Dissertation

MICROSTRUCTURAL ANALYSIS OF SUBCHONDRAL BONE IN KNEE OSTEOARTHRITIS

submitted by

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Dedicated to all my beloved ones who influenced my way of life and thinking.

Declaration

I hereby declare that this dissertation is my own original work and that I have fully acknowledged by name all of those individuals and organisations that have contributed to the research for this dissertation. Due acknowledgement has been made in the text to all other material used. Throughout this dissertation and in all related publications I followed the guidelines of "Good Scientific Practice".

Graz, 18.04.2015

Signature

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1. List of Abbreviations

ACL	Anterior Cruciate Ligament
a.p.	anterior-posterior
BMI	Body Mass Index
BS	Bone Surface
BSA/BV	Bone Surface Area / Bone Volume
BS/BV	Specific Bone Surface
BS/TV	Bone-surface-to-volume Ratio
BS/TV	Bone Surface Density
BV	Bone Volume
BV/TV	Bone Volume Fraction
BMD	Bone Mineral Density
Cm	Centimeter
COX-2	Prostaglandin-endoperoxide synthase 2
CT	Computed Tomography
Ct.Ar/Ct.Ar	Cortical Bone Area Fraction
Ct.Th.	Cortical Thickness
Ct.Ar.	Cortical Bone Area
DMOADs	Disease-modifying Osteoarthritis Drugs
DXA	Dual-energy X-ray Absorptiometry
FEA	Finite Element Analysis
HA	Hydroxyapatite
IGF	Insulin-like Growth Factor
IL-1	Interleukin-1
IL-6	Interleukin-6
JSN	Joint Space Narrowing

JSW	Joint Space Width
K&L	Kellgren & Lawrence Scale
Kg	Kilogram
KSS	Knee Society Score
lat.	lateral
MACI	Matrix-induced Autologous Chondrocyte Implantation
Mikro-CT	Mikro Computed Tomography
µCT	Micro-Computed Tomography
MMPs	Metalloproteinases
MRI	Magnetic Resonance Imaging
NSAIDs	Non-steroidal anti-inflammatory drugs
OA	Osteoarthritis
OPG	Osteoprotegerin
OR	Odds Ratio
PCL	Posterior Cruciate Ligament
PTH	Parathormone
RANK	Receptor Activator of Nuclear Factor kappa-B
RANKL	Receptor activator of nuclear factor kappa-B ligand
ROI	Region of Interest
SERMs	Selective Estrogen Receptor Modulator
SI	International Systems of Units (Système International d'Unités - SI)
TGF β	Transforming Growth Factor β
THA	Total Hip Arthroplasty
TJR	Total Joint Replacement
TKA	Total Knee Arthroplasty
TMD	Tissue mineral density
TNF- α	Tumor Necrosis Factor-alpha
Tr.N.	Trabecular Number

Tb. Pf.	Trabecular Pattern Factor
Tr.Th.	Trabecular Thickness
Tr.Sp.	Trabecular Separation
Tt.Ar.	Total Cross-sectional Area
TV	Total Volume of Interest
UKA	Unicondylar Knee Arthroplasty
VEGF	Vascular Endothelial Growth Factor
VOI	Volume of Interest
WOMAC	Western Ontario and McMaster Universities
µCT	Micro-Computed Tomography

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Figure 14. Saggital plane of a representative bone sample. As second step, the first coronal slice of each predefined VOI was manually adjusted in the sagittal plane and automatically extended over the range of 4 mm.

Figure 15. Mean subchondral bone fraction volume in the five analysed locations: medial meniscus (MT) (1), medial tibial compartment (MT) (2), anterior cruciate ligament (ACL) (3), lateral tibial compartment (LT) (4), lateral meniscus (LM) (5).

Figure 16. Mean subchondral bone fraction volume in the 5 analysed locations in respect to intact (green line) and subluxated or luxated (blue line) medial menisci. Location 1 is submeniscal medial, whereas location 2 is the medial tibial plateau.

Figure 17. Mean subchondral bone fraction volume in the 5 analysed locations in respect to intact (green line) and subluxated or luxated (blue line) lateral menisci. Location 1 is submeniscal lateral, whereas location 2 is the lateral tibial plateau.

Figure 18. Mean subchondral bone fraction volume in 5 analysed locations in respect to knee joint alignment. Varus (blue line) and valgus alignment (green line).

