75 % [43]. The three most frequent medical issues were congestive heart failure, pneumonia, and delirium [43]. The surgical complications include screw cut-out phenomenon, implant fracture or loosening and advancement of coxarthrosis [10]. Varus collapse and lag-screw migration in the femoral head, has been reported to be as high as 5.7 % of patients within 3 months [44].

Failures due to loss of fixation is thus- a prominent risk. Failure is either caused by bone related problems such as inferior quality of the cancellous bone around the implant material due to osteoporosis or surgery related such as insufficient repositioning or sub-optimal placement of the fixation system. An additional reoperation in an already fragile patient, reduces quality of life and most importantly, increases the risk of premature death. Rate of failure that requires revision surgery is about 3–7 % depending on the fracture type [14, 15]. In addition, around 2 % of the cases are reported to have a postoperative infection [16, 17]. The net risk of reoperation due to surgical site infections

within 1 year is reported as 2.7 % for all types of hip fractures. The highest risk of revision due to infections is observed for patients treated with a foreign material [45]. The proximal femur fracture patients in general are fragile and the 1-year mortality in patients >60 years of age ranges between 10 and 40 %, predominantly determined by age and comorbidities [46]. If a surgical site infection ensues, a 1-year mortality rate of 50 % has been reported compared to 24 % in a matched patient cohort without an infection [16, 17].

Because of this, elderly patients with osteoporosis who typically exhibit low bone quality, comminute fractures, and unstable fracture patterns are more likely to experience an early mechanical failure and hence exhibit a need for bone augmentation [47].

2.6. Fracture augmentation

Cement augmentation of internal fixation of hip fracture has been reported to increase the stability and consequently reduce the risk of the cut out of sliding screw being cut through the femoral head [48].

The bone quality, i.e., the degree of osteoporosis, is associated with the failures [49]. Augmentation increasing mechanical strength of cancellous bone in osteoporotic hip fractures lowers the burden of revision, which may outweigh the related additional cost [28]. Different techniques and materials used in fragile bone were described in in vivo studies biomechanical studies, computer models and clinically [49].

To improve the mechanical anchorage of the screw and prevent reoperations, the fragile osteoporotic bone has been reinforced with:

- polymer based injectable material like PMMA.
- CaP cements.

Both materials have proven to improve fracture stability in cadaver studies, observational clinical studies, and small randomized clinical trials but strong evidence from larger randomized controlled trials is missing [47, 48, 50–55]. Stramazzo et al. [48] in a review suggests that cement augmentation in the treatment of trochanteric fractures provides good clinical results and prevents failure of fixation with greater post operative mobility.

Increased mechanical stability with cement augmentation also helped shorten the hospitalization period due to earlier mobilization, with an earlier return performing daily activities [56].

2.7. Polymethyl methacrylate

PMMA was first used in the 1950s when Sir John Charnley successfully adapted it for the cementation of an orthopaedic prosthesis [57]. PMMA, a

polymer acrylic resin, is created by combining MMA, a monomer, and pre-polymerized polymethyl methacrylate particles at room temperature with an initiator, activator, and stabilizer [58].

PMMA is a used in osteoporotic fractures, oncology, vertebroplasty, and above all in cemented arthroplasty because of its high biocompatibility, simple handling, high strength, and in-situ moldability [48, 58–61].

Rompen et al. [61] performed a meta-analysis to compare cement augmentation to no augmentation in fixation of trochanteric femur fractures in the elderly patients (>65 years) following low energy trauma. A total of four RCT's and three observational studies were included with a total number of 730 patients of which 369 received cement augmentation following fixation. The result of the analysis showed that the duration of hospital stay was shorter in the augmented groups, the patients reported less pain and there was a significant difference in functional hip scores favoring augmentation.

Disadvantages using PMMA is the exothermic (generates temperatures of 66 to 120 °C) [62] setting reaction which can lead to necrosis of the femoral head and its inability to remodel into living bone [63]. The bone cement implantation syndrome is linked to joint arthroplasty, especially after cervical hip fractures. Clinical signs of hypoxia include hypotension, cardiac arrhythmias, and occasionally cardiovascular collapse [59, 64].

2.8. Calcium sulphate/hydroxyapatite

CaS/HA is the combination of Hydroxyapatite and Calcium Sulphate [9]. Owing to a balanced material degradation rate of the CaS/HA biomaterial, it has previously been demonstrated that the biomaterial can promote efficient bone healing. CaS/HA also has been studied as a drug delivery system for antibiotics and other medications [65]. The release of matrix-bound bone morphogenic proteins from cancellous bone or from an overlaying muscles has a stimulating effect [66, 67].

The CaS/HA biomaterial's powder phase is pre-mixed at a 60:40 (CaS: HA) weight ratio [68]. The CS hemihydrate particles transform into dihydrate after mixing with an aqueous solution, enabling the biomaterial to solidify into a hard mass with HA particles incorporated into the composite material [68]. Due to its osteoconductive properties, the material promotes bone formation by resorbing calcium sulphate, raising the local Ca2+ ion concentration, and creating room for bone ingrowth [66]. It drives both osteoblastic proliferation and differentiation, and the locally elevated Ca2+-ion concentration gives a apatite precipitation and contributes to cell recruitment and a favourable biological reaction [66]. The resorption of the CaS results in the formation of

a protein formed interlinked apatite matrix with a porosity that promotes the ingrowth of new bone [65].

More than 150 preclinical and clinical publications on the CaS/HA material have been published. Good results have been reported both in normal and osteoporotic bone and in other indications as presented by Nilsson et al. [66] and in distal radius as presented by Abramo et al. [69].

When used in vertebroplasty for fragility fractures, pain and QoL scores were similar as found with PMMA, but with a significant lower incidence of new adjacent level fractures [66].

The rate of resorption is a significant benefit of injectable biphasic CaS/HA bone substitutes. It is crucial for most applications that the material be replaced within years in accordance with the host own general bone turn over and biomechanical status [66]. Since it is injectable, the application is minimally invasive, easy – and precise. The CaS/HA material has been effectively employed loaded with antibiotics or other medications [66].

2.9. Safety and feasibility aspects of biomaterial augmentation in fracture fixation

The safety and feasibility aspects of the CaS/HA material for use in trochanteric hip fractures have been evaluated in three studies. Stravinskas et al. [70] performed a study to investigate the CaS/HA material containing gentamycin, CERAMENT G, with the aim to study the antibiotic elution from the material. Gentamicin release was measured in a total of 32 patients in a range of clinical situations (trochanteric hip fractures, uncemented hip revisions, etc.). Collection of wound drainage, serum and urine showed high initial levels of gentamicin, followed by a decreasing concentration over the next days. No systemic toxic effects were observed.

The antibiotic elution from the CaS/HA material containing vancomycin, (CERAMENT V), was evaluated in 9 patients with trochanteric hip fractures [71]. All patients were treated with internal fixation using a DHS and CaS/HA was used to augment the bone defects at implantation. The material was injected into the bone defect in the trochanteric region after placing the DHS but before plate implantation. The vancomycin elution was followed by simultaneously collecting drain fluid, blood, and urine up to 7 days post-operatively. The study showed initial high targeted local vancomycin levels and with complete release at 3 weeks. Systemic concentrations were well below toxic levels.

Stravinskas et al. [49] also performed a prospective, observational study in 20 patients with trochanteric fracture (treated with internal fixation) and aseptic revision of THA. The CaS/HA material containing gentamycin

(CERAMENT G) was used in both groups. For the fracture patients, CERAMENT G was injected into the bone defect in the trochanteric region after placing the dynamic screw but before plate implantation. In the revision of THA cases, CERAMENT G was injected into visible bone defects around the proximal part of the prosthesis after distal fixation of the uncemented stem. Radiological analysis of trochanteric fractures showed that reposition of bone fragments was satisfactory and bone healing was observed in patients available for follow-up, and CDD angles were like the healthy contralateral hip. A low level of dynamic screw migration, with an average of 3 mm at 3 months was observed. At 3 months and 1 year follow-up, 7 patients were able to walk independently, with no need for walking aids, while one patient had some walking problems. Radiological visualizing of CERAMENT G was possible on postoperative x-rays but at 3 months and 1 year follow-up the material was difficult to differ from new bone growing into the apatite scaffold. None of the patients had impaired hearing, kidney function or any allergic reactions to gentamicin. One [1] patient had hardware removal due to local discomfort of the DHS after 1 year. No other complications were registered.

Gadegone et al. [72] evaluated augmented PFN in patients with unstable trochanteric fractures, and they could demonstrate biomechanical stability with better functional and radiological outcomes.

In one of study by Hofmann et al. [73] compared the effectiveness of CaS/HA or autologous bone grafts (the gold standard in bone defect reconstruction) in fracture fixation treating bone deficiencies caused by tibial plateau fractures in terms of quality of life, pain, and radiological results. The study found that there were no statistically significant differences between the two groups.

2.10. Zoledronic acid

Globally, bisphosphonates are the most used (frequently used as a first-line medication [74]) drugs to treat bone resorption disorders, especially osteoporosis, and in bone oncology mainly in myeloma [75]. Single administration of ZA every year is an approved treatment for slowing down postmenopausal osteoporosis and reducing fracture risk [75]. ZA is a third-generation nitrogen-containing bisphosphonate that induces osteoclast apoptosis and inhibits bone resorption by inhibiting the mevalonate pathway [76].

In a large, well-designed, long-term study, intravenous ZA 5 mg once a year was effective in treating postmenopausal osteoporosis [75].

Annual infusions of zoledronic acid after a hip fracture decrease mortality from any cause by 28 % in addition to lowering future fracture risk [77].

The most typical side effects were mild cases of pyrexia, myalgia, arthralgia, and influenza-like illness [78]. There is a risk of hypocalcaemia and secondary hyperparathyroidism with all bisphosphonates [79]. There is no difference in nephrotoxicity between zoledronic acid and placebo, according to many large trials [77]. Jaw osteonecrosis is the worst side effect of bisphosphonate therapy, however it is uncommon and mainly affects oncology patients [80]. A rare side effects from long term ZA include atypical femur fractures [78].

2.11. Calcium sulphate/hydroxyapatite and local zoledronic acid

A pilot in vivo study by Širka et al. [68] indicated that local delivery of a bisphosphonate, zoledronic acid (ZA), using the CaS/HA biomaterial as a carrier enhanced bone formation in the femoral neck canal of severely osteoporotic rats. It is however important to mention that local delivery of ZA has a profound effect on cancellous bone regeneration in healthy as well as osteoporotic bone while the effect on cortical bone is low [68].

Using tibia bone defect models [81–83], it was further shown that CaS/HA could serve as a carrier for local delivery of ZA, resulting in an anabolic effect in cancellous bones. Findings that were verified using micro-CT and histology. An effect that could not be observed in cortical bone [81, 82]. In a more clinically relevant model, using an implant integration model, the above results were verified [84]. Significantly higher bone volume was seen around implants combined with CaS/HA and local delivery of ZA.

2.12. Unmet medical need

A standardized surgical method to augment fixation devices in unstable trochanteric fractures and the subsequent improvement of the immediate mechanical anchorage of fixation devices can be beneficial in establishing a standardized treatment approach that can aid in preventing failure of fixation.

The long-term success can be increased if the implanted CaS/HA material transforms into living bone and regenerates bone in the vicinity to the implant. A pharmacological biomodulation with systemic bone active drug bioactivating the material will be a significant step forward [81].

2.13. Health-economics

Patients who suffer fractures due to fragility costed the National Healthcare Systems in the United Kingdom £4.4 billion in 2018, in Europe €56 billion in 2019, and in the United States $57 billion in 2018 [85]. The economic burden of hip fractures is estimated to account for 72 % of the total cost of all fractures [86]. The average costs of reoperation per patient were 31 % higher

than those of the primary operation, and reoperations caused nearly 20 % of the overall direct costs of hip fractures [14].

Based on a RCT and using a cost-utility model that simulated clinical events, costs, and utilities over one year after index surgery, Joeris at al. [87] assessed the cost-effectiveness of treating unstable trochanteric fractures with and without cement augmentation from a German and US healthcare payer's perspective. The model showed that augmentation was associated with lower costs (–€105 and –$163.6/patient, respectively) and higher effectiveness measured in quality-adjusted life years compared to treatment without augmentation. Major influential parameters for the incremental cost-effectiveness ratio were mortality rate, revision rate, augmentation costs, and revision surgery costs.

Our own health economy calculation that is based on the assumption of a reoperation of a trochanteric fracture is calculated to 25000 US in US [88], Mortality doubles by the reoperation QALY difference between re-operation and no re-operation – 2,89. QALY gained per treatment if treatment reduces re-operation from 7 % to 3,5 % – 0,101. Value of treatment in USD based on QALY alone – 5062. Total USD value of treatment – 30 062.

3. METHODS

3.1. Pre-clinical studies

3.1.1. Screw fixation in osteoporotic human bone and synthetic saw bones

3.1.1.1. Study design

We examined the initial mechanical anchoring of a lag-screw in Sawbones- with PMMA and CaS/HA biomaterials injected to bone interface. Furthermore, the surgical procedure and distribution of the CaS/HA biomaterial were studied, using donated femoral heads from osteoporotic patients having a total hip replacement [89].

3.1.1.2. Distribution of CaS/HA in osteoporoic sawbones blocks

Blocks (SAWBONES Europe AB, Sweden) with 15 % bone volume fraction (BV/TV) open cells were used. Each block was predrilled with 8 mm burr to a depth of 3 cm. A stainless-steel lag screw with a length of 11.5 cm, a thread diameter of 1.25 cm, and a shaft diameter of 0.8 cm was partially inserted into the previously drilled hole to a depth of around 0.5 cm [89].

Following the manufacturer's instructions, the CaS/HA biomaterial was prepared and injected (Fig. 3.1.1.2.1). First, biomaterial was combined with a radiopaque contrast agent and mixed for 30 s. After that, the CaS/HA paste was poured into a 5-cc injection syringe. The luer lock adapter on a specially constructed titanium cannula (h = 16 cm, diameter = 2.5 mm) was attached to the syringe carrying the pre-mixed CaS/HA substance. The titanium cannula was passed through the cannulated metal screw and positioned at the most distal end of the pre-drilled channel in the block at t = 2.5 min after the mixing process began. To fill the pre-drilled bone void/channel 2 mL of the CaS/HA paste, was then progressively injected while the cannula was slowly retracted [89]. This procedure was carried out on 9 specimens. A similar procedure was carried out on samples containing PMMA (n = 7) and lag-screws without the biomaterial augmentation were used as controls (n = 8).

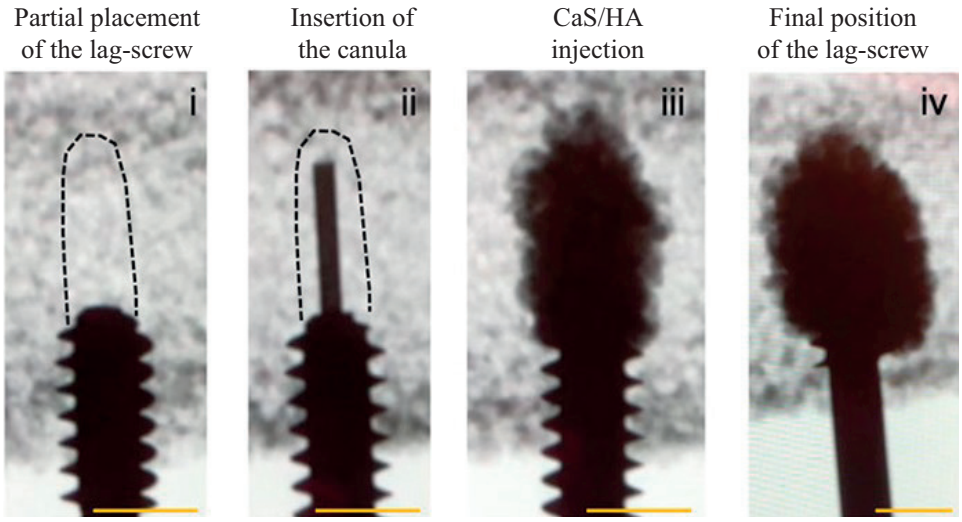

Fig. 3.1.1.2.1. *In an osteoporotic Sawbones model, a lag-screw was augmented using a CaS/HA biomaterial. The radiological time-lapse photography gives a general overview of the experimental stages, beginning with (i) the partial installation of the lag-screw. (ii) the insertion of the injection device through the lag-screw. (iii) CaS/HA biomaterial filled in the pre-drilled canal; (iv) the lag-screw's final position surrounded by the CaS/HA material; In (i) and (ii), dashed black lines show roughly where the pre-drilled zone is located. Scale bar equals roughly 1.3 cm [90].*

3.1.1.3. Spreading of the CaS/HA in donated femoral heads

The tissue bank was used to get two femoral heads from osteoporotic patients having hip replacement surgery after a low-energy cervical neck fracture. Under fluoroscopic guidance, a 3 cm long hole was drilled in the femoral head using an 8 mm burr. A 11.5 cm long lag-screw was only partially placed into the pre-drilled hole, around 0.5 cm. At this point, 2.5 mL of CaS/HA material was injected into the canal in front of the screw. The screw was then tightened and positioned in the final, planned location, which was confirmed by fluoroscopy. A micro-CT scanner was used to take an image of the augmented femoral head to assess how the CaS/HA material was distributed all around the screw-bone interface. The lag-screw was physically removed from the bone the next day after being left at –20 °C overnight after the CT scan was finished. To establish the presence of the CaS/HA biomaterial at the bone-lag-screw interface, the bone minus the lag-screw was then thawed once more. Pictures of the femoral heads were then taken [89].

3.1.1.4. Spreading of the CaS/HA in-vivo (surgical method)

Pertrochanteric fracture patients (Fig. 3.1.1.4.1(i)) receiving surgery were treated with DHS (Fig. 3.1.1.4.2 (ii)) augmented with the CaS/HA biomaterial. A canal of 0.8 cm was drilled all the way to the final position of the screw. A lag-screw was put into the bone 2.5 cm from its final planned position. The cannula connected to the injection syringe was inserted into the cannulated lag-screw after the guide wire was taken out of it (Fig. 3.1.1.4.1 (iii)). At t = 2.5 min after the start of mixing the biomaterial, the CaS/HA biomaterial was administered under fluoroscopic guidance. 2.5 mL of the material had been injected into the drilled space in front of the lag-screw and the surrounding cancellous bone (Fig. 3.1.1.4.1 (iv)). The femoral plate was then joined to the lag-screw after it had been placed in its final position (Fig. 3.1.1.4.1 (v)).