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5. Abstract

Background: Subcondral bone changes seem to contribute to the progression of osteoarthritis (OA). In early-stage OA there is a high rate of bone remodelling in the subchondral bone, whereas in late-stage disease this process slows down and subsequently bone formation increases. This results in subchondral bone thickening that is evident in X-rays of advanced stage disease. Whether these changes are the initiation or the result of cartilage degradation is not clear yet. Bone and cartilage are seen as a functional unit in which damage in one tissue will affect the other and vice versa, respectively. Microstructural analysis of late-stage OA of the hip demonstrated an increased density of subchondral in areas beneath cartilage damage.
This study aimed to analyse subchondral bone microstructure in specimens of late-stage knee OA in respect to articular cartilage damage, meniscus integrity and knee joint alignment.

Materials & methods: 30 proximal tibiae of 30 patients (20 female and 10 male) with late-stage OA that were retrieved during total knee arthroplasty (TKA) were scanned using a high-resolution Micro-Computed Tomography (µCT). The scans were semi-automatically segmented into five regions of interest (ROIs). The ROIs were than further analysed using a commercially available software. The degree of articular cartilage damage was assessed semiquantitatively by magnetic resonance imaging (MRI) before surgery.

Results: The mean bone fraction volume in the medial compartment was significantly higher compared to the mean bone fraction volume in the lateral compartment (62,07 ± 12,53 vs. 52,57 ± 13,33). The differences were statistically significant (p=0,007). The submeniscal mean bone fraction volume in the medial compartment was statistically significantly higher compared to submeniscal mean bone fraction volume in the lateral compartment (56,76 ± 12,8 vs. 47,36 ± 14,97; p=0,015). There was a significantly lower bone fraction volume in the medial submeniscal subchondral bone compared to the subchondral bone fraction volume from the medial tibial plateau compartment (p=0,041). There was a significant difference in the lateral submeniscal subchondral bone fraction volume compared to the subchondral bone fraction

volume from the lateral tibial plateau compartment (p=0,024). The bone fraction volume in all weight bearing locations (medial meniscus, medial tibial plateau, lateral meniscus, lateral tibial plateau) was significantly higher compared to the non weight-bearing reference point below the ACL (p=0,000).
In intact menisci, there was a significantly lower subchondral bone fraction volume compared to subluxated or luxated meniscus in the medial (p=0,020) and lateral compartment (p=0,005).
Varus alignment had a significantly higher subchondral bone fraction volume in the the medial compartment than valgus, whereas valgus alignment had a significantly higher subchondral bone fraction volume in the lateral compartment (p=0,011).

Conclusion: The results of this study show significant differences of subchondral bone microstructural parameters in respect to cartilage damage, meniscus' structural integrity and knee joint alignment. Therefore, subchondral microstructural bone changes seem to be a secondary process in the late-stage OA of knee caused by mechanical changes and the proper management of these changes might prevent or stop the progression of OA.

Furthermore, during preparation of this study an intensive literature search and subsequent analysis (citation analyses) in the field of bone, joint and arthroplasty was performed that resulted in two publications of most cited research papers in arthroplasty as well as osteoporosis and associated diseases.

5. Abstract in German (Zusammenfassung)

Hintergrund: Veränderungen im Bereich des subchondralen Knochens scheinen an der Progression der Arthrose beteiligt zu sein. In der Frühphase der Arthrose besteht ein hohe Knochenumbaurate, während sich in der Spätphase der Erkrankung die Umsatzrate reduziert und in weiterer Folge die Knochenformation überwiegt. Diese Veränderungen sind auch in konventionellen Röntgenbildern von Patientinnen mit fortgeschrittener Arthrose bemerkbar. Ob diese Änderungen der Auslöser oder das Resultat der Knorpeldegeneration sind ist bislang noch unklar. Knochen und Knorpel scheinen eine funktionelle Einheit zu sein, in welcher Änderungen bzw. Schäden des einen Gewebes das Andere beeinflussen kann. Mikrostrukturelle Analysen bei fortgeschrittener Coxarthrose zeigten eine erhöhte Dichte des subchondralen Knochens unterhalb von Arealen mit Knorpelschäden.
Das Ziel dieser Studie war die Analyse der subchondralen Mikrostruktur des Knochens Gewebsproben von Patinetinnen mit fortgeschrittener Gonarthrose in Hinblick auf Knorpelschaden, Meniskusintegrität Kniegelenksalignment.

Material und Methoden: 30 proximale Tibiae, die im Rahmen Implantation von Knietotalendoprothesen reseziert wurden, wurden mittels eines hochauflösenden Micro CTs gescannt. Die CT Scans wurden in weiterer Folge mittels einer kommerziellen Software analysiert. Der Grad der Knorpelschädigung wurde anhand präoperativer MR Bilder evaluiert.