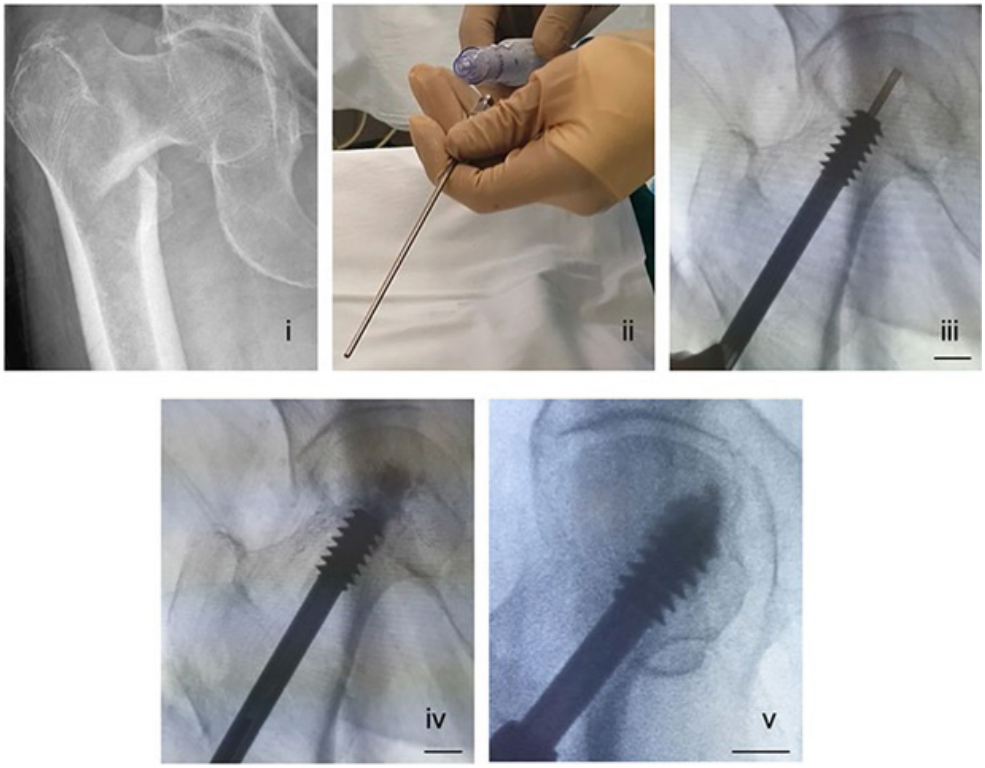

Fig. 3.1.1.4.1. *A method for using a cannulated lag-screw during surgery to administer a CaS/HA biomaterial to a patient having their pertrochanteric fracture treated.*

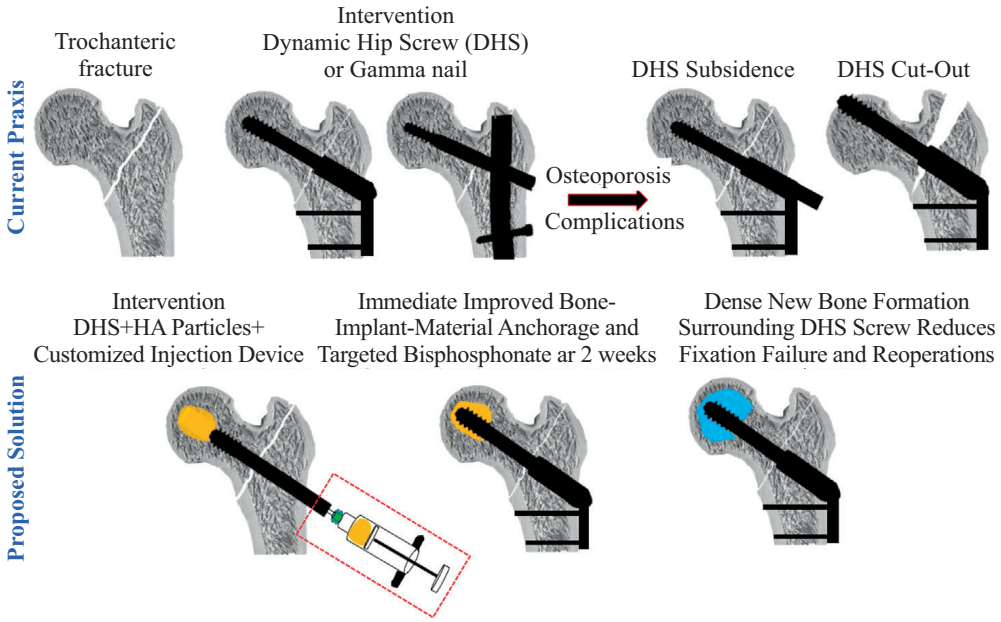

Fig. 3.1.1.4.2. *An illustration showing the current procedure for treating pertrochanteric fractures during surgery, the challenges as well as the proposed solution [89].*

3.1.1.5. Statistical analysis

Using the Shapiro-Wilk normality test, the data distribution from the mechanical testing experiment was examined. The comparison of the test groups with the control group was done using a one-way ANOVA with Dunnett's post hoc test on normally distributed data. The examination of non-parametrically distributed data utilized the Kruskal-Wally's test along with Dunn's post hoc procedure. The cut off for statistical significance was $p < 0.05$. Prism v9 (GraphPad, USA) was used for all statistical analyses.

3.1.2. Rat study

This research is divided into three smaller experiments to better understand how systemic bisphosphonate ZA therapy and peri-implant CaS/HA augmentation affect peri-implant bone growth. In the first section of this work, we examine implant integration and peri-implant bone growth in a clinically utilized stainless steel screw in the rat proximal tibia utilizing an osteoporotic rat model. The screw was either enhanced with CaS/HA or left untreated, and the subsequent investigation looked at how systemically administered ZA affected the peri-implant bone. In the second phase of the

investigation, we sought to clarify the variations in ZA accumulation as a function of administration time around a CaS/HA biomaterial implanted in the proximal tibia of rats. Fluorescently labelled ZA and in-vivo imaging system-based fluorescence imaging were used to explore this phenomenon. The purpose of the third significant sub-study was to comprehend how the timing of bisphosphonate administration affected the production of peri-implant bone in a model comparable to the one described in sub-study.

3.1.2.1. Implant integration in osteoporotic rats

We used a total of 38 ovariectomized (OVX) and four SHAM operated Sprague-Dawley female rats, which were procured from Janvier Labs (France). The animals were divided into four groups, designated as groups G1, G2, G3, and G4, at the time of operation (age at intervention = 24-weeks, average weight = 419 ± 29 g). A CaS/HA biomaterial (as CERAMENT™ BVF from Bone Support AB, Sweden) was used for screw augmentation. Isoflurane anesthesia (2–2.5 % combined with oxygen) was used during surgical procedures. Antibiotic prophylaxis (Penicilin-Streptocilin VET, Apoteket AB) was given 15 minutes before surgery. Buprenorphine (Temgesic, 0.03 mg/kg) was injected subcutaneously once to ease perioperative discomfort.

Each OVX animal underwent surgery approximately 5 mm under the tibial growth plate in the right tibia. Without damaging the MCL, a 1.8 mm unicortical burr-hole was made on the medial portion of the tibia, parallel to its insertion. The burr-hole was plugged with a stainless-steel screw (Trimed Ortho, Sweden) that had a 2.3 mm diameter and an 8 mm height. Groups G1 and G2 each had n = 11 rats, and the screws were not augmented in either group. After cleaning the burr-hole in groups G3 (n = 11) and G4 (n = 5), 50 µL of the CaS/HA material was added using a spatula 7 min after the start of mixing in those groups. Two weeks after surgery, all animals in groups G2 and G3 received a sub-cutaneous injection of bisphosphonate ZA (Novartis, Switzerland) at a dose of 0.1 mg/kg. Rats (G1–4) and the SHAM animals, which were neither ovariectomized not surgically treated, were sacrificed 6 weeks after surgery.

Micro-CT imaging was done on harvested tibiae from the defect side.

One representative sample from each of the groups G1–G4 was chosen for a more detailed imaging of the bone-implant interface using neutron imagining at the Institut Laue-Langevin (NeXT beamline, Grenoble, France). All remaining samples (except samples used for neutron imaging and undecalcified histology) were used for decalcified histology.

3.1.2.2. Timing of ZA injection and ZA absorption in the CaS/HA material

As stated in section 3.1.2.1. above, the proximal tibia was operated on in a total of 25 male Sprague-Dawley rats (average weight = 442 ± 23 g). N = 5 rats did not receive any CaS/HA material – control group. In n = 20 rats, the burr-hole was filled with 50 μL of the CaS/HA material. They were then separated into 4 groups depending on the day of fluorescent ZA (FZA) administration: on day 1, on day 3, on day 7 and on day 14. FZA was administered sub-cutaneously at a dose of 0.1 mg/kg. In order to optimize the signal-to-noise ratio during imaging, 10 % (weight) of Alexa-fluor 647 labelled ZA was first mixed with 90 % (weight) of unconjugated ZA. Animal sacrifices were performed 24 hours following the FZA injection. The defect and the contralateral tibiae from each animal were scanned on an In-Vivo Imaging System (IVIS Spectrum, Perkin Elmer, USA). The CaS/HA material was also taken out of the burr-hole and scanned independently to demonstrate that the signal did, in fact, come from the implanted material.

3.1.2.3. Effect of injection timing on peri-implant bone formation

Using the male Sprague-Dawley rats, we reproduced the implant integration scenario described in section 3.1.2.1. A total of 35 rats were divided into five experimental groups (n = 7/group) as follows; G1) CaS/HA, G2) CaS/HA+ Systemic ZA on day 1, G3) CaS/HA+ Systemic ZA on day 3, G4) CaS/HA+ Systemic ZA on day 7 and G5) CaS/HA+ Systemic ZA on day 14. At the predetermined time points, ZA (0.1 mg/kg) was subcutaneously injected into each animal in groups G2-G5. N = 4 rats were also used in a control group without the CaS/HA material surrounding the screw. According to earlier accounts, the animals in the control group were given ZA at a dose of 0.1 mg/kg subcutaneously 14 days after implantation. All animals were sacrificed 6-weeks post-surgery. Tibiae implanted with the metal screw were harvested and subjected to micro-CT imaging and histological analysis.

3.1.2.4. Is biomodulation with systemic ZA applicable to other calcium phosphate materials such as β-tricalcium phosphate

In all of our experiments so far, we used a CaS/HA biomaterial and therefore the goal of this sub-study was to evaluate whether other calcium phosphate materials, more specifically β-tricalcium phosphate (β-TCP) could also biologically activated by systemically circulating ZA. In order to only modify one variable, we decided to replicate the CaS/HA material composition by only replacing the HA component with β-TCP. We therefore pre-mixed

60 % weight CaS (VWR, Sweden) and 40 % weight β-TCP (Sigma Aldrich, Germany) both having a purity of >98 %. To this pre-mixed powder, 0.43 mL of Iohexol (Omnipaque, 180 mg I/mL, Apoteket AB, Sweden) was mixed to form a paste. In a 1.8 mm burr-hole in the proximal tibia of six male Sprague-Dawley rats 50 μL of the paste was the implanted followed by insertion of a stainless-steel screw (TriMed Ortho, Sweden) of 2.3 mm diameter and 8 mm height. One-week post-surgery, all six animals were injected with ZA (dose 0.1 mg/kg) sub-cutaneously. The animals were finally sacrificed six weeks post-surgery and the tibiae harvested for the analysis of peri-implant bone formation using micro-CT.

3.1.2.5. Statistical analysis

In the first experiment i.e. implant integration in osteoporotic rats, the primary outcome variable was micro-CT based peri-implant bone volume. Normality of data was tested using Shapiro-Wilk normality test. Data was further analyzed using a non-parametric ANOVA (Kruskal-Wallis test) with a Dunn's multiple comparison post-hoc test.

In the second experiment regarding the distribution of FZA around the CaS/HA material as a function of time, the CaS/HA leg was compared with the non-CaS/HA leg using a paired sample t-test since the data emanated from the same animal. The fluorescence signal in the CaS/HA treated legs followed by ZA injection at days 1, 3, 7 and 14 was then compared with the control group (i.e. animals that did not receive CaS/HA but were administered FZA systemically) using a Dunnett's ANOVA. The uptake of FZA in the remaining groups CaS/HA groups was compared using an ordinary one-way ANOVA with Tukey's post-hoc. Similar was done to evaluate the ZA uptake in the contralateral limbs that did not receive the CaS/HA material.

In the 3rd experiment involving the study of the effect of ZA administration time on peri-implant bone formation at a later time point, micro-CT based analysis of bone volume was used as a primary outcome variable. A total of 5 experimental groups were used and compared statistically; 1) CaS/HA, 2) CaS/HA+ Systemic ZA on day 1, 3) CaS/HA+ Systemic ZA on day 3, 4) CaS/HA+ Systemic ZA on day 7, and 5) CaS/HA+ Systemic ZA on day 14. CaS/HA group without ZA administration was used as a control and therefore the groups that received ZA (2–5) were compared with group 1 using a Dunnett's ANOVA. The remaining groups (2–5) that received both CaS/HA and systemic ZA were compared to each other using an ordinary one-way ANOVA with Tukey's post-hoc test.

In all statistical tests, statistical significance was set at $p < 0.05$.

3.1.3. Ethics

All animal experiments were approved by the Swedish Board of Agriculture (Permit number:152888/2019). All personnel involved in animal experimentation were trained as per FELASA guidelines and ARRIVE guidelines were followed in reporting the animal experiments. Animals had free access to regular food pellets and water during the entire course of the experiment. Animals were always housed at least 2/cage and were maintained in temperature and humidity-controlled rooms with 12 h light/dark cycles.

3.2. A study for identifying patients with a high risk of bone fractures who are most likely to benefit from bone augmentationt surgeries using the FAME index

3.2.1. Ethics

Each patient's or their family members signed an informed consent form. The principles of the Helsinki Declaration were followed in the conduct of the study.

3.2.2. FAME index

FAME index (Fig. 3.2.2.1), a tool for identifying patients who are most likely to benefit from augmentation surgeries and have a high risk of fracture but low risk of death. The FRAX and Sernbo classifications were used to classify the patients into low, medium, and high-risk groups for both indices, yielding a total of 9 combined categories for the FAME index [45]. According to treatment thresholds for FRAX, the colors red, yellow, and green represent high, medium, and low fracture risk, respectively [91].

Fracture and Mortality Evaluation (FAME) Index			
Patient ID:			
Age(*) [≥80(*2), <80(*5)]:			
Gender:			
Weight (kg):			
Height (cm):			
Living(*):		Own home(*5)	Sheltered home or frequent home assistance(*2)
Walking aids(*):		None, or one stick(*5)	Two sticks or walking frame(*2)
Mental status(*):		Alert(*5)	Slight confusion(*2)
Previous fracture Adult, (age +16) low energy		No	Yes
Parent had hip fracture		No	Yes
Current smoking		No	Yes
Glucocorticoids (Current or previous at least 3 months of usage)		No	Yes
Rheumatoid arthritis (Diagnosed)		No	Yes
Secondary osteoporosis (Early menopause, liver disease, endocrinological disease) Other disease (note here):		No	Yes
Alcohol (3 or more units per day)		No	Yes
FRAX Score: Hip: .....% Major Osteoporotic: .....%		10-year probability of major osteoporotic fracture (%) 40 30 20 10 0 40 50 60 70 80 90 Age (years)	
(*) **Sernbo Score**:	Low Risk (20 and 17)	Intermediate Risk (14)	High Risk (11 and 8)

Fig. 3.2.2.1. *Fracture and Mortality Evaluation (FAME index) [28].*

3.2.3. Patients and inclusion criteria

Two studies were performed based on the FAME index in two academic orthopaedic facilities from Kaunas, Lithuania, and Ankara, Turkey. A study by Sezgin, Markeviciute et al. [28] was started in November 2018 with 100 patients with osteoporotic fractures of the cervical or trochanteric region of the femur were admitted for surgery and enrolled in the study – 50 from each of the two centers.

At the end of 12 months based on FAME index classification, hospital records were reviewed to identify fractures on any site sustained during the follow-up period, reoperations, and mortality [29].

A form designed for the FAME index (Fig. 3.2.2.1) was used to collect all the information needed to calculate the FRAX without BMD and Sernbo scores.

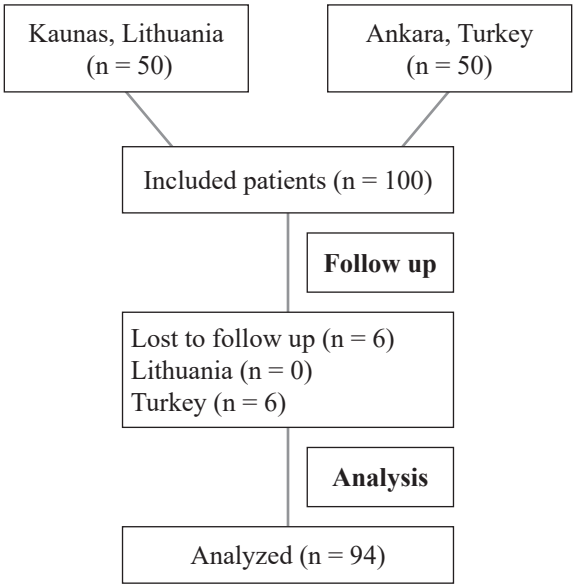

Fig. 3.2.3.1. *CONSORT diagram for FAME study.*

3.2.4. Statistical analysis

SPSS version 22 (IBM Chicago, IL, USA) was used for the statistical analysis. Shapiro-Wilk Normality Test was used to evaluate normality. The two centers differences were put to the test. Using the Chi-square test for categorical data and the independent-samples t-test or Mann-Whitney U test for continuous variables, the categories of the FAME Index, sex, age, height, and weight were evaluated. Statistics were considered significant at p-values less than 0.05.

3.3. A randomized controlled study to evaluate dynamic hip screw system augmented with a CaS/HA combined with systemic bisphosphonate for trochanteric femur fracture

3.3.1. Ethics

A clinical trial of lag-screw augmentation in TFs was approved by the Institute Review Board at the Kaunas University Hospital, Kaunas, Lithuania (Ethical permit number: P1 BE-2-76/2019) and the study details can be found at ClinicalTrials.gov (Identifier: NCT04498715). Before being enrolled in the clinical study, each participant signed an informed consent form.

3.3.2. Patients

Patients with a high risk of fracture but a low risk of death were included based on the FAME index.

Enrolled patients in the study: 20 (2 treatment groups: 10 patients in each group) (Table 3.2.2.1).

Table 3.3.2.1. *Patient demographics and Follow-up*

Patient ID	Follow up	Age	Sex	Height	Weight	CaS/HA	FAME	
							FRAX	SERNBO
1	Finished	95	Female	167	70	Yes	High risk	Low risk
2	Finished	85	Female	168	84	No	High risk	Low risk
3	Finished	80	Female	165	69	Yes	High risk	Low risk
4	Finished	84	Female	162	87	Yes	High risk	Low risk
5	Finished	78	Female	162	70	No	High risk	Low risk
6	Finished	72	Female	158	50	Yes	High risk	Low risk
7	Finished	86	Female	160	61	Yes	High risk	Low risk
8	Finished	78	Female	162	72	Yes	High risk	Low risk
9	Finished	95	Female	150	50	No	High risk	Low risk
10	Lost follow up	74	Female	167	100	No	High risk	Low risk
11	Lost follow up	67	Male	175	75	Yes	High risk	Low risk
12	Dead	84	Female	160	70	No	High risk	Low risk
13	Lost follow up	83	Female	160	76	No	High risk	Low risk
14	Finished	57	Male	175	73	Yes	High risk	Low risk
15	Dead	76	Male	175	71	No	High risk	Low risk
16	Finished	89	Female	168	70	No	High risk	Low risk
17	Finished	70	Female	168	82	Yes	High risk	Low risk
18	Finished	54	Male	175	95	No	High risk	Low risk
19	Finished	77	Female	167	70	No	High risk	Low risk
20	Lost follow up	52	Male	175	80	Yes	High risk	Low risk

3.3.3. Inclusion criteria

Patients 52–95 years of age; patient with unilateral proximal hip fracture (AO/OTA: A1 and A2) caused by low energy trauma (physical condition eligible for surgery with dynamic hip screw or proximal femoral nail fixation); informed consent is obtained and signed before any study-related activities (study-related activities are any procedures that would not have been performed during normal management of the patient, i.e. standard of

care), patient with a communicative ability to understand the procedure and participate in the study and the follow-up program.

3.3.4. Exclusion criteria

Patients >96 years of age; previous hip fractures on either side; concurrent oral treatment with corticosteroids, irreversible coagulopathy or bleeding disorder; note regarding reversible coagulopathies: patients on coumadin or other anticoagulants may participate; investigators should follow routine practices for perioperative discontinuation and reinitiation of anticoagulants; concurrent dialysis-dependent renal insufficiencies; active treatment due to metastatic malignancy including ongoing or completed radiotherapy involving the pelvis/hip area; fractures involving socket or acetabulum; active severe systemic infection or local skin infection at the puncture site; known hyperthyroidism or autonomous thyroid adenoma; history of serious reaction to iodine-based radio contrast agents; local infection at the site of implantation; pregnancy and nursing.

3.3.5. Information on CERAMENT™|BONE VOID FILLER

Based on BONESUPPORT AB and CERAMENT™ BVF Instructions for use: A synthetic, injectable, osteoconductive BVF is CERAMENT™|BVF. CERAMENT™|C-TRU (Iohexol 180 mg iodine/mL), a radio-opacity-enhancing component, is added to the biphasic formula of 60 % calcium sulfate and 40 % hydroxyapatite to allow bone ingrowth. CERAMENT™'s excellent injectability enables transcortical administration and guarantees effective intraosseous spread. The host bone that surrounds the osteoconductive hydroxyapatite grows in conjunction with the resorption of the calcium sulfate component with time. Like healthy cancellous bone, CERAMENT™ has the same compressive strength. The staff has not experienced any negative effects because of CERAMENT™. The CERAMENT™|CMI mixing apparatus reduces the chance of contamination during the mixing process. No hazardous reactions or temperature rise are brought on by the combined product. A non-ionic component that improves radiopacity is called CERAMENT™|C-TRU (Iohexol 180 mg/mL). Iohexol, the active component, has been utilized as an imaging agent for radiological exams for more than ten years. Under several brand names, such as GE Omnipaque™, iohexol has been used as a contrast medium for angiographic, intrathecal, and oral/body cavity investigations. Iohexol is appealing to employ since it has a low osmolality and is less toxic than traditional radio contrast agents like barium sulfate or zirconium oxide. The iohexol molecule is water soluble, thus it is delivered as an aqueous solution and added directly to the CERAMENT™ powder to begin the

calcium sulfate component of the product's setting process. Iohexol will provide adequate visualization of the artificial bone during the crucial weeks following injection. CERAMENT^{TM}|C-TRU releases a significant portion of its content in the first few weeks. The iohexol molecule has a very low affinity for serum albumin and exhibits very little metabolism, de-iodination, or biotransformation. The extracellular fluid compartment contains iohexol, which is then eliminated unchanged by glomerular filtration.

3.3.6. Information on zoledronic acid

All patients received an intravenous injection of ZA. 100 mL of sterile 0.9 % sodium chloride was used to dilute the ZA 4 mg/5 mL solution. Solution was given as an infusion 7–14 days following surgery that lasted at least 15 minutes.

3.3.7. Investigational design

This is an open-labelled pilot study to investigate the bone augmentation and remodelling around dynamic hip screw or proximal femoral nail using drugs that affects bone metabolism (CERAMENT and Zoledronic acid) (Fig. 3.3.7.1)

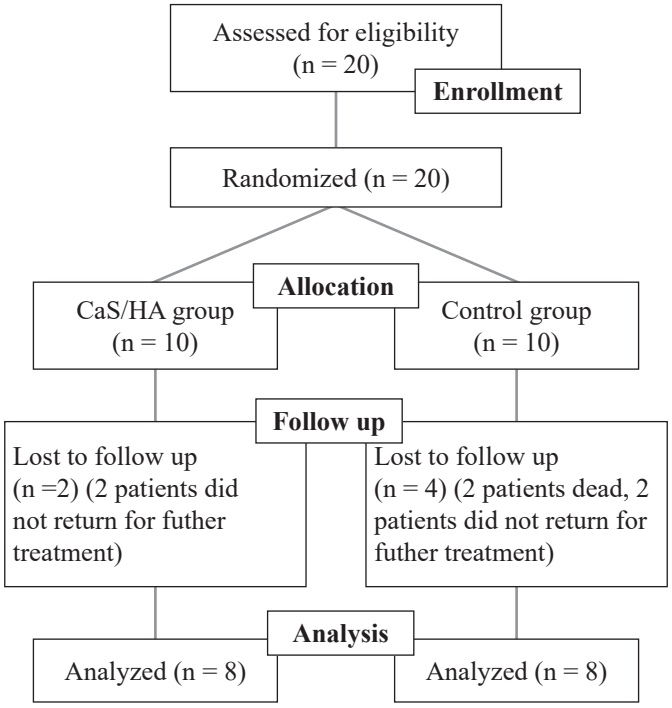

Fig. 3.3.7.1. *CONSORT diagram for clinical trial.*

3.3.8. Rationale for investigational design

A follow-up schedule of 6 months has been chosen as this period will allow for a proper assessment of the primary endpoint and secondary endpoints and for assessment of protocol safety (Table 3.3.8.1).

Table 3.3.8.1. *Visit schedule*

Visit No	1	2	3	4	5
Visit Window	**Screening**	**Hip surgery**	**6 weeks**	**3 months**	**6 months**
Informed consent	X				
Incl/ cl criteria	X				
Check Incl/Excl Criteria		X			
Demography	X				
Medical history	X				
FAME index	X				
Physical examination	X	X	X	X	X
Body weight	X				X
Height	X				
Vital signs (pulse and blood pressure)		X	X	X	X
X-ray (AP and lateral)	X		X	X	X
CT		X[1]		X	X
DEXA					X
Concomitant medication		X	X	X	X

[1] – CT scan will be performed postoperatively day 3 (±1 days).

3.3.9. Treatment of patients

All eligible patients will undergo surgery to treat their hip fracture and, in addition, receive treatment with systemic ZA with/without CERAMENT™ (depends on consecutive group) in their femoral head region. The post-procedural follow-up period is 6 months. The total number of follow-up visits are 3.

3.3.10. Treatment groups

1. Osteosynthesis + systemic ZA (4 mg i/v, 1–2 weeks post-surgery) – 10 patients.
2. Osteosynthesis + Local CERAMENT™ BVF (10 mL) + systemic ZA (4 mg i/v, 1–2 weeks post-surgery) – 10 patients.

3.3.11. Surgery procedure

The patient is supine with the fractured leg positioned in traction table. The biplanar x-ray device is moved and adjusted for proper simultaneous AP and lateral view. Hip region is washed and dressed in sterile draping. Using standard proximal femur osteosynthesis technique 2.0 mm diameter Kirschner wire is placed in the middle zone of the femoral neck in AP and lateral view. The canal is opened using 8 mm drill bit and measured length of dynamic hip screw is placed in the femoral head and neck. 2.5 mL of CERAMENT BVF only (1 Group) will be injected through the cannulated canal of the dynamic screw. In systemic ZA (1 and 2 groups) standard dose of the ZA was injected intravenously 1–2 weeks after surgery, surgical procedure performed as previously mentioned. An injectable CaS/HA biomaterial was prepared by following the manufacturers (Bonesupport AB, Sweden) guidelines and transferred into a 10-cc injection syringe. A long titanium cannula with a luer lock was then connected to the injection syringe. The cannula connected to the injection syringe was inserted into the cannulated lag-screw after the guide wire was taken out of it. At t = 2.5 min after the commencement of mixing, the CaS/HA biomaterial was administered under fluoroscopic guidance with the injection being started most distally, or at the final desired position of the lag-screw. When the drilled space in front of the lag-screw and the surrounding cancellous bone were filled with 2.5 cc of the material, the cannula was progressively retracted. The lag-screw was advanced to its final position at t = 3.5 minutes. Using a fluoroscope, the materials' spread and positioning around the dynamic screw head in the femoral head and neck was checked. The material was let to set for 12–13 min following which the plate was placed on the dynamic screw and secured with additional screws in distal part. A post operative X-ray was also taken.

3.3.12. Imaging studies

X-ray for AP and lateral before surgery, 1–3 days, 6 weeks, 3 months, and 6 months after surgery. CT of both femoral heads and necks were performed for evaluation 1–3 days, 3 months, and 6 months after surgery. DEXA of both femoral heads and necks were performed for evaluation 6 months after surgery.

3.3.13. DEXA analysis

We created our own DEXA evaluation protocol. First, we conducted a DEXA test after removing the metal. R1, R2 and R3 are defined regions of interest (Fig. 3.3.13.1). All three regions of interest were within the femoral

head. R1: This region is down/medial from screw. R2: This region is up/lateral from screw. R3: This region is front/proximal from screw. We manually sketch a region of interest box without including the metallic part of the screw. R1–3 areas had BMD (g/cm^2) evaluations. Hologic's Horizon DXA equipment was used by us for evaluations.

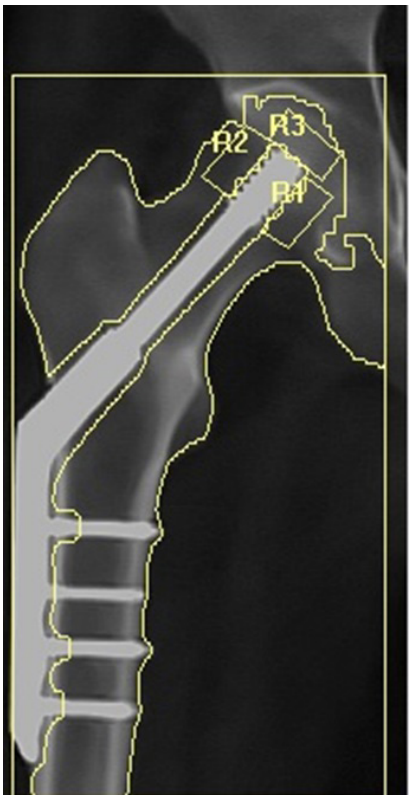

Fig. 3.3.13.1. *Identified areas (R1–3) of interest for DEXA analysis.*

4. RESULTS

4.1. Pre-clinical studies

4.1.1. Screw fixation method for osteoporotic bone

4.1.1.1. Spreading of the CaS/HA in osteoporoic sawbones blocks

The lag-screw was drawn directly out of the Sawbones blocks without any augmentation, and no remaining Sawbones were found to be attached to the lag-screw when the pull-out was finished (Fig. 4.1.1.1.1). The biomaterials prevented the Sawbones from failing at the direct contact of the lag-screw in the cases of CaS/HA and PMMA [89].

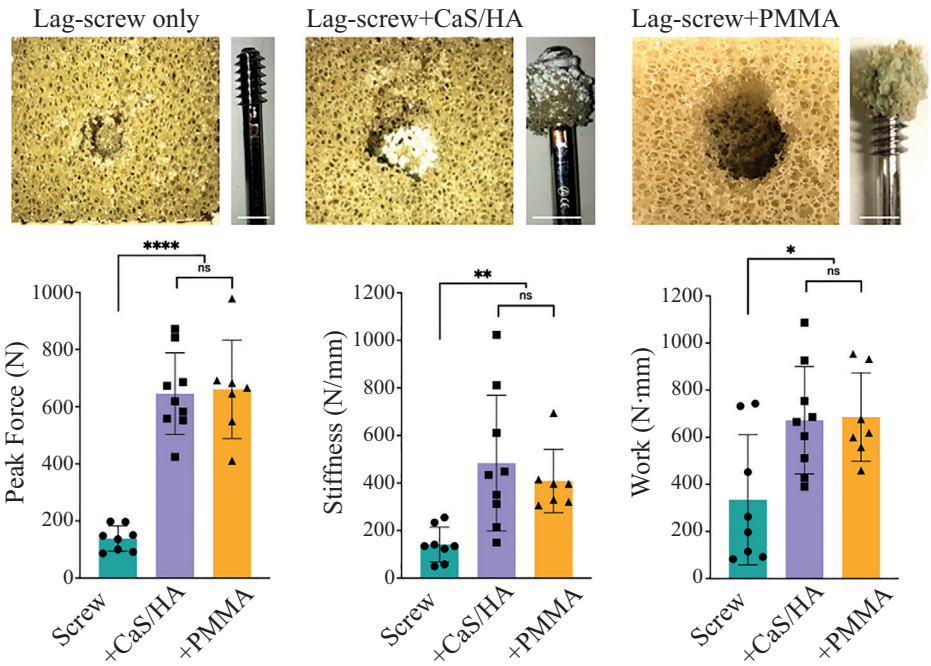

Fig. 4.1.1.1.1. *Mechanical effects of CaS/HA and PMMA augmentation on lag-screw anchorage in Sawbones (Top) Shows photographs of the Sawbones block and the lag-screw after the pull-out test. Following pull-out testing, see the CaS/HA material spreading throughout the pre-drilled hole and the presence of both CaS/HA and PMMA biomaterial-Sawbones composite on the lag screws (Bottom).*

No difference was observed in any of the measured parameters between the CaS/HA and the PMMA augmented lag-screw, but the peak force, stiffness,

and work were all significantly higher in the CaS/HA and PMMA augmented lag-screws than in the un-augmented lag-screw ($p < 0.0001$, $p < 0.01$, and $p < 0.05$, respectively) [89].

(Fig. 4.1.1.1.1) [89] Scatter plots depicting the mean peak force needed to remove the screw, the stiffness, and the amount of effort needed before the screws failed in the Sawbones model. ns stands for not significant. *Indicates $p < 0.05$, ** Indicates $p < 0.01$ and **** Indicates $p < 0.0001$. The scale bar shows 1.3 cm.

4.1.1.2. Spreading of the CaS/HA in ex-vivo human bones

The cadaver femoral head's showed a uniform distribution of the CaS/HA biomaterial around the screw threads. Micro-CT pictures made it abundantly evident that the CaS/HA material was present both in the bone around the lag-screw threads and in the area between them. (Fig. 4.1.1.2.1) After removing the lag-screw from the femoral head, the presence of the CaS/HA material was also confirmed [89]. The scale bar shows 0.5 cm.

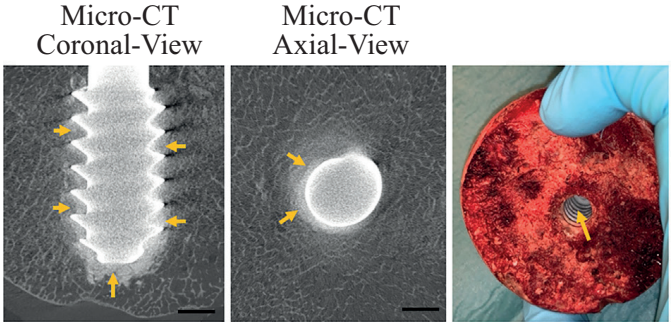

Fig. 4.1.1.2.1. *Micro-CT imaging of a cadaver femoral head and a digital photo of the femoral head after removal of the lag screw revealed CaS/HA spreading across the lag-screw and bone interface.*

4.1.1.3. Spreading of the CaS/HA in-vivo (surgical method)

In both AP and lateral views (Fig. 4.1.1.3.1), radiopaque CaS/HA material was seen at the lag-screw and bone interface as well as the lag-screw's tip once the lag-screw was in final position.

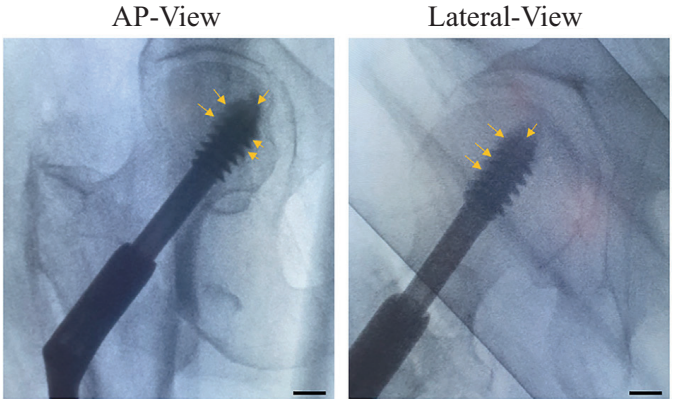

Fig. 4.1.1.3.1. *Patients having pertrochanteric fracture fixation with a sliding lag-screw and a femoral plate experienced CaS/HA spreading around the lag-screw. The radio dense CaS/HA material along the lag-screw-bone interface is indicated by arrows. The scale bar shows 1.3 cm.*

4.1.2. Rat study

4.1.2.1. Implant integration in osteoporotic rats

First and foremost, the osteoporotic rat model was verified by micro-CT imaging and there was a significant loss of cancellous bone in the OVX rats compared with SHAM animals. The bone volume/tissue volume (BV/TV) fraction was significantly lower in the OVX rats when compared with SHAM animals. Furthermore, OVX animals had a significant larger trabecular separation, which is a typical feature of osteoporosis.

In the implant integration study, the animals were divided into four groups; G1) Empty, G2) Un-augmented implant followed by systemic administration with ZA@2-weeks, G3) CaS/HA followed by systemic ZA@2-weeks and G4) CaS/HA without systemic ZA. Using micro-CT imaging, we could demonstrate that the BV in the proximal region of interest was significantly higher in the CaS/HA groups, irrespective of the ZA treatment. However, CaS/HA+systemic ZA group had a significantly higher BV compared with group 2. No difference in BV between G2 and G4 could be observed. In the distal ROI, both CaS/HA groups had significantly higher BV than groups G1 and G2. Micro-CT based calculation of BV relies on x-ray adsorption by the material and consequent gray scale values and therefore it is challenging to separate the living bone from the CaS/HA material.

From the qualitative CT images (Fig. 4.1.2.1.1), it was quite evident that G3 had large amounts of peri-implant cancellous bone while G4 only showed

the presence of resorbing CaS/HA material. This finding was further verified by performing resin embedded histology of the bone containing the implants.

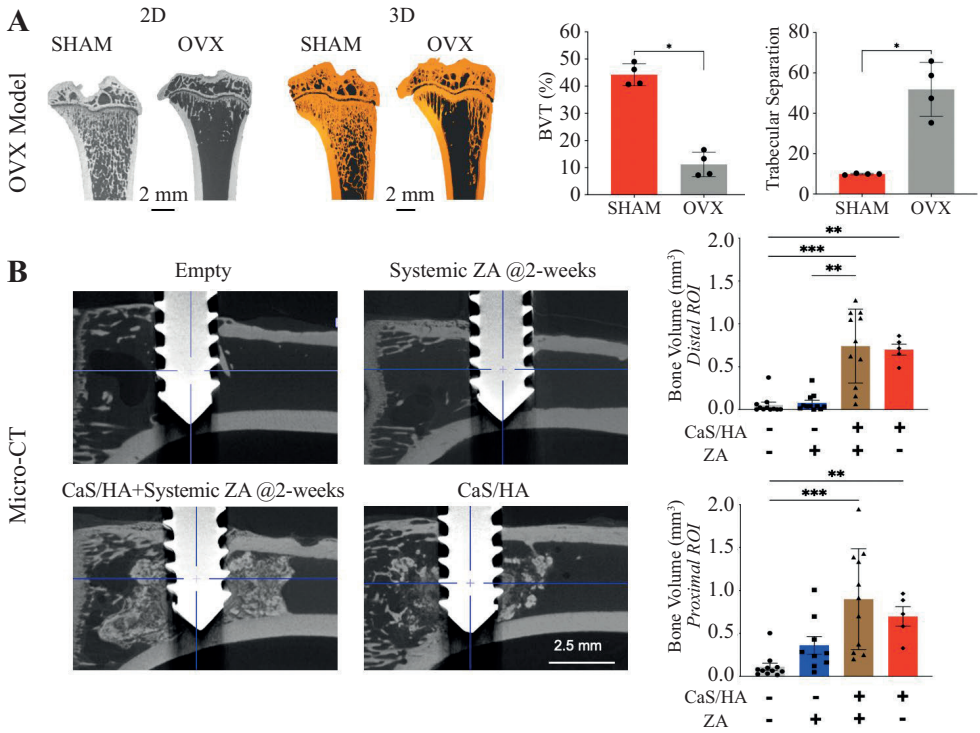

Fig. 4.1.2.1.1. *Development and characterization of an osteoporotic rat model and the evaluation of implant integration. A) Shows the micro-CT based characterization of the osteoporotic model as 2D slices (left), 3D slices (middle) and quantification of important bone parameters (right). B) Shows the results from the implant integration model after 6-weeks. Images in the left show representative CT slices from each treatment group while the plots on the right show the micro-CT based bone volume (BV) measured in the area proximal and distal to the screw.*

As evidenced from the histology images (Fig. 4.1.2.1.2), both groups G1 and G3 had minimal bone formation around the screw threads. On the contrary, the screw threads in G3 were filled with bone which extended at least 1 mm outwards from the screw threads. The amount of bone seen in the screw threads of G4 was significantly lower than in G3 and remnants of the CaS/HA material could be observed in close vicinity to the implant.

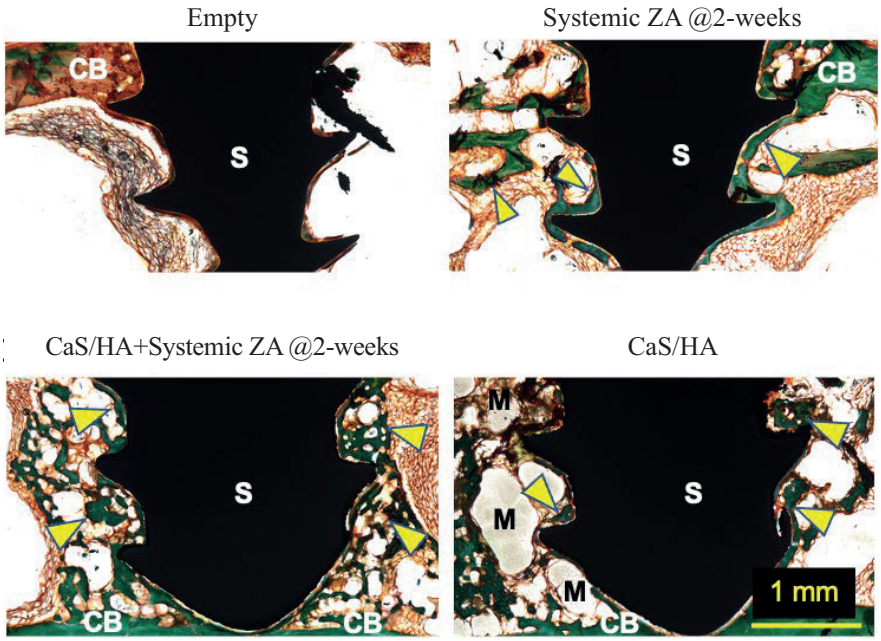

Fig. 4.1.2.1.2. *Resin embedded histology of intact bone with the implant inside. CB indicates cortical bone, S indicates stainless steel screw, M indicates CaS/HA material and the yellow arrows indicate bone.*

4.1.2.2. Timing of ZA injection and ZA absorption in the CaS/HA material

In this rat study, CaS/HA material was implanted in the right tibia of all experimental animals while the left tibia did not contain any material. At different time points (day 1, 3, 7 and 14), the animals received one systemic injection of fluorescent ZA and the animals were sacrificed 24 h after ZA injection. We also used a control group of animals wherein a hole was drilled but was left empty. The animals in the control group also received fluorescent ZA. After sacrifice, the tibiae from all animals were harvested and imaged using an in-vivo imaging system.

In all experimental animals (Fig. 4.1.2.2.1), the uptake of the FZA was significantly larger in the CaS/HA implanted leg when compared to a similar anatomical region in the contralateral leg.