Ergebnisse: Die mittlere bone fraction volume im medialen Gelenkskompartment war signifikant höher verglichen zur mittleren bone fraction volume im lateralen Gelenkskompartment (62,07 ± 12,53 vs. 52,57 ± 13,33). Die Unterschiede waren statistisch signifikant (p=0,007). Die submeniscale bone fraction volume im medialen Gelenkskompartment war signifikant höher verglichen zur mittleren submeniskale bone fraction volume im lateralen Gelenkskompartment (56,76 ± 12,8 vs. 47,36 ± 14,97; p=0,015). Es zeigten sich eine signifikant niedrigere submeniscale bone fraction volume im medialen Gelenkskompartment verglichen zur bone fraction volume im medialen Gelenkskompartment (p=0,041). Es zeigten sich eine signifikant niedrigere submeniskale bone fraction volume im lateralen Gelenkskompartment verglichen zur bone fraction volume im lateralen Gelenkskompartment (p=0,024). Die

bone fraction volume in sämtlichen Gewichtsbelasteten Lokalisationen (medialer Meniskus, mediales Tibiaplateau, lateraler Meniskus, laterales Tibiaplateau) war signifikant höher verglichen zum unbelasteten Referenzmesspunkt unterhalb des vorderen Kreuzbandes (p=0,000).
Bei intakten Menisci zeigte sich eine signifikant niedrigere subchondrale bone fraction volume verglichen zu subluxierten oder luxierten Menisci, sowohl im medial (p=0,020) als auch im lateralen Gelenkskompartment (p=0,005).
Bei varischer Achsfehlstellung zeigte sich signifikant höhere suchondrale bone fraction volume verglichen zu valgischer Achsfehlstelung im medialen Gelenkskompartment. Valgische Achsfehlstellung wies eine signifikant höhere subchondrale bone fraction volume im lateralen Gelenkskompartment auf (p=0,011).

Conclusio: Die Ergebnisse dieser Studie zeigen signifikante Unterschiede der subchondralen Mikrostrukturparameter in Bezug auf Chondropathie, struktureller Integrität der Menisci und Kniegelenksachse. Daher, scheinen die Änderungen im subchondralen Knochen bei fortgeschrittener Gonarthrose sekundäre Prozesse zu sein, die durch mechanische Einflüsse verursacht werden. Die Vermeidung dieser mechanischen Einflüsse scheint daher ein potenzieller Angriffspunkt um der Entwicklung einer Gonarthrose vorzubeugen beziehungsweise das Fortschreiten dieser zu verhindern.

Darüber hinaus wurde in Vorbereitung dieser Studie eine intensive Literatursuche und darauffolgende Zitierungsanalyse im Bereich von Knochen- und Gelenksforschungs sowie Hüft- und Knietotalendoprothetik durchgeführt. Diese Analysen resultierten in zwei Publikationen zu Zitierungsanalysen von Literatur im Bereich von Osteoporose und assoziierten Erkrankungen sowie von Literatur im Bereich der Endoprothetik.

6.1. Osteoarthritis

Osteoarthritis (OA) is a long-term chronic disease that is characterized by the deterioration of cartilage in joints resulting in stiffness, pain, and impaired range of motion (ROM). OA most commonly affects the joints of the hip, knee and feet as well shoulder and hands. OA is a disease that is associated with ageing. However there is also a variety of intrinsic and extrinsic factors that predispose patients for OA e.g. obesity, lack of exercise, genetical aberrations, bone density, occupation, trauma and gender [1].

OA is classified in two groups: (1) Primary OA that can occur localized or systematically and (2) Secondary OA that has an underlying cause such as obesity, inflammatory arthritis or trauma.

OA is the most common form of arthritis [1, 2, 3, 4, 5]. The World Health Organization estimates that globally 25% of adults over the age of 65 years have clinically symptomatic osteoarthritis of any joint [3]. It ranks fourth in health impact in women and eighth in men in the civilized western world. OA ranks second only to cardiovascular disease as a cause of disability (e.g. walking or stair climbing) [1, 2]. OA affects patients and health care systems worldwide. Due to the socio-demographic changes in the civilized western world preventive measures are becoming increasingly important [2, 4].

6.1.1. Clinical Features and Symptoms

Usually patients with OA are over the age of 50 years and present with pain and stiffness in the affected joint, that generally increases with activity and decreases by rest. Early morning stiffness is a common symptom that can be present for up to half an hour [5, 6].

In the clinical examination tenderness of the joints and crepitus on movement can be seen. Swelling is common. It might result from effusion caused by increased synovial fluid production and accumulation or result from architectural bone changes due to osteophytes. Various specific questionnaires and scales exist that allow to evaluate the stage of OA clinically.

Blood samples or laboratory parameters are generally in normal range and therefore have no value in diagnosis.

Joint puncture can be useful in the differential diagnosis of septic or rheumatoid arthritis [6].
Diagnosis of OA relies on medical history and X-rays. Radiographic changes occur late in the disease and are largely irreversible. Theoretically molecular markers may be able to detect early osteoarthritic changes. A variety of potential markers exist. These reflect remodelling of bone, cartilage and synovium. However none of these markers have been found to be specific for OA [6].