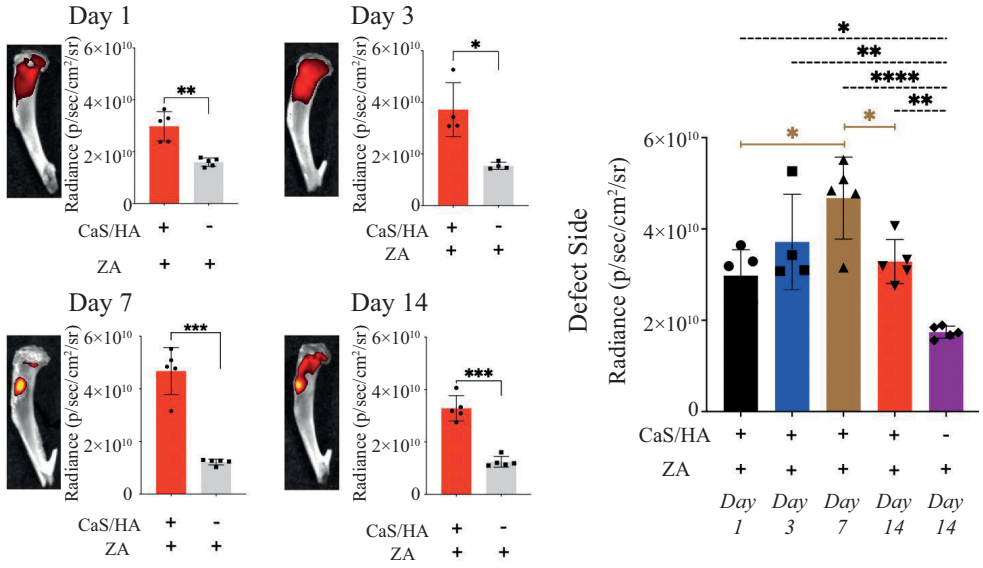

Fig. 4.1.2.2.1*. Biological distribution of systemically administered ZA and its uptake in the CaS/HA material as a function of administration time. Images on the left show the fluorescence from a representative CaS/HA containing tibia at days 1, 3, 7 and 14. The graph next to the fluorescent image shows the uptake of ZA in the CaS/HA implanted leg compared with the contralateral leg that did not contain any CaS/HA material. Finally, the graph on the right shows the total ZA absorption in the defect leg of all experimental animals compared with the control as well as with time.*

All CaS/HA treated experimental animals had a significantly higher uptake of ZA when compared to the control animals that did not receive the CaS/HA material. Finally, when comparing the CaS/HA treated animals as a function of time, the uptake of ZA in the animals that received the injection at day 7 was higher than the animals that received the ZA injection on day 1 or day 14. No statistical differences were observed in animals that received ZA on day 3 or 7.

4.1.2.3. Effect of injection timing on peri-implant bone formation

A total of 35 rats were divided into five experimental groups (n = 7/ group) as follows; G1) CaS/HA, G2) CaS/HA+ Systemic ZA on day 1, G3) CaS/HA+ Systemic ZA on day 3, G4) CaS/HA+ Systemic ZA on day 7 and G5) CaS/HA+ Systemic ZA on day 14. At the predetermined time points, ZA (0.1 mg/kg) was subcutaneously injected into each animal in groups G2–G5.

Animals were sacrifice after 6-weeks and the analysis of peri-implant bone formation was performed using micro-CT.

It was evident from the micro-CT images (Fig. 4.1.2.3.1) that G1 without systemic ZA did not lead to much cancellous bone formation around the implant and could be seen as a chunk of resorbing CaS/HA material in the marrow cavity.

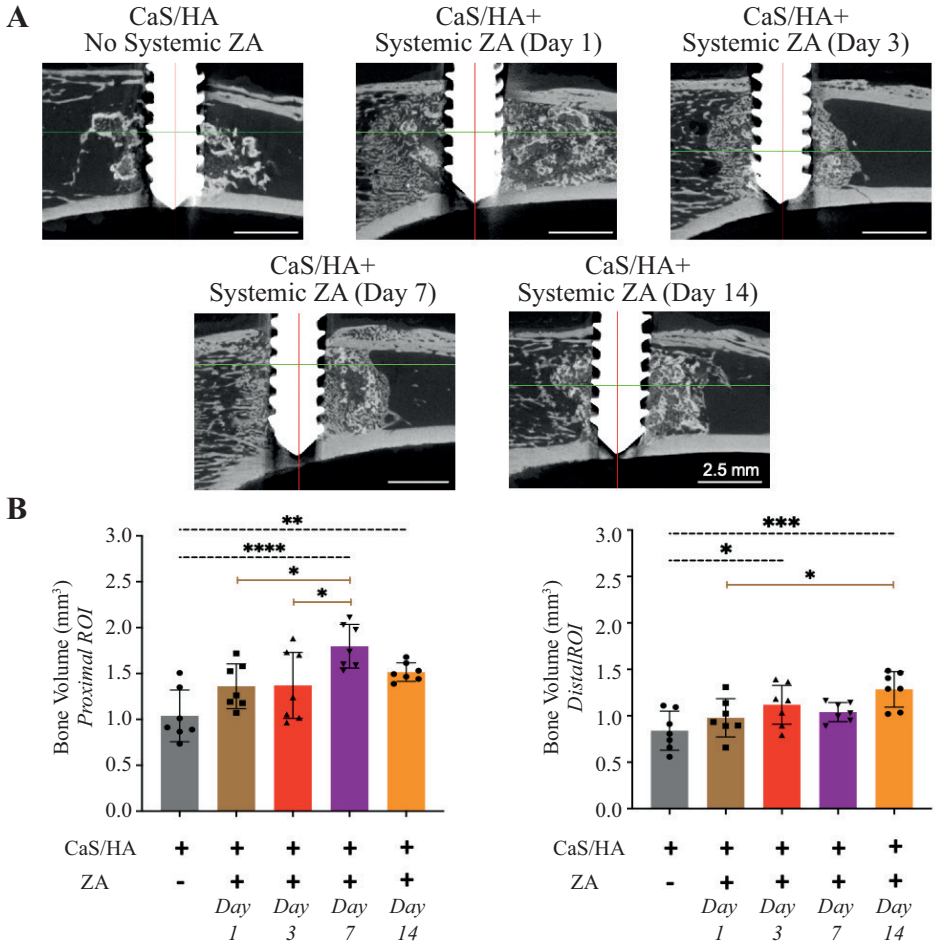

Fig. 4.1.2.3.1. *Micro-CT based analysis of peri-implant bone formation to evaluate the effect of ZA administration time on peri-implant bone formation.*

In the proximal ROI, compared to the control group G1 (no ZA), groups G4 (ZA at day 7) and G5 (ZA at day 14) had a significantly larger bone volume (BV) while the BV in groups G2 and G3 was not different than the

BV in G1. Comparing all groups that received ZA, only group G4 (ZA at day 7) had a greater BV than groups G2 and G3.

In the distal ROI, compared to the control group G1 (no ZA), groups G3 (ZA at day 3) and G5 (ZA at day 14) had a significantly larger bone volume (BV) while the BV in groups G2 and G4 was not different than the BV in G1. Comparing all groups that received ZA, only group G5 (ZA at day 14) had a greater BV than group G2.

Based on the micro-CT data, we can conclude that the optimal time for ZA administration in order to biologically activate peri-implant CaS/HA particles is between post-operative days 7 and 14 in this model.

4.1.2.4. Biomodulation of other calcium phosphate materials such as β-tricalcium phosphate

In this sub-study, we aimed to evaluate whether other calcium phosphate materials, more specifically β-tricalcium phosphate (β-TCP) could also biologically activated by systemically circulating ZA. We repeated the same implant integration model and administered ZA one week post implant placement.

Using micro-CT we could successfully demonstrate that even β-tricalcium phosphate could be biologically activated using a systemic administration of ZA, which therefore expands the choice of resorbable materials that can be used for improved implant anchorage.

4.2. A study for identifying patients with a high risk of bone fractures who are most likely to benefit from bone augmentationt surgeries using the FAME index

In the first study [28] we included 100 hip fragility fracture patients (22 males, 78 females; mean age 78.9 years; range, 45 to 100 years). 50 patients from each institution had data available, and there was no difference in the distribution of patients across the various FAME Index categories between the centers (Table 4.2.1). Patients from the two centers did not differ statistically significantly in terms of gender, age, or weight. The patients were divided into nine subgroups using a combination of FRAX and Sernbo score questions. The subgroup of the FAME index that potentially would benefit the most from cancellous bone augmentation following internal fixation of a fragility hip fracture accounted for 18 % of all patients and had a high fracture risk and low mortality risk (Table 4.2.2).

Table 4.2.1. *Demographic comparisons between the facilities' patient populations*

		Turkey			Lithuania			
		n	**Mean**	**95 %**	**n**	**Mean**	**95 %**	**p**
Gender	Male	9			13			0.3
	Female	41			37			
Mean age (year)			78	74.9–81.2		79.7	77.2–88.3	0.4
Mean weight (kg)			65.3	61.5–69.2		67.2	62.7–71.7	0.5
Mean height (cm)			159.6	157.5–161.7		164.9	162.5–167.3	0.001

Table 4.2.2. *Classification of patients at both centers based on the FAME index*

	Sernbo Low			Sernbo intermediate			Sernbo High			Total		
	Total	**TR**	**LT**	**Total**	**TR**	**LT**	**Total**	**TR**	**LT**	**Total**	**TR**	**LT**
Frax Low	24	13	11	5	3	2	2	1	1	31	17	14
Frax intermediate	24	7	17	2	1	1	5	2	3	31	10	21
Frax high	18	9	9	10	9	1	10	5	5	38	23	15
Total	66	29	37	17	13	4	17	8	9	100	50	50

In the second our study [29] we included 94 patients (20 males, 74 females; mean age: 79.3±8.9 years; range, 57 to 100 years). The identification of refractures (reoperations, implant failure, and new fragility fractures) and death at one year involved reviewing hospital records. The patients were categorized into low, medium, and high-risk groups based on the FRAX and the Sernbo classification, yielding nine combined categories according to the FAME index.

In comparison to other categories, FRAX-H was substantially related with greater mortality and refracture rates (Table 4.2.3) and (Table 4.2.4). The overall rates of refracture and death were respectively 20.2 % and 33 % [29]. Statistical significance at $p < 0.05$

Table 4.2.3. *Statistics data for refracturing at one year*

	No re-fracture			Re-fracture			All					
	n	**%**	**Mean ± SD**	**n**	**%**	**Mean ± SD**	**n**	**%**	**Mean ± SD**	**OR**	**95 % CI**	**p**
Mean age (year)			78.8 ± 9.3			81.3 ± 6.6			79.3 ± 8.9	1.04	1–1.1	0.3
Sex										1	0.3–3.5	0.9
Male	16	80		4	20		20	21.3				
Female	59	79.7		15	20.3		74	78.7				
Hospital of follow up										0.8	0.3–2	0.6
Turkey	34	77.3		10	22.7		44	46.8				
Lithuania	41	82		9	18		50	53.2				
Mean BMI (kg/m^2)			24.8 ± 5.1			26.1 ± 5.5			25 ± 5.2	1.05	1–1.2	0.3
Fracture risk										2.9	1–8.2	**0.037**
Rest of the categories	51	86.4		8	13.6		59	62.8				
FRAX-H	24	68.6		11	31.4		35	37.2				
Mortality risk										0.4	0.2–1.2	0.1
Sernbo-L	54	84.4		10	15.6		64	68.1				
Rest of the categories	21	70		9	30		30	31.9				
FAME category										2.7	0.8–8.6	0.1
FAME - HL	11	64.7		6	35.3		17	18.1				
Rest of the categories	64	83.1		13	16.9		77	81.9				

Table 4.2.4. *Statistics data for mortality at one year*

	Alive			Dead			All					
	n	**%**	**Mean ± SD**	**n**	**%**	**Mean ± SD**	**n**	**%**	**Mean ± SD**	**OR**	**95 % CI**	**p**
Mean age (year)			78.8 ± 9.4			81.3 ± 7.4			79.3 ± 8.9	1.04	1–1.1	0.2
Sex										3.5	1–12.8	0.054
Male	17	85		3	15		20	21.3				
Female	46	62.2		28	37.8		74	78.7				
Hospital of follow up										0.9	0.4–2.2	0.8
Turkey	29	65.9		15	34.1		44	46.8				
Lithuania	34	68		16	32		50	53.2				
Mean BMI (kg/m^2)			24.8 ± 5			25.5 ± 5.8			25 ± 5.2	1.03	1–1.1	0.6
Fracture risk										3.7	1.5–9.3	**0.003**
Rest of the categories	46	78		13	22		59	62.8				
FRAX–H	17	48.6		18	51.4		35	37.2				
Mortality risk										0.4	0.2–1.02	0.053
Sernbo–L	47	73.4		17	26.6		64	68.1				
Rest of the categories	16	53.3		14	46.7		30	31.9				
FAME category										1.1	0.4–3.4	0.8
FAME – HL	11	64.7		6	35.3		17	18.1				
Rest of the categories	52	67.5		25	32.5		77	81.9				

4.3. A randomized controlled study to evaluate dynamic hip screw system augmented with a CaS/HA combined with systemic bisphosphonate for trochanteric femur fracture

We included 20 patients in our clinical study: control group n = 10, CaS/HA -group n = 10. 7–14 days following surgery, all 20 patients received systemically administered ZA (4 mg). We have already completed the full six-month follow-up for 14 patients: CaS/HA group n = 8 and control group n = 6. Six patients' follow-up was lost: control group n = 4, CaS/HA group n = 2. Two patients in the control group died, and 4 patients did not return for further treatment. Based on the FAME index, all included patients were with a high risk of fracture but a low risk of death. For all patients who completed their follow-up care, n = 14 (CaS/HA = 8, control = 6), we compared the DEXA results in both groups (Table 4.3.1). Patients in the CaS/HA group had improved outcomes in all areas (R1–3), and the R1–3 average was 1.12 times higher compared to the control group.

No complications (loosening, infection, mechanical) were observed in any of the patients in either group.

Table 4.3.1. *CaS/HA group (left) and control group (right) DEXA results*

CaS/HA group		
R1 (down)	R2 (up)	R3 (front)
0.708	0.753	1.077
0.849	0.591	1.317
1.425	1.249	1.586
1.493	1.238	1.602
0.922	0.717	0.992
0.965	1.367	1.408
1.017	1.009	1.484
1.629	1.155	1.992
Average: 1.126	Average: 1.009875	Average: 1.43225
R1+2+3	1.208368421	

Control group		
R1 (down)	R2 (up)	R3 (front)
0.814	0.935	0.996
0.79	0.86	0.909
1.027	0.83	1.139
1.406	1.02	1.846
1.306	1.052	1.129
1.066	1.064	1.279
Average: 1.0682	Average: 0.96	Average: 1.216
R1+2+3	1.081555556	

5. DISCUSSION

Fracture augmentation should be considered in patients with osteoporosis with focus on unstable trochanteric fractures. The main benefits of augmentation include shorter hospital stay, lower reoperation rates and enhanced mobility [56]. In clinical studies Plang et al. [92], Chang et al. [93], Dall'Oca et al. [52], and Goodnough et al. [94] reported that patient with trochanteric fracture who had augmentation had better functional results, and improved health-related quality of life.

A standardized surgical method to augment fixation devices and the subsequent improvement of the immediate mechanical anchorage of fixation devices can be beneficial and aid in preventing fixation failure. The long-term success can be increased if the implanted material transforms into living bone. Additionally, it was suggested in cost-utility models based on a RCT that treating unstable trochanteric fractures with cement augmentation was more affordable and effective compared to no augmentation [87].

One of the risks associated with bone augmentation regardless of the type of material used, is the potential leakage of the delivered material into the joint, which could be controlled with fluoroscopy, specialized injection techniques using a delivery device.

However, there appears to be a lack of commonly acknowledged consistent surgical methods for the application of an augmentation material at the bone-implant interface. Previous studies have indicated:

- prefilling of the entire reamed canal in the femoral head with PMMA before inserting the fixation device [53, 95];
- injecting CaP into entire reamed lag-screw canal [50];
- injecting CaP into smaller canal parallel to the reamed lag-screw canal [50];
- injecting CaP through fenestrated lag-screw [50, 51, 55, 95].

Therefore, in the case of trochanteric fractures, neither the volume of the augmentation material to be used is standardized nor is the surgical technique.

Kok et al. [96] further investigated if addition of CaS/HA could increase the fixation of a DHS. This study was divided into two parts. In the first part, synthetic bone blocks in three subgroups were investigated: cannulated empty, cannulated, and fenestrated with injected material. Synthetic bone blocks used were of low- and high-density mimicking osteoporotic and healthy bones, respectively. In the second part, femoral heads were harvested from patients that were undergoing hip replacement surgery. The same three subgroups as for the synthetic bone blocks were used. In both synthetic bone blocks and the femoral heads cannulated groups, the DHS was partly inserted, and the

CaS/HA material (2 mL) was injected whereafter the screw was further inser-ted to its final position. In the fenestrated groups the DHS was inserted completely whereafter the CaS/HA material was injected. Bone volume fraction, insertion angle, and head diameter were measured. Pull-out tests were performed, and peak force, stiffness, and the work to peak force were measured. The approach of injecting a CaS/HA biomaterial through a cannulated screw led to a 46 % strength increase in the low-density synthetic bone and 65 % in the high-density bone. In the tests with the synthetic bone blocks, it was shown that the injection of CaS/HA through the fenestrated screw resulted in the highest increase in pull-out strength compared to no injection in both low-density (103 % increase) and high-density (72 % increase) material. In the test with human femoral heads, no differences were observed between the groups.

In one of our studies, we repeated the experiments performed by Kok et al. [96] and the novel method to deliver the CaS/HA biomaterial for augmenting lag-screws was described. Osteoporotic sawboneses were used for the mechanical testing to verify the immediate increase in the anchorage of the lag-screws using the suggested technique. The mechanical testing indicated that the CaS/HA based biomaterial increased the peak extraction force of the lag-screw by 4 times compared with un-augmented lag-screws. No difference was observed in any of the measured parameters between the CaS/HA and the PMMA. X-ray images from the patient series showed that it was possible to inject the CaS/HA material in the predrilled void without applying additional pressure and the CaS/HA material spreading was observed at the interface of the lag-screw threads and the bone. The spreading of the CaS/HA material based on micro-CT imaging indicated that the entire length of the lag screw threads was covered with the CaS/HA biomaterial.

PMMA has drawbacks, such as exothermic effect, a high elastic modulus, and high compressive strength [97]. Other resorbable materials such tricalcium phosphate have also been used for instance, in distal radius and tibial plateau fractures, however, clinical follow-up radiographs show essentially no resorption or remodelling, with the material still being present largely unchanged after 30 months in the case of tibial plateau fractures [98]. A downside of using calcium phosphate cement in clinical settings is that it has lower initial mechanical strength than PMMA and possible imbalance between bone ingrowth and biodegradation [99].

Lyles et al. [77] in a randomized study found a relationship between a yearly infusion of zoledronic acid within 90 days of the healing of a low-trauma hip fracture and a decreased incidence of future clinical fractures and increased survival. The risk of death was found to be 28 % lower in the zoledronic acid group. In the ZONE study [100] authors demonstrated a lower risk of

new vertebral fractures after a yearly intravenous ZA infusion. The BMD was increased at all femoral regions in this study, including the femoral neck, intertrochanteric region, and shaft, when zoledronic acid was given [101]. Additionally, it enhanced the cortical width determined by CT scans (CT data was gathered at baseline, 12 and 24 months later) [101].

A number of studies has been done on ZA administration and fracture healing, but not a lot is known about the timing of ZA infusion and the impact on augmentation and implant integration.

In the clinical studies Kim et al. [102] prospective pilot study, Gao et al. [103] performed a meta-analysis involving over 5000 patients, Colon-Emeric et al. [104] in randomized – the HORIZON Recurrent Fracture trial, Jalan et al. [105] in randomized trial, Duckworth et al. [106] in randomized trial – reveals no timing-related differences. Amanat et al. [107] demonstrated that a single systemic dosage of ZA administered at the appropriate time is critical for controlling the callus characteristics in a pre-clinical rat fracture model. In comparison to the control group, mechanical strength increased by 30 % after receiving an injection of ZA at the time of fracture. The systemic dose given 1 or 2 weeks after fracture increased mechanical strength by 44 % and 50 %, respectively, compared to control group.

The pre-clinical investigations demonstrate a stronger fracture callus when ZA is administered at later time periods. Due to the apparent limitations of not being able to undertake a destructive test to examine peri-implant bone, such as high-resolution CT, biomechanical testing, and histology, clinical data on this phenomenon is sparse. Our study demonstrated that timing is important, and that the two highest ZA uptakes were observed after 7 (highest) and 14 days (second highest). Therefore, this was a major factor in our decision when to administer ZA in the clinical RCT study.

Raina et al. [82] evaluated the in-vivo release of rhBMP-2 and ZA from CaS/HA and investigated peri-implant bone regeneration using an implant integration model in a tibia defect model. The implant with CaS/HA and local ZA group, displayed a pull-out force that was 44 % higher and an energy absorption that was 84 % higher compared to the empty group. Also, Raina et al. [90] investigated whether a systemically administered ZA could seek the CaS/HA placed around a PEEK implant in a rat model. Peak force and peri-implant bone volume were significantly larger in the implant containing HA particles than in the empty implant. They further demonstrated bisphosphonate ZA's ability to induce bone formation in cancellous bone. In a clinical setting, this combination could potentially be equally efficient or superior to present techniques for augmenting osteoporotic trochanteric fractures. When comparing local ZA and CaS/HA with systemic ZA and CaS/HA, the biggest differences are the regulatory hurdles when locally combining two approved

products. Currently there are no regulatory approval for incorporation of ZA in CaS/HA material, – however both systemic ZA and local CaS/HA in combination with fracture fixation are approved, and are widely used. Based on the existing approvals, there should be no major regulatory barriers for implementing the developed technique for fragility fracture treatment.

Taken together, our in-vivo, and ex-vivo biomechanical studies have demonstrated that implants augmented with CaS/HA material in osteoporotic bone enhances pull-out force and that activation of HA particles by systemically administered ZA leads to new bone formation.

It is not clearly explained why bone regeneration are almost similar even though it is clear from the fluorescence study that time is crucial and that the two highest ZA uptakes were shown after 7 and 14 days. We agree that the largest ZA uptakes occurred at a certain time, but it's possible that to activate the biomaterial, a lower ZA dose would be enough which is not shown by the fluorescence analysis due to limitations in the sensitivity of the method. It indicates that there may be possibilities to provide systemic ZA immediately following surgery so that the patient won't need to stay in the hospital for 7–14 days, or that we may be able to use lower dose of ZA giving less adverse drug reactions.

The least risk of patient harm is necessary to translate the preclinical findings into clinical practice. Two studies have been performed to investigate the feasibility of a new method to identify patients that would benefit most from augmentation [28, 29]. A standardized automated digital approach employing QCT may also be used to create the stratification of hip fractures, which would indicate which sort of fixtion device or augmentation is optimal for certain hip fracture patients [28]. Arguments can be made that patients with high fracture risks and low mortality risks would benefit from an augmentation operation far more than those with low fracture risks and high mortality risks [28]. We presented evidence supporting the viability of a FRAX-SERNBO combined into a FAME index when utilized in a clinical emergency environment, and we provided a list of patients distributed across three risk groups using either tool.

By utilizing a simple form, the FAME Index was successfully applied in the acute setting before the operation, during history taking by well-informed medical staff in less than 10 minutes [28].

According to study by Sezgin et al. [28] the FAME index subgroup that potentially would benefit the most from cancellous bone augmentation following internal fixation of a fragility hip fracture accounted for 18 % of all patients, with high fracture risk and low mortality risk. These two studies have been the base in identifying patients that would benefit most from augmentation [28, 29].

As failure of internal fixation of fractures in osteoporotic bone typically occurs through breakage of the bone that surrounds the implant, rather than the implant itself, our strategy includes augmentation of the bone in the vicinity of the hip screw in patients with trochanteric fractures. Kim et al. [54] investigated augmentation to increase the stability of osteoporotic hip fractures and found intertrochanteric fractures treated with a PFN were much more stable after being reinforced with calcium phosphate cement, which also decreased the failure rates overall. Clinical studies from Mitsuzawa et al. [108], or Fernandez et al. [109] observed no complications such as cut-out or cut-through in any of the patients in the augmentation group.

Iwata et al. [110] investigated whether the use of HA tubes affects the intraoperative insertion torque of lag screws during intertrochanteric femoral fracture surgery. A positive correlation was observed between BMD and mean and maximum torque in both groups. The HA tubes did not affect the mean and maximum intraoperative insertion torque of the lag screw. The mean torque/BMD ratio was significantly higher in the HA group than in the control group. The amounts of telescoping of the lag screw in the HA group were significantly lower than that in the control group.

Based on the pre-clinical data and limited clinical studies it could be concluded that our approach fulfilled requirements to start a study on both immediate mechanical anchorage and bone forming potential.

Therefore, the next step was a limited clinical RCT (ClinTrials.gov NCT04498715), to confirm the performance, both regarding enhanced implant anchorage and bone formation. In this study, 20 patients with high fracture risk and low mortality based on the FAME index, undergoing treatment for trochanteric fracture with a DHS and femoral plate were included and randomized into two groups (CaS/HA+Systemic ZA or only systemic ZA). 20 patients have been included. Bone density and remodelling around the screw are investigated by CT scan at 1–3 days, 3 and 6 months and DXA scan at 6 months.

Results from this study show that the peri-implant bone density increases but due to the small size of the study, we cannot comment on clinically important efficacy, - Ideally, however if we could reduce the reoperation of unstable fractures from 6–7 %, with 50 %, with our method combining local augmentation with systemic pharmacological biomodulation this will be a significant advantage for the patient and the healthcare system. It is estimated that a sample size of 1000 patients per group adequately powered would be required. A nested study within a national registry on hip fractures are now planned.

CONCLUSIONS

1. We demonstrate an optimised surgical method to apply a CaS/HA material (or any other injectable material) at the interface of a lag-screw and osteoporotic bone without the use of addition pressure or material leakage in the surrounding vasculature.
2. Pre-clinical data demonstrated that biomechanical advantages can be achieved from CaS/HA augmentation of lag-screws in osteoporotic bone blocks. Peak force, stiffness, and work were all significantly higher in the CaS/HA augmented lag-screw compared to the unaugmented lag-screw.
3. Using pre-clinical animal model data, we demonstrate that CaS/HA material around a metallic implant can be biologically activated by a systemic injection of ZA. Biological activation leads to new cancellous bone formation around the implant.
4. Although the timing of bisphosphonate infusion appears to be important for the overall uptake of ZA in the CaS/HA material, no large effects of timing could be observed on the overall peri-implant bone formation. We verify that the material is biologically activated when ZA is injected within 1–2 weeks of operation as seen by an increase in peri-implant bone mineral density.
5. Apart from the combination of CaS/HA, other calcium phosphate materials, in particular, beta tricalcium phosphate can be bioactivated with systemically administered ZA.
6. FAME index, a tool for identifying patients who are most likely to benefit from augmentation surgeries and have a high risk of fracture but low risk of death.
7. In a proof-of-concept clinical trial in patients undergoing trochanteric fracture fixation using a dynamic hip screw system, we demonstrate the feasibility of injecting the CaS/HA material for augmentation. To show real clinical benefits of the developed approach, a larger clinical study with different fracture fixation devices such as PFN and gamma nail is required.

SUMMARY OF FINDINGS AND CLINICAL SIGINIFICANCE

In brief, we describe a surgical procedure that is designed to apply material to an implant without the requirement for extra pressure or material leakage.

Our research on rats and a review of other authors' studies on the CaS/HA material demonstrated that the CaS/HA biomaterial based strengthening of weak cancellous bone will result in a composite that is biomechanically stronger.

In both pre-clinical and clinical research, we show that postoperative biomodulation, which involves administering a systemic bisphosphonate ZA that seeks for and biologically activates the calcium-enriched HA, will stimulate bone growth around the implant.

From the clinical point of view, the creation of FAME index could allow doctors to identify patients who are most likely to benefit from augmentation treatments.

The main clinical significance of our research is that it clearly demonstrated that the benefits of bone augmentation for the patients would include shorter hospital stay, lower reoperation rates and enhanced mobility. A standardized surgical method to augment fixation devices and the subsequent improvement of the immediate mechanical anchorage of fixation devices can be beneficial and aid in preventing fixation failure. The long-term success can be increased if the implanted material transforms into living bone.

FUTURE WORK

We think that our approach, which combines local augmentation with systemic pharmaceutical biomodulation, can reduce the current reoperation rates (6–7 %) by 50 %. However, it is necessary to conduct a larger clinical trial, with a sample size of at least 1000 patients in each group.

Before beginning the use of systemic ZA and local CaS/HA for trochanteric fracture, we must do more feasibility clinical trials using other devices, such as PFN and Gama nails.

Clinically widespread use of systemic ZA and local CaS/HA in combination with fracture fixation requires more studies conducted at other anatomical locations, such as the proximal tibia and distal radius etc.

One more clinical study using a lower ZA dose to evaluate whether using less ZA dosage can activate the bone substitute.

Artificial intelligence may be able to predict mortality and fracture risks for the next five years and select the best surgical and systemic therapy options. It's feasible that artificial intelligence may be able to detect the possibility of a hip or other body part breaking easily, allowing us to administer preventative augmentation before a fracture occurs.

By the time we're done, we have a variety of materials, each with advantages and disadvantages. I believe it is conceivable for artificial intelligence to select the optimum method of augmentation and surgical procedure for the patient.

SANTRAUKA

ĮVADAS

Pasaulio sveikatos organizacija (PSO) osteoporozę apibrėžia, kaip skeleto sistemos ligą, kuriai būdingas mažas kaulų tankis bei kaulinio audinio mikroarchitektūros blogėjimas, dėl ko padidėja kaulų trapumas ir didėja lūžių tikimybė [1]. Osteoporozės ir nepakankamumo lūžių atvejų skaičius su amžiumi didėja, o didėjantį šių atvejų kiekį tik iš dalies sustabdo pirminės ir antrinės prevencinės programos. Osteoporoze jau dabar serga daugiau nei 200 milijonų žmonių, o iki 2050 numatomas šio skaičiaus padvigubėjimas [2]. Tai yra dažniausiai nustatoma metabolinė kaulų liga visame pasaulyje [3]. Remiantis naujausiais statistiniais duomenimis, viena iš trijų vyresnių nei 50 metų moterų ir vienas iš penkių vyresnių nei 65 metų vyrų patiria lūžį dėl osteoporozės, tai įrodo, kokio masto problema tai yra viso pasaulio sveikatos priežiūros sistemoje [4]. Prognozuojama, kad dėl visuomenės senėjimo per ateinantį dešimtmetį lūžių skaičius dėl osteoporozės toliau tik didės.

Dinaminė pusiausvyra tarp kaulų susidarymo ir rezorbcijos yra būtina normaliam kaulų metabolizmui [1]. Senėjimas, estrogenų trūkumas ir ilgalaikis nejudrumas yra tik keli veiksniai, keičiantys įprastą apoptozę, autofagiją ir uždegimą. Šie faktoriai lemia, kad atsiranda neįprastas osteoklastų aktyvumas, kuris skatina kaulų rezorbciją ir slopina naujo kaulinio audinio formavimąsi [1]. Osteoporozės rizikos veiksnius galima suskirstyti į modifikuojamus (svoris, rūkymas, alkoholio vartojimas, fizinio aktyvumo stoka, kalcio trūkumas maiste ir ilgalaikis gliukokortikoidų vartojimas) ir nemodifikuojamus (lytis, amžius, rasė ir genetinės savybės) [5].

Osteoporozę taip pat galima suskirstyti į du tipus pirminę ir antrinę. Pirminė – pasireiškia abiem lytims, bet kuriame amžiuje, tačiau dažnausia pasireiškia moterims dėl menopauzės, o vyram pasireiškia vėliau. Antrinė – kitų veiksnių – vaistų (pvz., gliukokortikoidų), ar kitų medicininių būklių ir ligų pasekmė [6]. Osteoporozė diagnozuojama remiantis paciento ligos istorija, kruopščia fizine apžiūra, FRAX indeksu, krūtinės ląstos ir juosmeninės stuburo dalies rentgenogramomis, kaulų mineralinio tankio (KMT) nustatymu ir laboratoriniais tyrimais [4, 8]. Klinikinėje praktikoje osteoporozei gydyti dažniausiai naudojama farmakologinė terapija, tokia kaip kalcis, vitaminas D, pakaitinė hormonų terapija, bisfosfonatai ir kt. [1].

Dažnas ligos pasireiškimas yra ryškus KMT sumažėjimas ir trabekulinio kaulo poringumo padidėjimas [6]. Dėl to lūžių fiksacija įprastomis priemonėmis, kaip sraigtai – osteoporoze sergantiems pacientams tampa iššukiu kasdieninėje ortopedų praktikoje.

Kaip hipertenzija bei didelis cholesterolio kiekis yra insulto ir širdies ligų rizikos veiksniai, atitinkamai osteoporozė yra pagrindinis lūžių rizikos faktorius [3]. Neretai ligos pradžia yra asimptominė, kitaip dar vadinama „tyliąja“ osteoporoze. Neretai osteoporozė nustatoma atsiradus pirmiesiems lūžiams, kurie sukelia papildomus sunkumus sveikatos priežiūros sistemai [9]. Tipiniai osteoporotiniai lūžiai yra stuburo slankstelių kompresiniai lūžiai, šlaunikaulio proksimalinės dalies lūžiai, proksimaliniai žastikaulio ir distaliniai stipinkaulio lūžiai – laikomi osteoporozės „indikatoriniais lūžiais“ [10]. Tikėtina gyvenimo trukmė po šlaunikaulio proksmalinės dalies lūžio sutrumpėja beveik 25 proc., lyginant pagal amžių ir lytį atitinkančią populiaciją.

Maždaug 20 proc. pacientų, po šlaunikaulio proksimalinės dalies lūžio, reikalinga ilgalaikė slauga, o net 60 proc. nebegrįžta į prieš lūžį buvusią fizinę būklę [11]. Pacientai po lūžio susiduria su judėjimo apribojimu, varginančiu skausmu bei sumažėjusia autonomija, ko pasekoje išsivysto psichosocialinės problemos, tokios kaip liūdesys, žema savivertė, motyvacijos reabilitacijai stoka [3].

Net jei lūžis ir yra didelės energijos traumos pasekmė, dauguma vyresnio amžiaus žmonių lūžių, vistiek iš dalies yra sumažėjusios kaulų masės rezultatas [3]. Apskaičiuota, kad beveik 2,5 milijono žmonių kasmet patiria proksimalinės šlaunikaulio dalies lūžį ir numatoma, kad 2050 m. šis skaičius pasieks 5 milijonus [2, 12]. 1 metų mirtingumas po šlaunikaulio lūžio yra 8,4–36 % didesnis lyginant su bendra populiacija, o vyrų mirtingumas yra didesnis nei moterų [13].

Darbo naujumas ir aktualumas

Osteoporozė yra dažna liga, kuriai būdinga maža kaulų masė su kaulinio audinio mikroarchitektūros pokyčiais bei padidėjęs kaulų trapumas. Komplikacijų dėl nepakankamos fiksacijos rizika yra didesnė, kuomet yra prastesnė kaulinio audinio kokybė. Taip pat įtakoja su operacija susiję veiksniai, kaip prastesnė akytojo kaulo kokybė aplink implantą arba nepakankama repozicija ar nepakankamai tiksli fiksuojančio implanto vieta. Pakartotinė operacija vyresnio amžiaus pacientui pablogina gyvenimo kokybę ir svarbiausia, padidina ankstyvos mirties riziką. Literatūros duomenimis, pakartotinės operacijos rizika yra 3–7 proc., priklausomai nuo lūžio tipo [14, 15]. Taip pat literatūros duomenimis, apie pooperacinę infekciją yra pranešama maždaug 2 proc. atvejų [16, 17].

Pooperacinės komplikacijos, kaip pakartotiniai lūžiai, dažniausiai yra susijusios su fiksacija, kurią pablogina osteoporotinis kaulas, o ne pats implantas, todėl pacientams, su trochanteriniais lūžiais turėtų būti svartoma augmentacijos galimybė siekiant stabilizuoti implantą.

Vis dėlto, šiuo metu nėra standartizuotas nei reikalingas medžiagos tūris augmentacijai, nei operacinė technika jos implantavimui. Mūsų pagrindinis tikslas yra sukurti naują augmentacijos metodą, kuris pagerintų sraigto fiksaciją kaule, tam pasitelkiant kalcio sulfato/hidroksiapatito (CaS/HA) medžiagą.

Iki šiol sisteminiai bisfosfonatai nebuvo naudojami, kaip biomedžiagos aktyvatoriai, skatinantys naujo kaulo formavimąsi.

Pagrindiniai studijų metu keliami klausimai:

1. Ar CaS/HA medžiaga aplink metalinį implantą, gali padidinti tiesioginį mechaninį implanto tvirtinimasi su kaulu?
2. Ar CaS/HA medžiagą gali biologiškai aktyvuoti sistemiškai vartojami bisfosfonatai?
3. Kaip efektyviausiai atrinkti pacientus, sumažinant riziką pacientams iki minimalios, siekiant perkelti ikiklinikinio tyrimo rezultatus į klinikinį tyrimą?
4. Koks yra sukurto metodo klinikinis efektyvumas gydant pertrochanterinius lūžius labiau pažeidžiamoje ir vyresnio amžiaus žmonių populiacijoje?

TIKSLAS IR UŽDAVINIAI

Hipotezė

Naudojant naują chirurginę techniką – suleidžiant CaS/HA medžiagą aplink implantą – bus pagerintas mechaninis kaulo tvirtinimas tarp implanto ir kaulo.

Biomedžiaga CaS/HA sutvirtins trapų akytąjį kaulą ir sudarys biomechaniškai stipresnę struktūrą.

Pooperacinė biomoduliacijos metu naudojama zoledroninė rūgštis veikia sistemiškai, surasdama ir biologiškai suaktyvindama kalciu praturtintą HA, to pasekoje padidindama kaulo regeneraciją ir formavimąsi aplink implantą.

Tikslai

Šio darbo tikslas – sukurti metodą, kurį naudojant bus pagerintas mechaninis kaulo tvirtinimas tarp implanto ir kaulo, operacijos metu suleidžiant CaS/HA ir ją biologiškai suaktyvinant sistemine ZA periimplantui moduliuoti.

Uždaviniai

1. Surasti optimaliausią chirurginį metodą suleisti biomedžiagą nenaudojant spaudimo, kad biomedžiaga CaS/HA, kuri padidina tiesioginį mechaninį implanto tvirtinimąsi su kaulu, nenutekėtų į aplinkinę kraujotaką.

2. Ištirti, ar implantuota CaS/HA medžiaga aplink metalinį implantą, gali padidinti tiesioginį biomechaninį implanto tvirtinimasi su kaulu osteoporotinio kaulo modelyje.
3. Ištirti in vivo implantų integraciją ir kaulo regeneraciją bei nustatyti ar CaS/HA biomedžiagą galima biologiškai suaktyvinti sistemiškai suleidus zoledroninę rūgštį (ZA).
4. Nustatyti optimalų ZA suleidimo laiką po operacijos, remiantis kaulo formavimusi aplink implantą.
5. Ištirti, ar kalcio sulfato ir fosfatų deriniai gali būti suaktyvinti.
6. Naujas indeksas – FAME, apjungiantis FRAX ir SERNBO (didelė lūžių ir didelio mirtingumo rizika) indeksus buvo sukurtas siekiant ikiklinikinius duomenis pervesti į klinikinę praktiką. Ištirti FAME indekso pritaikymą klinikinėje praktikoje bei pateikti pacientų įvertintų FAME indeksu vienerių metų stebėjimo rezultatus
7. Patikrinti ikiklinikinių tyrimų rezultatus randomizuotame klininikiame tyrime. Dokumentuoti ir palyginti – klinikinius rezultatus, bei naujo kaulo formavimąsi bei regeneraciją apie sraigta pacientams kuriems buvo skirta sistemiškai ZA ir suleista CaS/HA su pacientais kuriems skirta tik sisteminė ZA. Kaulo remodeliavimas buvo vertinamas naudojant pooperacinę ir 3 mėnesių kompiuterinę tomografiją (KT), bei galutiniam įvertinimui po 6 mėn buvo atliekama kaulų densitomerijos tyrimas (DEXA) ir KT tyrimai. Tyrimai vertinami su neoperuotu klubo sąnariu.

METODAI

Ikiklinikiniai ekspermentiniai tyrimai atlikti Lundo Universitetinės ligoninės Biomedicininių tyrimų cente. Klinikinis tyrimas FAME indekso vertinimui ir validizacijai atlikti Gazi universiteto Medicinos fakulteto Ortopedijos ir traumatologijos klinikoje, Antalijoje, Turkikoje ir Lietuvos sveikatos mokslų univesiteto ligoninėje, Kauno klinikose, Ortopedijos ir traumatologijos skyriuje. Klinikinis randomizuotas tyrimas atliktas Lietuvos sveikatos mokslų universiteto ligoninėje, Kauno klinikose, Ortopedijos ir traumatologijos skyriuje. Ikiklinikiniai ir klinikiniai tyrimai vykdyti 2018–2023 metų laikotarpyje.

Tyrimai atlikti gavus Švedijos žemės ūkio valdybos komiteto leidimą (leidimo nr: 152888/2019.). Kauno universitetinės ligoninės etikos komiteto leidimą, (leidimo numeris: P1 BE–2–76/2019). Tyrimų protokolai parengti vadovaujantis Pasaulio medicinos asociacijos etikos kodeksu (Helsinkio deklaracija). Visi tiriamieji pasirašė informuoto asmens sutikimo formą daly-

vauti tyrime. Tyrimuose su gyvūnais tyrėjai buvo išlaikę Felasa C kategorijos egzaminą moksliniems tyrimams su tiriamaisiais gyvūnais.

Tyrimas šiuo metu įtrauktas į ClinicalTrials.gov (Identifikacijos numeris: NCT04498715).

Tyrimo struktūra

Tyrimas buvo suskirstytas į kelis etapus. Pirmiausia įvertinome mechaninį tvirtumą tarp sraigto ir sintetinio kaulo,– buvo naudojamos dvi skirtingos medžiagos – PMMA ir CaS/HA. Sekančiu etapu išbandėme chirurginę procedūrą ir įvertinome CaS/HA biomedžiagos pasiskirstymą donorinėse osteoporotinių šlaunikaulių galvose, paskutiniu etapu chirurginį metodą išbandėme pacientui su pertrochanteriniu lūžiu. Jam buvo atlikta osteosintezės operacija, kurios metu buvo suleidžiama CaS/HA biomedžiaga.

Antras tyrimo etapas buvo suskirstytas į tris mažesnius eksperimentus, siekiant geriau suprasti, kaip kaulo regeneraciją ir formavimąsi veikia sisteminės ZA ir injekuotos CaS/HA medžiagos kombinacija. Pirmo tyrimo metu mes analizavome implanto intergraciją ir aplink implantą esančio kaulo formavimąsi osteoporotiniame žiurkės modelyje, naudojant nerūdijančio plieno sraigtus proksimaliniame blauzdikaulyje. Sraigto integracijai ir kaulo formavimuisi paskatinti daliai žiurkių skirta ZA sistemiškai. Tikslas buvo išsiaiškinti ar ZA paveikė implanto integraciją ir kaulo formavimasi aplink sraigtą. Antrajame tyrimo etape bandėme išsiaiškinti, ZA kaupimosi aplink CaS/HA medžiagą skirtumus, skiriant skirtingu pooperaciniu laiku. Tyrimo metu buvo naudojama fluorescenciniu būdu pažymėta ZA. Trečio tyrimo metu analizavome, kaip aplink sraigtą esančio kaulo formavimąsi įtakoja sisteminės ZA skyrimo laikas.

Trečia ir ketvirta studija buvo skirta naujo FAME indekso įvertinimui ir pritaikymui. Visų pirmą jis reikalingas norint ikiklinikinių tyrimų rezultatus pervesti į klinikinę studiją. Taip pat FAME indeksas reikalingas, padėti nustatyti asmenis, kurie gautų daugiausiai naudos iš augmentacijos operacijos metu. Tai yra pacientai, kurie turi didelę pakartotinių lūžių riziką, bet yra maža mirties rizika. Pirmos studijos metu, mes vertinome FAME indeksą klinikinėje praktikoje. Po vienerių metų buvo atlikta antroji studija, remiantis FAME indekso klasifikacija, ligoninių įrašai buvo peržiūrėti, siekiant nustatyti pakartotinius lūžius bet kurioje vietoje, pakartotines operacijas ir mirtingumą.

Paskutinės studijos metu norėjome patikrinti ikiklinikinių tyrimų rezultatus randomizuotame klininikiame tyrime. Pacientams su pertrochanteriniais lūžiais po mažos energijos traumos atlikta operacija naudojant dinaminę klubo plokštelės ir sraigto (DHS) sistemą, sisteminius bisfosfonatus bei priklausomai nuo tyrimo grupės papildomą augmentaciją CaS/HA medžiaga.

Ikiklinikinės studijos

CaS/HA pasiskirtstymas sintetiniame osteoporotinio kaulo modelyje

Tyrime buvo naudojami sintetinio kaulo modeliai (SAWBONES, Europa AB, Švedija) su 15 proc. kaulo tūrio (BV/TV) celėse. Kiekvienas sintetinio kaulo blokas buvo pragręžtas 8 mm gražtu iki 3 cm gylio. Nerūdijančio plieno sraigtas (11,5 cm ilgio, 1,25 cm sriegio ir 0,8 cm skersmens) buvo įsriegtas į anksčiau išgręžtą skylę iki 0,5 cm gylio.

Pagal gamintojo nurodymus buvo paruošta ir sušvirkšta CaS/HA biomedžiaga. Iš pradžių biomedžiaga buvo sumaišyta su rentgenokontrastine medžiaga ir maišyta 30 s. Po to CaS/HA pasta buvo perpilta į 5 cm^3 injekcinį švirkštą. Adapterio pagalba speciali titaninė kaniulė (aukštis = 16 cm, skersmuo = 2,5 mm) buvo sujungta su švirkštu, kuriame jau sumaišyta CaS/HA medžiaga. Titaninė kaniulė buvo įstatyta į tolimiausią kanalo dalį – išgręžta iš anksto 2,5 min nuo maišymo pradžios. Į distaliausią kanalo ertmę buvo iš lėto suleista 2 ml CaS/HA medžiagos. Ši procedūra atlikta su 9 sintetiniais osteoporotiniais kaulo modeliais, dar 7 mėginiai atlikti naudojant tą patį sintetinį kaulą, tačiau injekuojant PMMA, ir 8 mėginiai buvo naudojami kontrolei be injekuotos medžiagos.

CaS/HA medžiagos pasiskirstymas žmogaus kaule ex-vivo tyrimas

Iš audinių banko buvo panaudotos 2 osteoporoze sergančių pacientų šlaunikaulių galvos. Tai yra pacientai, kuriems buvo atlikta klubo sąnario endoprotezavimo operacija dėl šlaunikaulio kaklo lūžio po mažos energijos traumos. Rentgeno kontrolėje šlaunikaulio galvoje buvo išgręžtas 3 cm ilgio kanalas, naudojant 8 mm gražtą. 11,5 cm ilgio sraigtas buvo ne pilnai įstatytas į iš anksto išgręžtą kanalą, maždaug 0,5 cm. Į ertmę sraigto distalinėje dalyje buvo įšvirkšta 2,5 ml CaS/HA medžiagos. Po to sraigtas įsuktas į galutinę poziciją, rentgeno kontrolėje. Abiem šlaunikaulio galvom po procedūros atlikta mikro – KT įvertinti, kaip pasiskirstė mežiaga aplink implantą ir kaulą, jų tvirtinimosi vietoje. Kitą dieną sraigtai buvo pašalinti – po KT tyrimo šlaunikaulių galvos laikytos –20 °C per naktį.

Norint nustatyti CaS/HA biomedžiagos pasiskistymą tarp kaulo ir sraigto, šlaunikaulių galvos pakartotinai atšildytos ir nufotografuotos.

CaS/HA medžiagos pasiskirstymas in-vivo tyrimas (chirurginė metodika)

Pacientui su pertrochanteriniu lūžių buvo atlikta operacija naudojant DHS sistemą, o operacijos metu taip pat buvo suleidžiama CaS/HA biomedžiaga.

DHS sistemos sraigtas į šlaunikaulio galvą buvo įvedamas nepilnai. 2,5 min nuo CaS/HA maišymo pradžios, biomaterija buvo suleidžiama į ertmę prieš sraigtą, rentgeno kontrolėje. 2,5 ml medžiagos buvo sušvirkšta į išgrežtą ermę distalinėje sraigto dalyje ir į akytąjį kaulą aplink sraigtą. Sraigtas įsuktas į galutinę padėtį.

Implanto integracija osteoporotinėse žiurkėse

Tyrime naudotos 24 savaičių amžiaus 38 Sprague-Dawley žiurkių patelės, kurioms pašalintos kiaušidės ir 4 SHAM kontrolinėje grupėje. Gyvūnai prieš operaciją buvo suskirstyti į 4 grupes G1, G2, G3, G4. Sraigtų augmentacijai buvo naudojama CaS/HA biomedžiaga (CERAMENTTM BVF iš Bone Support AB, Švedija). Operacijos metu buvo taikoma anestezija izofluranu (2–2,5 proc. kartu su deguonimi). Antibiotiko profilaktika buvo atliekama 15 minučių iki operacijos (Penicilinas-Streptocilinas VET, Apotek AB). Buprenorfinas (Temgesic 0,03 mg/kg) buvo suleidžiamas subkutaniškai vienkartinai, pooperaciniam nuskausminimui.

Visiem gyvūnams operacija buvo atlikta dešinėje pusėje, blauzdikaulių proksimalinėje dalyje, 5mm žemiau augimo plokštelės. Operacijos metu buvo išgrežiama skylė monokortikaliai. Į skylę buvo įsukamas nerūdijančio plieno sraigtas (Trimed Ortho, Švedija), sraigto skersmuo 2,3 mm, ilgis 8 mm G1 ir G2 grupėse buvo n = 11 žiurkių, tačiau nei vienoje iš šių grupių sraigtai nebuvo naudoti. G3 (n = 11) ir G4 (n = 5) grupėse prieš įsukant sraigtą buvo panaudota defekto srityje po 50 µl CaS/HA medžiagos. Visos G2 ir G3 grupių žiurkės gavo sistemiškai ZA 2 savaitės po operacijos. G1–G4 grupių ir SHAM žiurkėm 6 savaitės po operacijos buvo atlikta eutanazija. Blauzdikauliai buvo eksplantuoti, jiems atliktas mikro–KT tyrimas. Iš kiekvienos grupės paėmus po 1 pavyzdį buvo atliktas neutronų vaizdinis tyrimas ir nekalcifikuota histologija Laue–Langevin institute (Prancūzijoje), kiti visi mėginiai buvo naudojami dekalcifikuotai histologijai.

Laiko reikšmės vertinimas tarp ZA injekcijos ir kaupimosi CaS/HA medžiagioje

25 Sprague–Dawley žiurkių patinams atliktos operacijos proksimaliniuose blauzdikauliuose, operacijos metodika nurodyta 3.1.2.1. skirsnyje. N = 5 žiurkės negavo CaS/HA medžiagos – panaudota, kaip kontrolinė grupė. Operuotų žiurkių n = 20 defektai užpildyti 50 µl CaS/HA. Operuotos žiurkės buvo suskirstytos į 4 grupės pagal fluoresencinės zoledroninės rūgšties (FZA) injekcijos dieną. Injekcijoje atlikos 1, 3, 7 ir 14 dieną po operacijos. FZA buvo suleidžiama po oda 0,1 mg/kg dozėmis. Siekiant optimizuoti signalo intensyvumą ir išvengti paklaidos pirmiausia buvo sumaišyta 10 proc.

Alexa-fluor 647 pažymėtos ZA su 90 proc. nekonjuguotos ZA. Gyvūnam buvo atlikta eutanazija praėjus 24 valandom po FZA injekcijos. Visų žiurkių operuotiems ir sveikiems blauzdikauliams buvo atliktas vaizdinis tyrimas – „In–Vivo“ vaizdo gavimo sistema (IVIS Spectrum, Perkin Elmer, JAV). CaS/HA medžiaga taip pat buvo pašalinta iš defekto ir įvertinta nepriklausomai, siekiant parodyti, kad signalas iš tikrųjų kilo iš implantuotos medžiagos.

Kaip priklauso kaulo formavimasis aplink implantą nuo zoledroninės rūgšties injekcijos laiko

Sprague–Dawley žiurkių patinams atkartojome 3.1.2.1. skirsnyje aprašytą operaciją. 35 žiurkės, suskirstytos į 5 grupes (n = 7/grupė): G1 – CaS/HA; G2 – CaS/HA+sisteminė ZA 1 diena po operacijos; G3 – CaS/HA + sisteminė ZA 3 dienos po opercijos G4 – CaS/HA + sisteminė ZA 7 dienos po operacijos ir G5 – CaS/HA + sisteminė ZA 14 dienų po operacacijos. ZA (0,1mg/kg) buvo suleidžiama atitankamu laiku kiekvienam gyvūnui po oda. N = 4 žiurkės buvo panaudotos kontrolei, joms operacijos metu nebuvo injekuota CaS/HA aplink sraigtą, o ZA (0,1 mg/kg) suleista 14 dienų po operacijos. Visiems gyvūnams buvo atlikta eutanazija 6 savaitės po operacijos. Operuotiems blauzdikauliams su sraigtais ir medžiaga buvo atlikta mikro–KT ir histologiniai tyrimai, prieš tai juos eksplantavus.

Klinikinės studijos

FAME studija, skirta identifikuoti pacientus, kuriems būtų vertingiausia atlikti kaulų augmentaciją operacijos metu

Dvejose universitetinėse ligoninėse, ortopedijos skyruose – Kaune (Lietuva) ir Ankaroje (Turkija) vadovaujantis FAME indeksu, atliktos dvi studijos. Sezgimo, Markevičiūtės ir kt., pirmoji studija buvo pradėta 2018 m. lapkričio mėn., studijoje dalyvavo 100 pacientų po 50 iš kiekvieno centro. Į tyrimą buvo įtraukti pacientai, kuriems buvo taikomas chirurginis gydymas dėl osteoporotinių šlaunikaulio kaklo arba trochanterininės dalies lūžiu. Po 12 mėnesių, buvo atlikta antroji studija, remiantis FAME indekso klasifikacija, ligoninių įrašai buvo peržiūrėti, siekiant nustatyti pakartotinius lūžius, bet kurioje vietoje, pakartotines operacijas ir mirtingumą – vienerių metų laikotarpyje. Tyrimas atliktas naudojant specialią FAME indekso formą, norint surinkti visą informaciją, reikalingą apskaičiuoti FRAX indeksą be KMT ir Sernbo indekso balus.

Randomizuotas klinikinis tyrimas įvertinti augmentacijos naudą CaS/HA kartu su sistemine ZA operacijos metu, pacientams su pertrochanteriniais lūžiais naudojant DHS sistemą

Remiantis FAME indeksu – esant didelei lūžių tikimybei ir mažai mirties rizikai. Į studiją įtraukti 20 pacientų (po 10 pacientų į kiekvieną grupę). Į tyrimą įtraukti pacientai 19–95 amžiaus, kuriems dėl diagnozuoto šlaunikaulio pertrochanterinis lūžio (AO A1/A2), buvo taikomas operacinis gydymas ir sutinkantys dalyvauti tyrime.

Neįtraukti pacientai kurie:

- anksčiau turėjo lūžius klubo srityje, kurioje nors pusėje,
- buvo atlikta kito klubo endoprotezavimo operacija,
- vartoja kortikosteroidus,
- turi nekoreguojamą koagulopatiją ar kraujavimo sutrikimą,
- turintys inkstų nepakankamumą, kurio gydymui reikalingos dializės,
- aktyviai gydomi dėl metastazavusio piktybinio naviko,
- anamnezėje buvo taikyta radioterapija dubens / klubo srityje,
- nustatyti lūžiai apimantys gūžduobę,
- sergantys sunkia aktyvia sistemine infekcija,
- sergantys hipertiroze arba turi autonominę skydliaukės adenomą,
- diagnozuota alergija kontrastinei medžiagai,
- diagnozuota vietinė infekcija operacijos vietoje,
- nėščios moterys,
- slaugomi pacientai.

Pacientai randomizuoti į dvi grupes. Visiems pacientams buvo atliekama įprasta šlaunikaulio proksimalinės dalies osteosintezės operacija panaudojant DHS sistemą. Tiriamajai grupei operacijų metu papildomai rentgeno kontrolėje į šlaunikaulio galvą ir kaklą buvo suleidžiama biomedžiaga CaS/HA ir po 7–14 dienų nuo operacijos visiem pacientam intraveniškai buvo suleidžiama zoledroninė rūgštis. Kontrolinei grupei buvo atliekama ta pati operacija, tačiau operacijos metu nebuvo suleidžiamas CaS/HA, taip pat kaip ir tiriamųjų grupei po 7–14 dienų nuo operacijos buvo skiriama zoledroninė rūgštis. Visi į tyrimą įtraukti pacientai bus gydomi pagal klinikoje nustatytą standartinį algoritmą (operacijos pobūdis, tromboembolijų profilaktika, reabilitacija, pooperacinė priežiūra). Sekimo laikotarpis – 6 mėnesiai. Visiems pacientams buvo atliktas radiologinis ištyrimas: rentgenogramos prieš operaciją, 1–3 dienos po operacijos, po 6 savaičių, ir po 3 ir 6 mėnesių, kompiuterinė tomografija buvo atliekama 1–3 dienos po operacijos, po 3 ir 6 mėnesių, bei DEXA tyrimas po 6 mėnesių. Kompiuterinė tomografija bei DEXA kaulų densitometrija buvo atliekama specialiais rėžimais, neutralizuojant metalo artefaktus

bei vertinama pagal mūsų sukurtą protokolą. Atlikus tyrimus buvo vertinamas lūžio gijimas bei kaulo regeneracija. Visą gydymo laikotarpį buvo sekami ir dokumentuojami, bet kokie nepageidaujami reiškiniai, nusiskundimai.

REZULTATAI

Ikiklinikinės studijos

CaS/HA pasiskirtstymas sintetiniame osteoporotinio kaulo modelyje

Sraigtas iš osteoporotinio kaulo modelio be augmentacijos buvo pašalintas lengvai, ant sraigto nebuvo stebėta jokių osteoporotonio kaulo modelio likučių. Osteoporotinio sintetinio kaulo modeliuose, kai buvo naudojama CaS/HA ir PMMA sraigtai tiesioginio kontakto su osteoporotiniais kaulų modeliais neturėjo. Jokio skirtumo nestebėta osteoporotiniame kaulo modelyje naudojant CaS/HA ir PMMA, tačiau stabilumas, tvirtumas ir didžiausia jėga buvo statistiškai reiškimgai didesni augmentuotose modeliuose (CaS/HA ir PMMA) lyginant su kontroline grupe (atitinkamai $p < 0,0001$, $p < 0,01$ ir $p < 0,05$).

CaS/HA medžiagos pasiskirstymas žmogaus kaule ex-vivo tyrimas

Kadaverinėse šlaunikaulių galvose CaS/HA biomedžiaga išsisklaidė panašiai kaip ir osteoporotinio kaulo modeliuose. Mikro–KT vaizdai aiškiai parodė, kad CaS/HA medžiaga buvo tiek kaule aplink sraigto griovelius, tiek tarp jų. Iš šlaunikaulio galvos išėmus sraigtą, jame taip pat buvo rasta CaS/HA medžiagos.

Implanto integrazija osteoporotinėse žiurkėse

Atliktame tyrime su osteoporotinėmis žiurkėmis mikro KT vaizduose buvo palyginta implanto integracija ir naujo kaulo formavimasis. Vaizduose buvo stebimi akivaizdūs skirtumai, naujo kaulo formavimasis aplink implantą, G3 grupėje, kurioje buvo naudojama CaS/HA su sistemine ZA.

Laiko reikšmės vertinimas tarp ZA injekcijos ir kaupimosi CaS/HA medžiagoje

Visuose gyvūnuose, kuriems naudota CaS/HA buvo stebimas intensyvesnis ZA kaupimasis. Lyginant gyvūnus su CaS/HA, kaip laiko funkciją, intensyviausias ZA pasisavinimas ir aktyvūmas stebėtas 7 dieną.

Kaip priklauso kaulo formavimasis aplink implantą nuo zoledroninės rūgšties injekcijos laiko

Remiantis mikro KT vaizdais stebėjome, kad optimalus ZA skyrimo laikas, siekiant biologiškai aktyvuoti CaS/HA, yra 7–14 dienų po operacijos.

Klinikinės studijos

FAME studija, skirta identifikuoti pacientus, kuriems būtų vertingiausia atlikti kaulų augmentaciją operacijos metu

Į pirmą tyrimą buvo įtraukta 100 pacientų (po 50 iš kiekvieno centro), kurie patyrė šlaunikaulio proksimalinės dalies lūžį dėl osteoporozės po mažos energijos traumos. Pacientų pasiskirstymas įvairiose FAME indekso kategorijose tarp centrų nesiskyrė. Pacientai iš dviejų centrų statistiškai reikšmingai nesiskyrė nei lytimi, nei amžiumi, nei svoriu. Pacientai buvo suskirstyti į devynis pogrupius, naudojant FRAX ir Sernbo balų klausimų derinį. 18 proc. visų pacientų sudarė FAME indekso pogrupis, kurie daugiausiai naudos gautų iš augmentacijos operacijos metu. Tai pacientai, kurie turėjo didelę lūžių, bet mažą mirtingumo riziką.

Antrojoje studijoje dalyvavo 94 pacientai. Po vienerių metų buvo peržiūrimi pacientų ligoninės išrašai ir pacientai pakartotinai buvo įvertinami dėl pakartotinių lūžių (pakartotinės operacijos, implantų mechaninės komplikacijos ir nauji lūžiai) ir išgyvenamumas po vienerių metų. Remiantis FRAX ir Sernbo klasifikacija, pacientai buvo suskirstyti į mažos, vidutinės ir didelės rizikos grupes, pagal FAME indeksą gautos devynios kombinuotos kategorijos. Palyginti su kitomis kategorijomis, FRAX-H buvo iš esmės susijęs su didesniu mirtingumu ir lūžių dažniu. Bendras lūžių ir mirties dažnis buvo atitinkamai 20,2 proc. ir 33 proc. Statistinis reikšmingumas, kai $p < 0,05$.

Randomizuotas klinikinis tyrimas įvertinti augmentacijos naudą CaS/HA kartu su sistemine ZA operacijos metu, pacientams su pertrochanteriniais lūžiais naudojant DHS sistemą

Į klinikinį tyrimą įtraukėme 20 pacientų: kontrolinė grupė n = 10, CaS/HA grupė n = 10. Praėjus 7–14 dienų po operacijos, visi 20 pacientų gavo sisteminę ZA 4 mg. Pilnas pacientų sekimas – šešių mėnesių stebėjimas pabaigtas 14 pacientų: CaS/HA grupė n = 8 ir kontrolinėje grupėje n = 6. Šešių pacientų sekimas buvo nutrauktas: kontrolinėje grupėje n = 4, CaS/HA grupėje n = 2. 2 pacientai kontrolinėje grupėje mirė ir 4 pacientai negrįžo tolesniam gydymui. FAME indeksu, visi įtraukti pacientai turėjo didelę lūžių riziką, bet mažą mirties riziką. Visų pacientų, kurie baigė tolesnę priežiūrą,

n = 14 (CaS/HA = 8, kontrolinė = 6), palyginome DEXA rezultatus abiejose grupėse. CaS/HA grupės pacientų rezultatai pagerėjo visose srityse (R 1–3), o R1–3 vidurkis buvo 1,12 karto didesnis nei kontrolinės grupės. Jokių komplikacijų (implantų nestabilumo, infekcijų, mechaninių komplikacijų ar kt.) nebuvo pastebėta nė vienam iš pacientų nei vienoje grupėje. Nė vienoje pacientų grupėje kliniškai reikšmingo skirtumo nepastebėta.

IŠVADOS

1. Mes pademonstravome naują chirurginį metodą, kaip pritaikyti CaS/HA medžiagą (ar bet kurią kitą injekcinę medžiagą) sraigto paviršiaus osteoporozinio kaulo sąsajoje, nenaudojant papildomo slėgio ir be medžiagos nutekėjimo į aplinkinę kraujotaką.
2. Ikiklinikinio tyrimo metu panaudojant osteoporozinių kaulų modelius, nustatėme, kad CaS/HA pagerina biomechaninį kaulo tvirtinimasi tarp implanto ir kaulo.
3. Naudodami ikiklinikinių tyrimų su gyvūnais modelio duomenis, mes parodėme, kad CaS/HA medžiaga aplink implantą gali būti biologiškai suaktyvinta sistemine ZA injekcija. Biologinis medžiagos aktyvinimas skatina naujo kaulo formavimąsi aplink implantą. Nors atrodo, kad bisfosfonato infuzijos laikas yra svarbus bendram ZA įsisavinimui CaS/HA medžiagoje, didelio laiko poveikio bendram periimplantuoto kaulo formavimuisi nepastebėta.
4. Mes pademonstravome, kad medžiaga yra biologiškai aktyvuojama, kai ZA suleidžiama per 1–2 savaites po operacijos. Tai galima stebėti iš padidėjusio periimplantinio kaulo mineralinio tankio viename iš eksperimentų.
5. Sistemiškai vartojama ZA gali būti biologiškai aktyvuojamos ir kitos kalcio fosfato medžiagos, ypač beta trikalcio fosfatas.
6. FAME indeksas – skirtas identifikuoti pacientus, kuriems bus didžiausia nauda iš augmentacijos operacijos metu. Tai pacientai, kurie turi didelę pakartotinių lūžių riziką, tačiau mažą mirties riziką.
7. Klinikiniame tyrime su pacientais mes pademonstravome, kad įmanoma suleisti ir kaip reikėtų suleisti CaS/HA medžiagą augmentacijai, atliekant pertrochanterinių lūžių fiksaciją naudojant DHS sistemą.

PRAKTINĖS REKOMENDACIJOS IR ATEITIES PLANAI

Sukurtas FAME indeksas, leidžia gydytojams identifikuoti pacientus, kuriems tikslingiausia būtu operacijos metu papildomai taikyti augmentaciją.

Pagrindiniai augmentacijos privalumai pacientams: trumpesnė hospitalizacija, mažesnis pakartotinių operacijų skaičius ir didesnis pacientų mobilumas. Standartizuotas chirurginis metodas, skirtas sustiprinti fiksavimo prietaisus ir vėliau pagerinti tiesioginį mechaninį fiksavimo prietaisų tvirtinimą, gali būti naudingas ir padėti išvengti fiksavimo nestabilumo. Ilgalaikiai rezultatai turi potencialo būti geresniais, jei implantuota medžiaga virsta gyvybingu kaulu.

Mes manome, kad mūsų metodas, kuris apjungia lokalią augmentaciją su sistemine farmakologine biomoduliacija, gali sumažinti nestabilių lūžių pakartotinių operacijų dažnį iki 50 proc. Tačiau tam būtina atlikti didesnį klinikinį tyrimą, kuriame kiekvienoje grupėje būtų ne mažiau kaip 1000 pacientų.

Prieš pradėdami plačiai naudoti sisteminį ZA ir vietinį gydymą CaS/HA trochanteriniams lūžiams, turime atlikti dar kelis nors ir nedidelius klinikinius tyrimus, naudodami įvairius prietaisus, tokius kaip PFN ir Gama vinis.

Mūsų vienas iš tikslų ateityje klinikinėje praktikoje išplėsti sisteminės ZA ir vietinio CaS/HA naudojimą lūžių fiksacijoje. Tačiau, prieš tai turime atlikti tyrimus su kitomis anatominėmis vietomis, tokiomis kaip proksimalinis blauzdikaulis, distalinis stipinkaulis ir kt. Mes manome, kad aktyvuoti medžiagai užtektų mažesnės dozės ZA. Tam įrodyti, reikia atlikti dar vieną ekspermentą su mažesnėmis ZA dozėmis.

REFFERENCES

1. Zhang L, Zheng YL, Wang R, Wang XQ, Zhang H. Exercise for osteoporosis: A literature review of pathology and mechanism. Front Immunol. 2022;13:1005665.
2. Odén A, McCloskey EV, Kanis JA, Harvey NC, Johansson H. Burden of high fracture probability worldwide: secular increases 2010-2040. Osteoporos Int. 2015;26(9):2243-8.
3. LeBoff MS, Greenspan SL, Insogna KL, Lewiecki EM, Saag KG, Singer AJ, et al. The clinician's guide to prevention and treatment of osteoporosis. Osteoporos Int. 2022;33(10):2049-102.
4. Tarantino U, Iolascon G, Cianferotti L, Masi L, Marcucci G, Giusti F, et al. Clinical guidelines for the prevention and treatment of osteoporosis: summary statements and recommendations from the Italian Society for Orthopaedics and Traumatology. J Orthop Traumatol. 2017;18(Suppl 1):3-36.
5. Salari N, Ghasemi H, Mohammadi L, Behzadi MH, Rabieenia E, Shohaimi S, et al. The global prevalence of osteoporosis in the world: a comprehensive systematic review and meta-analysis. J Orthop Surg Res. 2021;16(1):609.
6. Osteoporosis prevention, diagnosis, and therapy. Jama. 2001;285(6):785-95.
7. Aoki TT, Grecu EO, Srinivas PR, Prescott P, Benbarka M, Arcangeli MM. Prevalence of osteoporosis in women: variation with skeletal site of measurement of bone mineral density. Endocr Pract. 2000;6(2):127-31.
8. Compston J, Cooper A, Cooper C, Gittoes N, Gregson C, Harvey N, et al. UK clinical guideline for the prevention and treatment of osteoporosis. Arch Osteoporos. 2017;12(1):43.
9. Ajaxon I, Acciaioli A, Lionello G, Ginebra MP, Ohman-Magi C, Baleani M, et al. Elastic properties and strain-to-crack-initiation of calcium phosphate bone cements: Revelations of a high-resolution measurement technique. J Mech Behav Biomed Mater. 2017;74:428-37.
10. Bombah FM, Diawara M, Ekani BY, Nana T, Mikiela A. Complications after Dynamic Hip Screw Osteosynthesis of Proximal Femoral Fractures at Army Instructional Hospital-Libreville. Case Reports in Orthopedics. 2021;2021:4177203.
11. Office of the Surgeon G. Reports of the Surgeon General. Bone Health and Osteoporosis: A Report of the Surgeon General. Rockville (MD): Office of the Surgeon General (US); 2004.
12. Gullberg B, Johnell O, Kanis JA. World-wide projections for hip fracture. Osteoporos Int. 1997;7(5):407-13.
13. Lewiecki EM, Ortendahl JD, Vanderpuye-Orgle J, Grauer A, Arellano J, Lemay J, et al. Healthcare Policy Changes in Osteoporosis Can Improve Outcomes and Reduce Costs in the United States. JBMR Plus. 2019;3(9):e10192.
14. Tiihonen R, Alaranta R, Helkamaa T, Nurmi-Lüthje I, Kaukonen JP, Lüthje P. A 10-Year Retrospective Study of 490 Hip Fracture Patients: Reoperations, Direct Medical Costs, and Survival. Scand J Surg. 2019;108(2):178-84.
15. Pyrhönen HS, Lagergren J, Wolf O, Bojan A, Mukka S, Möller M, et al. No Difference in Conversion Rate to Hip Arthroplasty After Intramedullary Nail or Sliding Hip Screw for Extracapsular Hip Fractures: An Observational Cohort Study of 19,604 Individuals. J Bone Joint Surg Am. 2022;104(19):1703-11.
16. Edwards C, Counsell A, Boulton C, Moran CG. Early infection after hip fracture surgery: risk factors, costs and outcome. J Bone Joint Surg Br. 2008;90(6):770-7.
17. Pollmann CT, Dahl FA, Røtterud JHM, Gjertsen JE, Årøen A. Surgical site infection after hip fracture - mortality and risk factors: an observational cohort study of 1,709 patients. Acta Orthop. 2020;91(3):347-52.

18. El Miedany Y. FRAX: re-adjust or re-think. Archives of Osteoporosis. 2020;15(1):150.
19. Kanis JA, Hans D, Cooper C, Baim S, Bilezikian JP, Binkley N, et al. Interpretation and use of FRAX in clinical practice. Osteoporosis International. 2011;22(9):2395-411.
20. Masud T, Binkley N, Boonen S, Hannan MT. Official Positions for FRAX® Clinical Regarding Falls and Frailty: Can Falls and Frailty be Used in FRAX®?: From Joint Official Positions Development Conference of the International Society for Clinical Densitometry and International Osteoporosis Foundation on FRAX®. Journal of Clinical Densitometry. 2011;14(3):194-204.
21. Kanis JA, Odén A, McCloskey EV, Johansson H, Wahl DA, Cooper C. A systematic review of hip fracture incidence and probability of fracture worldwide. Osteoporos Int. 2012;23(9):2239-56.
22. Kanis JA, Harvey NC, Johansson H, Lorentzon M, Liu E, Leslie WD, et al. FRAX. In: Pape H-C, Kates SL, Hierholzer C, Bischoff-Ferrari HA, editors. Senior Trauma Patients : An Integrated Approach. Cham: Springer International Publishing; 2022. p. 89-99.
23. Lewiecki EM. Osteoporosis: Clinical Evaluation. In: Feingold KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E, et al., editors. Endotext. South Dartmouth (MA): MDText.com, Inc.

24. Rogmark C, Carlsson A, Johnell O, Sernbo I. A prospective randomised trial of internal fixation versus arthroplasty for displaced fractures of the neck of the femur. Functional outcome for 450 patients at two years. J Bone Joint Surg Br. 2002;84(2):183-8.
25. Dawe EJ, Lindisfarne E, Singh T, McFadyen I, Stott P. Sernbo score predicts survival after intracapsular hip fracture in the elderly. Ann R Coll Surg Engl. 2013;95(1):29-33.
26. Mellner C, Eisler T, Börsbo J, Brodén C, Morberg P, Mukka S. The Sernbo score predicts 1-year mortality after displaced femoral neck fractures treated with a hip arthroplasty. Acta Orthop. 2017;88(4):402-6.
27. Mellner C, Hedström M, Hommel A, Sköldenberg O, Eisler T, Mukka S. The Sernbo score as a predictor of 1-year mortality after hip fracture: a registry study on 55,716 patients. Eur J Trauma Emerg Surg. 2021;47(6):2043-8.
28. Sezgin EA, Markevičiūtė V, Širka A, Tarasevičius Š, Raina DB, Isaksson H, et al. Combined fracture and mortality risk evaluation for stratifying treatment in hip fracture patients: A feasibility study. Jt Dis Relat Surg. 2020;31(2):163-8.
29. Sezgin EA, Tor AT, Markevičiūtė V, Širka A, Tarasevičius Š, Raina DB, et al. A combined fracture and mortality risk index useful for treatment stratification in hip fragility fractures. Jt Dis Relat Surg. 2021;32(3):583-9.
30. Zuckerman JD. Hip fracture. N Engl J Med. 1996;334(23):1519-25.
31. van Embden D, Rhemrev SJ, Meylaerts SA, Roukema GR. The comparison of two classifications for trochanteric femur fractures: the AO/ASIF classification and the Jensen classification. Injury. 2010;41(4):377-81.
32. Selim A, Beder FK, Algeaidy IT, Farhat AS, Diab NM, Barakat AS. Management of unstable pertrochanteric fractures, evaluation of forgotten treatment options. Sicot j. 2020;6:21.
33. Buckley RE, Moran CG, Apivatthakakul T. AO Principles of Fracture Management: AO Foundation; 2017.
34. Chen SR, Petersen TD, Turvey BR. Geriatric Femur Fractures: Implant Choice in Pertrochanteric Hip Fractures. Operative Techniques in Orthopaedics. 2023;33(2):101043.
35. Liang Y, Liu S, Zhong F. Outcome of unstable pertrochanteric fractures in high-risk geriatric treated with external fixators. European Journal of Orthopaedic Surgery & Traumatology. 2022;32(5):867-74.

36. Saul D, Riekenberg J, Ammon JC, Hoffmann DB, Sehmisch S. Hip Fractures: Therapy, Timing, and Complication Spectrum. 2019;11(6):994-1002.
37. Socci AR, Casemyr NE, Leslie MP, Baumgaertner MR. Implant options for the treatment of intertrochanteric fractures of the hip: rationale, evidence, and recommendations. Bone Joint J. 2017;99-b(1):128-33.
38. Sharma A, Sethi A, Sharma S. Treatment of stable intertrochanteric fractures of the femur with proximal femoral nail versus dynamic hip screw: a comparative study. Rev Bras Ortop. 2018;53(4):477-81.
39. Xu H, Liu Y, Sezgin EA, Tarasevičius Š, Christensen R, Raina DB, et al. Comparative effectiveness research on proximal femoral nail versus dynamic hip screw in patients with trochanteric fractures: a systematic review and meta-analysis of randomized trials. J Orthop Surg Res. 2022;17(1):292.
40. Schemitsch EH, Nowak LL, Schulz AP, Brink O, Poolman RW, Mehta S, et al. Intramedullary Nailing vs Sliding Hip Screw in Trochanteric Fracture Management: The INSITE Randomized Clinical Trial. JAMA Network Open. 2023;6(6):e2317164-e.
41. Whitehouse MR, Berstock JR, Kelly MB, Gregson CL, Judge A, Sayers A, et al. Higher 30-day mortality associated with the use of intramedullary nails compared with sliding hip screws for the treatment of trochanteric hip fractures: a prospective national registry study. The bone & joint journal. 2019;101-b(1):83-91.
42. Li S, Chang SM, Niu WX, Ma H. Comparison of tip apex distance and cut-out complications between helical blades and lag screws in intertrochanteric fractures among the elderly: a meta-analysis. J Orthop Sci. 2015;20(6):1062-9.
43. Flikweert ER, Wendt KW, Diercks RL, Izaks GJ, Landsheer D, Stevens M, et al. Complications after hip fracture surgery: are they preventable? Eur J Trauma Emerg Surg. 2018;44(4):573-80.
44. Morvan A, Boddaert J, Cohen-Bittan J, Picard H, Pascal-Mousselard H, Khiami F. Risk factors for cut-out after internal fixation of trochanteric fractures in elderly subjects. Orthop Traumatol Surg Res. 2018;104(8):1183-7.
45. Kristensen NK, Lange J, Frøslev T, Pedersen AB. Risk of reoperation due to surgical site infection in 74,771 hip fracture patients: a Danish nationwide cohort study. Acta Orthop. 2022;93:760-6.
46. Mattisson L, Bojan A, Enocson A. Epidemiology, treatment and mortality of trochanteric and subtrochanteric hip fractures: data from the Swedish fracture register. BMC Musculoskelet Disord. 2018;19(1):369.
47. Goodnough LH, Wadhwa H, Tigchelaar SS, DeBaun MR, Chen MJ, Graves ML, et al. Indications for cement augmentation in fixation of geriatric intertrochanteric femur fractures: a systematic review of evidence. Arch Orthop Trauma Surg. 2022;142(10):2533-44.
48. Stramazzo L, Ratano S, Monachino F, Pavan D, Rovere G, Camarda L. Cement augmentation for trochanteric fracture in elderly: A systematic review. J Clin Orthop Trauma. 2021;15:65-70.
49. Stravinskas M, Tarasevicius S, Laukaitis S, Nilsson M, Raina DB, Lidgren L. A ceramic bone substitute containing gentamicin gives good outcome in trochanteric hip fractures treated with dynamic hip screw and in revision of total hip arthroplasty: a case series. BMC Musculoskelet Disord. 2018;19(1):438.
50. Mattsson P, Larsson S. Unstable trochanteric fractures augmented with calcium phosphate cement. A prospective randomized study using radiostereometry to measure fracture stability. Scand J Surg. 2004;93(3):223-8.
51. Stoffel KK, Leys T, Damen N, Nicholls RL, Kuster MS. A new technique for cement augmentation of the sliding hip screw in proximal femur fractures. Clin Biomech (Bristol, Avon). 2008;23(1):45-51.

52. Dall'Oca C, Maluta T, Moscolo A, Lavini F, Bartolozzi P. Cement augmentation of intertrochanteric fractures stabilised with intramedullary nailing. Injury. 2010;41(11):1150-5.
53. Gupta RK, Gupta V, Gupta N. Outcomes of osteoporotic trochanteric fractures treated with cement-augmented dynamic hip screw. Indian J Orthop. 2012;46(6):640-5.
54. Kim SJ, Park HS, Lee DW, Lee JW. Is calcium phosphate augmentation a viable option for osteoporotic hip fractures? Osteoporos Int. 2018;29(9):2021-8.
55. Fuchs A, et al. (2019). Implant Augmentation for Trochanteric Fractures with an Innovative, Ready to Use Calcium-Phosphate-Cement. J Orthopaedics Bone Res 1: 104.
56. Mattsson P, Alberts A, Dahlberg G, Sohlman M, Hyldahl HC, Larsson S. Resorbable cement for the augmentation of internally-fixed unstable trochanteric fractures. A prospective, randomised multicentre study. J Bone Joint Surg Br. 2005;87(9):1203-9.
57. Arora M, Chan EK, Gupta S, Diwan AD. Polymethylmethacrylate bone cements and additives: A review of the literature. World J Orthop. 2013;4(2):67-74.
58. Bistolfi A, Ferracini R, Albanese C, Vernè E, Miola M. PMMA-Based Bone Cements and the Problem of Joint Arthroplasty Infections: Status and New Perspectives. Materials (Basel). 2019;12(23).
59. Jaeblon T. Polymethylmethacrylate: properties and contemporary uses in orthopaedics. J Am Acad Orthop Surg. 2010;18(5):297-305.
60. Sa Y, Yang F, Wang Y, Wolke JGC, Jansen JA. Modifications of Poly(Methyl Methacrylate) Cement for Application in Orthopedic Surgery. In: Chun HJ, Park CH, Kwon IK, Khang G, editors. Cutting-Edge Enabling Technologies for Regenerative Medicine. Singapore: Springer Singapore; 2018. p. 119-34.
61. Rompen IF, Knobe M, Link BC, Beeres FJP, Baumgaertner R, Diwersi N, et al. Cement augmentation for trochanteric femur fractures: A meta-analysis of randomized clinical trials and observational studies. PLoS One. 2021;16(6):e0251894.
62. Johnell O, Kanis JA. An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. Osteoporos Int. 2006;17(12):1726-33.
63. Whitehouse MR, Atwal NS, Pabbruwe M, Blom AW, Bannister GC. Osteonecrosis with the use of polymethylmethacrylate cement for hip replacement: thermal-induced damage evidenced in vivo by decreased osteocyte viability. Eur Cell Mater. 2014;27:50-62; discussion -3.
64. Wang L, Gardner AW, Kwek EB, Naidu GR. Retrograde cement arteriovenogram of nutrient vessels following hemiarthroplasty of the hip. Acta Orthop Belg. 2012;78(4):431-5.
65. Wang JS, Tägil M, Isaksson H, Boström M, Lidgren L. Tissue reaction and material biodegradation of a calcium sulfate/apatite biphasic bone substitute in rat muscle. J Orthop Translat. 2016;6:10-7.
66. Nilsson M, Zheng MH, Tägil M. The composite of hydroxyapatite and calcium sulphate: a review of preclinical evaluation and clinical applications. Expert Rev Med Devices. 2013;10(5):675-84.
67. Katagiri T, Watabe T. Bone Morphogenetic Proteins. Cold Spring Harbor perspectives in biology. 2016;8(6).
68. Širka A, Raina DB, Isaksson H, Tanner KE, Smailys A, Kumar A, et al. Calcium Sulphate/Hydroxyapatite Carrier for Bone Formation in the Femoral Neck of Osteoporotic Rats. Tissue Eng Part A. 2018;24(23-24):1753-64.
69. Abramo A, Geijer M, Kopylov P, Tägil M. Osteotomy of distal radius fracture malunion using a fast remodeling bone substitute consisting of calcium sulphate and calcium phosphate. J Biomed Mater Res B Appl Biomater. 2010;92(1):281-6.

70. Stravinskas M, Horstmann P, Ferguson J, Hettwer W, Nilsson M, Tarasevicius S, et al. Pharmacokinetics of gentamicin eluted from a regenerating bone graft substitute: In vitro and clinical release studies. Bone Joint Res. 2016;5(9):427-35.
71. Stravinskas M, Nilsson M, Vitkauskiene A, Tarasevicius S, Lidgren L. Vancomycin elution from a biphasic ceramic bone substitute. Bone Joint Res. 2019;8(2):49-54.
72. Gadegone WM, Shivashankar B, Lokhande V, Salphale Y. Augmentation of proximal femoral nail in unstable trochanteric fractures. Sicot j. 2017;3:12.
73. Hofmann A, Gorbulev S, Guehring T, Schulz AP, Schupfner R, Raschke M, et al. Autologous Iliac Bone Graft Compared with Biphasic Hydroxyapatite and Calcium Sulfate Cement for the Treatment of Bone Defects in Tibial Plateau Fractures: A Prospective, Randomized, Open-Label, Multicenter Study. The Journal of bone and joint surgery American volume. 2020;102(3):179-93.
74. Takada J, Sato S, Arai K, Kito Y, Oshita Y, Saito K. Safety and effectiveness of once-yearly zoledronic acid in Japanese osteoporosis patients: three-year post-marketing surveillance. Journal of Bone and Mineral Metabolism. 2023;41(2):268-77.
75. Deeks ED, Perry CM. Zoledronic Acid. Drugs & Aging. 2008;25(11):963-86.
76. Li X, Han J, Shi X, Bi Z, Liu J. Zoledronic acid and denosumab for periprosthetic bone mineral density loss after joint arthroplasty: a systematic review and meta-analysis of randomized controlled trials. Archives of Osteoporosis. 2023;18(1):37.
77. Lyles KW, Colón-Emeric CS, Magaziner JS, Adachi JD, Pieper CF, Mautalen C, et al. Zoledronic acid and clinical fractures and mortality after hip fracture. N Engl J Med. 2007;357(18):1799-809.
78. Kotian P, Boloor A, Sreenivasan S. Study of Adverse Effect Profile of Parenteral Zoledronic Acid in Female Patients with Osteoporosis. Journal of clinical and diagnostic research : JCDR. 2016;10(1):Oc04-6.
79. Do WS, Park JK, Park MI, Kim HS, Kim SH, Lee DH. Bisphosphonate-induced Severe Hypocalcemia - A Case Report. Journal of bone metabolism. 2012;19(2):139-45.
80. Woo SB, Hellstein JW, Kalmar JR. Narrative [corrected] review: bisphosphonates and osteonecrosis of the jaws. Annals of internal medicine. 2006;144(10):753-61.
81. Raina DB, Matuszewski LM, Vater C, Bolte J, Isaksson H, Lidgren L, et al. A facile one-stage treatment of critical bone defects using a calcium sulfate/hydroxyapatite biomaterial providing spatiotemporal delivery of bone morphogenic protein-2 and zoledronic acid. Sci Adv. 2020;6(48).
82. Raina DB, Qayoom I, Larsson D, Zheng MH, Kumar A, Isaksson H, et al. Guided tissue engineering for healing of cancellous and cortical bone using a combination of biomaterial based scaffolding and local bone active molecule delivery. Biomaterials. 2019;188:38-49.
83. Horstmann PF, Raina DB, Isaksson H, Hettwer W, Lidgren L, Petersen MM, et al. (*) Composite Biomaterial as a Carrier for Bone-Active Substances for Metaphyseal Tibial Bone Defect Reconstruction in Rats. Tissue Eng Part A. 2017;23(23-24):1403-12.
84. Raina DB, Larsson D, Sezgin EA, Isaksson H, Tägil M, Lidgren LJAb. Biomodulation of an Implant for Enhanced Bone-Implant Anchorage. 2019.
85. Alabdah F, Alshammari A, Hidalgo-Bastida A, Cooper G. A Review of Conventional and Novel Treatments for Osteoporotic Hip Replacements. Bioengineering (Basel). 2023;10(2).
86. Adeyemi A, Delhougne G. Incidence and Economic Burden of Intertrochanteric Fracture: A Medicare Claims Database Analysis. JB JS Open Access. 2019;4(1):e0045.
87. Joeris A, Kabiri M, Galvain T, Vanderkarr M, Holy CE, Plaza JQ, et al. Cost-Effectiveness of Cement Augmentation Versus No Augmentation for the Fixation of Unstable Trochanteric Fractures. J Bone Joint Surg Am. 2022;104(22):2026-34.

88. Amarilla-Donoso FJ, Roncero-Martin R, Lavado-Garcia JM, Toribio-Felipe R, Moran-Garcia JM, Lopez-Espuela F. Quality of life after hip fracture: a 12-month prospective study. PeerJ. 2020;8:e9215.
89. Raina DB, Markevičiūtė V, Stravinskas M, Kok J, Jacobson I, Liu Y, et al. A New Augmentation Method for Improved Screw Fixation in Fragile Bone. 2022;10.
90. Raina DB, Liu Y, Isaksson H, Tägil M, Lidgren L. Synthetic hydroxyapatite: a recruiting platform for biologically active molecules. Acta Orthop. 2020;91(2):126-32.
91. Esen E, Dur H, Ataoğlu MB, Ayanoğlu T, Turanlı S. Evaluation of proximal femoral nail-antirotation and cemented, bipolar hemiarthroplasty with calcar replacement in treatment of intertrochanteric femoral fractures in terms of mortality and morbidity ratios. Eklem Hastalik Cerrahisi. 2017;28(1):35-40.
92. Plang S, Dayan R, Khiami F, Preneau C, Barbier O, Choufani C. Cementation of proximal femoral nails of the very elderly subject in per-trochanteric fractures. Acta Orthop Belg. 2021;87(2):293-8.
93. Chang FC, Chuang PY, Lee CY, Lee CY, Chou YC, Huang TW, et al. The effects of bone-substitute augmentation on treatment of osteoporotic intertrochanteric fractures. Biomed J. 2021;44(6):717-26.
94. Goodnough LH, Wadhwa H, Tigchelaar SS, DeBaun MR, Chen MJ, Bishop JA, et al. Trochanteric fixation nail advanced with helical blade and cement augmentation: early experience with a retrospective cohort. Eur J Orthop Surg Traumatol. 2021;31(2):259-64.
95. Larsson S, Procter P. Optimising implant anchorage (augmentation) during fixation of osteoporotic fractures: is there a role for bone-graft substitutes? Injury. 2011;42 Suppl 2:S72-6.
96. Kok J, Širka A, Liu Y, Tarasevičius Š, Belickas J, Tägil M, et al. Augmenting a dynamic hip screw with a calcium sulfate/hydroxyapatite biomaterial. Med Eng Phys. 2021;92:102-9.
97. Gong Y, Zhang B, Yan L. A Preliminary Review of Modified Polymethyl Methacrylate and Calcium-Based Bone Cement for Improving Properties in Osteoporotic Vertebral Compression Fractures. 2022;9.
98. Jubel A, Andermahr J, Mairhofer J, Prokop A, Hahn U, Rehm KE. [Use of the injectable bone cement Norian SRS for tibial plateau fractures. Results of a prospective 30-month follow-up study]. Orthopade. 2004;33(8):919-27.
99. Lu Q, Liu C, Wang D, Liu H, Yang H, Yang L. Biomechanical evaluation of calcium phosphate-based nanocomposite versus polymethylmethacrylate cement for percutaneous kyphoplasty. Spine J. 2019;19(11):1871-84.
100. Nakamura T, Fukunaga M, Nakano T, Kishimoto H, Ito M, Hagino H, et al. Efficacy and safety of once-yearly zoledronic acid in Japanese patients with primary osteoporosis: two-year results from a randomized placebo-controlled double-blind study (ZOledroNate treatment in Efficacy to osteoporosis; ZONE study). Osteoporos Int. 2017;28(1):389-98.
101. Ito M, Sone T, Shiraki M, Tanaka S, Irie C, Ota Y, et al. The effect of once-yearly zoledronic acid on hip structural and biomechanical properties derived using computed tomography (CT) in Japanese women with osteoporosis. Bone. 2018;106:179-86.
102. Kim TY, Ha YC, Kang BJ, Lee YK, Koo KH. Does early administration of bisphosphonate affect fracture healing in patients with intertrochanteric fractures? J Bone Joint Surg Br. 2012;94(7):956-60.
103. Gao Y, Liu X, Gu Y, Song D, Ding M, Liao L, et al. The Effect of Bisphosphonates on Fracture Healing Time and Changes in Bone Mass Density: A Meta-Analysis. Front Endocrinol (Lausanne). 2021;12:688269.

104. Colón-Emeric C, Nordsletten L, Olson S, Major N, Boonen S, Haentjens P, et al. Association between timing of zoledronic acid infusion and hip fracture healing. Osteoporos Int. 2011;22(8):2329-36.
105. Jalan H, Perumal R, Prabhu S, Palanivelayutham S, Viswanathan VK, Rajasekaran S. Intravenous bisphosphonate therapy does not delay fracture healing in inter-trochanteric femur fractures - A randomised controlled study. J Clin Orthop Trauma. 2021;20:101472.
106. Duckworth AD, McQueen MM, Tuck CE, Tobias JH, Wilkinson JM, Biant LC, et al. Effect of Alendronic Acid on Fracture Healing: A Multicenter Randomized Placebo-Controlled Trial. J Bone Miner Res. 2019;34(6):1025-32.
107. Amanat N, McDonald M, Godfrey C, Bilston L, Little D. Optimal timing of a single dose of zoledronic acid to increase strength in rat fracture repair. J Bone Miner Res. 2007;22(6):867-76.
108. Mitsuzawa S, Matsuda S. Cement distribution and initial fixability of trochanteric fixation nail advanced (TFNA) helical blades. Injury. 2022;53(3):1184-9.
109. Fernandez M, David Y, Dubrana F, Di Francia R. Use of a Trochanteric Fixation Nail-Advanced (TFNA) with cement augmentation for treatment of trochanteric fractures in patients greater than sixty five years of age. Int Orthop. 2022;46(3):645-51.
110. Iwata H, Takada N, Kuroyanagi G, Ikuta K, Usami T, Sekiya I, et al. Effect of hydroxyapatite tubes on the lag screw intraoperative insertion torque for the treatment of intertrochanteric femoral fractures. Injury. 2021;52(11):3377-81.

LIST OF PUBLICATIONS

Publications related to the results of the dissertation

1. Sezgin EA, **Markevičiūtė V**, *Širka* A, Tarasevičius Š, Raina DB, Isaksson H, Tägil M, Lidgren L. Combined fracture and mortality risk evaluation for stratifying treatment in hip fracture patients: A feasibility study. Joint Disease and Related Surgery. 2020;31(2):163-168. doi: 10.5606/ehc.2020.73458.
2. Sezgin EA, Tor AT, **Markevičiūtė V**, *Širka* A, Tarasevičius Š, Raina DB, Liu Y, Isaksson H, Tägil M, Lidgren L. A combined fracture and mortality risk index useful for treatment stratification in hip fragility fractures. Joint Disease and Related Surgery. 2021;32(3):583-589. doi: 10.52312/jdrs.2021.382.
3. Raina DB, **Markevičiūtė V,** Stravinskas M, Kok J, Jacobson I, Liu Y, Sezgin EA, Isaksson H, Zwingenberger S, Tägil M, Tarasevičius Š, Lidgren L. New Augmentation Method for Improved Screw Fixation in Fragile Bone. A Front Bioeng Biotechnol. 2022 Mar 2;10:816250. doi:10.3389/fbioe.2022.816250.
4. **Vetra Markeviciute**, Manoj Puthia, Linnea Arvidsson, Yang Liu, Elin Törnquist, Alessandro Tengattini, Jintian Huang, Yiguang Bai, Corina Vater, Robertas Petrolis, Stefan Zwingenberger, Algimantas Krisciukaitis, Alfredas Smailys, Saulius Lukosevicius, Mindaugas Stravinskas, Hanna Isaksson, Sarunas Tarasevicius, Lars Lidgren, Magnus Tägil, Deepak Bushan Raina. Systemically administered zoledronic acid activates locally implanted synthetic hydroxyapatite particles enhancing peri-implant bone formation: A regenerative medicine approach to improve fracture fixation, Acta Biomaterialia (2024), doi: https://doi.org/10.1016/j.actbio.2024.03.005

Other publications

1. **Markevičiūtė V**, Markevičiūtė MŠ, Stravinskas M. Ollier Disease: A Case Series and Literature Review. Acta Medica Lituanica**.** 2021;28(1):181-188. doi: 10.15388/Amed.2021.28.1.8.
2. M. Stravinskas,**V. Markevičiūtė**, P.Bakutis. Osteosarkomos Diagnostikos Ir Gydymo Iššūkiai. Klinikinis Atvejis. Visuomenės Sveikata 29(3):63-68 DOI:10.5200/smhs.2019.038
3. Paškonienė, Aistė; Baltagalvienė, Renata; Lengvenis, Givi; Beleškienė, Vilma; Ivaška, Justinas**; Markevičiūtė**, Vėtra; Lesinskas, Eugenijus;

Mickevičienė, Vaiva. The importance of the temporal bone 3T MR imaging in the diagnosis of Meniere's disease. Otology and neurotology. Philadelphia: Lippincott Williams & Wilkins. ISSN 1531-7129. 2020, vol. 41, no. 2, p. 235-241. DOI: 10.1097/MAO.0000000000002471.

4. Paškonienė, Aistė; **Markevičiūtė, Vėtra**; Padvariškytė, Sigita; Lesinskas, Eugenijus. Diagnostinių tyrimų patikimumas diagnozuojant Menjero ligos tipus. Sveikatos mokslai. Vilnius. Sveikatos mokslai. 2019, t. 29, Nr. 4, p. 102-108. DOI: 10.35988/sm-hs.2019.064.
5. Paškonienė, Aistė; **Markevičiūtė, Vėtra;** Lengvenis, Givi; Baltagalvienė, Renata; Lesinskas, Eugenijus. Diagnostic features of the Meniere's disease Acta medica Lituanica. Vilnius : Lietuvos mokslų akademijos leidykla. DOI: 10.1097/MAO.0000000000002471
6. **Markevičiūtė V.,** Markevičiūtė M. Šilenė and Stravinskas M. Ankstyvoji metastazių kauluose diagnostika – patologinių lūžių prevencija. Internistas. Vilnius : "Baltijos idėjų grupė" ir partneriai, 2021, Nr. 3(208)5
7. **Markeviciute V,** Ramonaite J, Cekanauskas E, Stravinskas M. Surgical Management of Pediatric Osteosarcoma: Case Reports. Journal of Surgery. Lisle, IL : Gavin Publishers, 2022, vol. 7, no. 12 doi. org/10.29011/2575-9760.001538

LIST OF SCIENTIFIC CONFERENCES

The results of the dissertation were presented in the scientific conferences:

1. Petrolis, R., Markeviciute, V., Tarasevicius, Š., Raina, D., Lidgren, L., Lukosevicius, S. and Krisciukaitis, A. Mathematical Morphology Based Volumetric Analysis of Bone Density Around Implant in Post-Operational Follow-up of Per-Trochanteric Fractures. DOI: 10.5220/0011714600003414. In Proceedings of the 16th International Joint Conference on Biomedical Engineering Systems and Technologies (BIOSTEC 2023) - Volume 2: BIOIMAGING, pages 110-114 ISBN: 978-989-758-631-6; ISSN: 2184-4305
2. Vetra Markeviciute, Sarunas Tarasevicius, Saulius Lukosevicius, Mindaugas Stravinskas, Lars Lidgren, Deepak B Raina. Trochanteric femur fracture operated with dynamic hip screw system augmented with a biphasic apatite sulphate combined with local bisphosphonate. Nordic Orthopaedic Federation (NOF) Congress: 7-9 September 2022, Vilnius, Lithuania : Online abstract book / Editor Sarunas Tarasevicius ; : Eventas, 2022; ISBN 978-609-96167-6-6
3. Raina D, Markeviciute V, Arvidsson L, et al. A new method for improved mechanical and biological fixation of fracture fixation devices. Orthop Procs. 2023;105-B(SUPP_8):55-55. doi:10.1302/1358-992X.2023.8.055

APPENDIX A

LUNDS UNIVERSITET

Certificate of Course Completion
Laboratory Animal Science for Researchers – Rodents and Lagomorphs
May 12th 2023

Vetra Markeviciute
(1993-07-30)

has completed the full training in Laboratory Animal Science for Researchers – Rodents and Lagomorphs, 3 ECTS*, at Lund University Lund, Sweden.

The theoretical content of the course is in accordance with the FELASA (Federation of European Laboratory Animal Science Associations) recommendations of the theoretical education of persons carrying out experiments with laboratory animals (40 hours at a FELASA B equivalent level). The course is in accordance with Swedish and EU Legislation regulating the protection of animals used for scientific purposes.

Theoretical content online:
Ethics and Animal Use
Swedish Legislation, 2010/63/EU
Animal Records
Identification Methods
Biology
Husbandry
Animal Care and Supervision
Ethology
Humane Endpoints
Anaesthesia, Analgesia and Euthanasia
Diseases in Laboratory Animals
Animal Experimental Methodology
Genetically Modified Organisms
Alternative Methods

Practical Training (licensed species rats)
Handling and restraining
Injection i.p
Injection s.c.

Euthanasia

Approved Practical Training by:

Kim Arehov

Lena Uller, Education Officer and Course Director
Lund University

*only granted to PhD students

CURRICULUM VITAE

Name, Surname:	**Vėtra Markevičiūtė**
Address:	Hospital of Lithuanian University of Health Sciences Kauno klinikos, Eivenių 2, LT-50009 Kaunas, Lithuania
E-mail:	vetramarkeviciute@gmail.com

Education:

2018–2023	Lithuanian University of Health Sciences, Medical Academy, Postgraduate studies, licence of orthopeadic and traumatology surgeon
2012–2018	Vilnius University, Faculty of Medicine, Master`s degree of medicine

Are of activity: Orthopeadic and traumatology surgeon, active licence in Lithuania

Work Experience:

2023–present	Orthopeadic and traumatology surgeon, Department of Orthopaedic and traumatology, Hospital of Lithuanian University of Health Sciences, Kauno Klinikos
2018–2023	Resident doctor in orthopeadic and traumatology, Department of Orthopaedic and traumatology, Hospital of Lithuanian University of Health Sciences, Kauno Klinikos

PADĖKA

Esu labai dėkinga savo mokslinio darbo vadovui ir Draugui Deepak Bushan Raina už nuolatinę pagalbą, pozityvumą, profesionalumą, pastabas bei puikius patarimus.

Noriu padėkoti profesoriui Šarūnui Tarasevičiui už skirtą laiką, paramą bei tyrimo idėją.

Noriu padėkoti Lietuvos sveikatos mokslų universiteto ligoninės Kauno klinikų Ortopedijos traumatologijos klinikos kolektyvui už paramą ir ypatingai noriu padėkoti klinikos vadovui profesoriui Alfredui Smailiui už sudarytas sąlygas atlikti mokslinį darbą bei už nuolatinį skatinimą siekti geriausių rezultatų bei palaikymą.

Dėkoju gydytojams bei kolegoms iš Švedijos Lundo Universiteto, ypatingai profesorui Lars Lidgren, profesoriui Magnus Tagil bei Mathias Lidgren.

Dėkoju gydytojams bei kolegoms iš Lietuvos sveikatos mokslų universiteto ligoninės, Kauno klinikų, ypatingai profesoriui Sauliui Lukoševičiui, profesoriui Algimantui Kriščiukaičiui, Robertui Petroliui ir gydytojui Mindaugui Stravinskui.

Dėkoju gydytojams Valdui Dumbliauskui ir Arūnui Marcinkui už pagalbą priimant sprendimus gyvenimo kelyje ir nuolatinį palaikymą gydytojui Vytautui Kimčiui.

Dėkoju savo Mamai ir Tėčiui už palaikymą ir meilę, už visas gyvenimo pamokas. Draugams, už meilę ir palaikymą, nes be Jūsų palaikymo nebūčiau ten kur esu ir labai didelis AČIŪ – mano sesei, Medeinei Šilenei, už tai, kad esam komanda visur ir visada.