6.1.2. Pathogenesis

OA is a dynamic degenerative process that may progress episodically. It is viewed as an adaptive response of synovial joints to a variety of environmental, genetic and biomechanical stresses [7].
Cartilage's extracellular matrix is made of water (70%) and a collagen type II framework of proteoglycans and glycosaminoglycans (consisting of chondroitin and aggrecan), that are produced by chondrocytes. Proteoglycans bind to hyaluronate that allows stabilisation of the macromolecule. By diffusion, chondrocytes receive their nutrition from the synovium. The synovial fluid is circulated by joint motion. It has been hypothesized that if immobilisation occurs, chondrocytes lose their source of nutrition and therefore as a result cartilage repair ceases. Metalloproteinases (MMPs) are produced that catalyse collagen and proteoglycan degradation. It has been shown that the synovium can be inflamed in OA. Therefore the production and levels of interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) as well as cytokines activity that induces nitric oxide and MMP production increases. Interleukin-6 (IL-6) and mechanical loading of the joint induce catabolic activity of cytokine receptors. Cytokine receptors in turn bind IL-6 and TNF- α within cartilage causing further destruction [7].
Osteophytes and subchondral bone sclerosis are mainly believed to be secondary changes to cartilage loss. These changes are seen as reaction to compensate cartilage loss. However, there is also data that shows subchondral bone changes before cartilage deterioration. These bone changes are thought to cause further degradation of cartilage covering it (For detailed information see chapter Bone changes in Osteoarthritis) [7].

6.1.3. Risk factors

Age

The ageing process seems to cause laxity around the joints, reduced joint proprioception, cartilage calcification, reduced chondrocyte function as well as loss of water content, degradation of collagen and loss of glycoproteins in the extracellular matrix. All of these factors contribute to OA progression [8, 9, 10].

Trauma

Instability due to trauma of ligaments such as cruciate or collateral ligaments or structural changes of intraarticular objects such as meniscal tears lead to an increased risk of OA. Patients with a history of knee injury were at a 5-6 fold increased risk of developing OA. This usually occurs in a younger age group. Evidence is accumulating that meniscectomy results in an increased risk of developing knee osteoarthritis [8, 11].

Occupation

OA is more common in professions that have high physical demands. Knee bending, squatting or kneeling predisposes OA. Dockers or miners have been found to be more likely to get OA due to kneeling or repetitive use of their joints [12, 13].

Exercise

Professional sports persons that perform high impact sports have an increased risk of OA. This might results from injuries of tendon or ligaments around the joints or multiple impacts e.g. the metatarsophalangeal (MTP) I joint in soccer players [9, 12].

Gender and ethnicity

Generally women have a higher overall prevalence and incidence of OA than men. This gets more balanced after the age of 80 years. Hormonal changes (estrogen loss at menopause) are believed to be a trigger. In the age group of under 50 years of age incidence and prevalence is higher in men. Generally, the prevalence is higher in Caucasians than in people from other descent [14, 15].

Genetics

Data is accumulating that OA is linked to genetic changes. This could be shown in twin studies. Furthermore various genes have been linked with OA. Most concordance was shown for chromosome 2q, 4 and 16. Families with rare autosomal dominant patterns of inheritance of OA have been found. The defected genes often code for structural proteins of the extracellular matrix of joint and collagen proteins. Children of patients with early onset OA are at a higher risk to develop OA themselves [16].

Obesity

Body weight is the strongest extrinsic risk factor. During walking up to six times the body weight is transferred across the knee joint. The body weight has to be multiplied with this factor to estimate the forces and impacts in the knee joint of obese patients. A two unit increase of the body mass index (BMI) (~ 5 kg) increases the odds ratio (OR) to 1.36 for developing knee osteoarthritis. High body weight also increases the risk of contralateral knee OA development when one joint is already affected. Losing 5 kg of weight reduced the risk of symptomatic knee osteoarthritis by 50% in women with average height.
There is also a higher incidence of OA in obese patients in non-weight bearing areas such as the finger joints. This suggests that factors such as adipokines promote cartilage degeneration and subsequently osteoarthritis [10, 14].

Diet

A threefold risk of progression of knee OA was associated with low levels of vitamin C and vitamin D blood levels. However, no evidence supports the intake of vitamin C or Vitamin D to prevent from developing OA [8, 17].

Bone density

There is an inverse relationship between bone density and osteoarthritis. Increasing subchondral bone density may lead to increased loading through weight-bearing joint cartilage. More details can be seen below [12, 18]. (More detailed information can be found in chapter Bone Changes in Osteoarthritis)

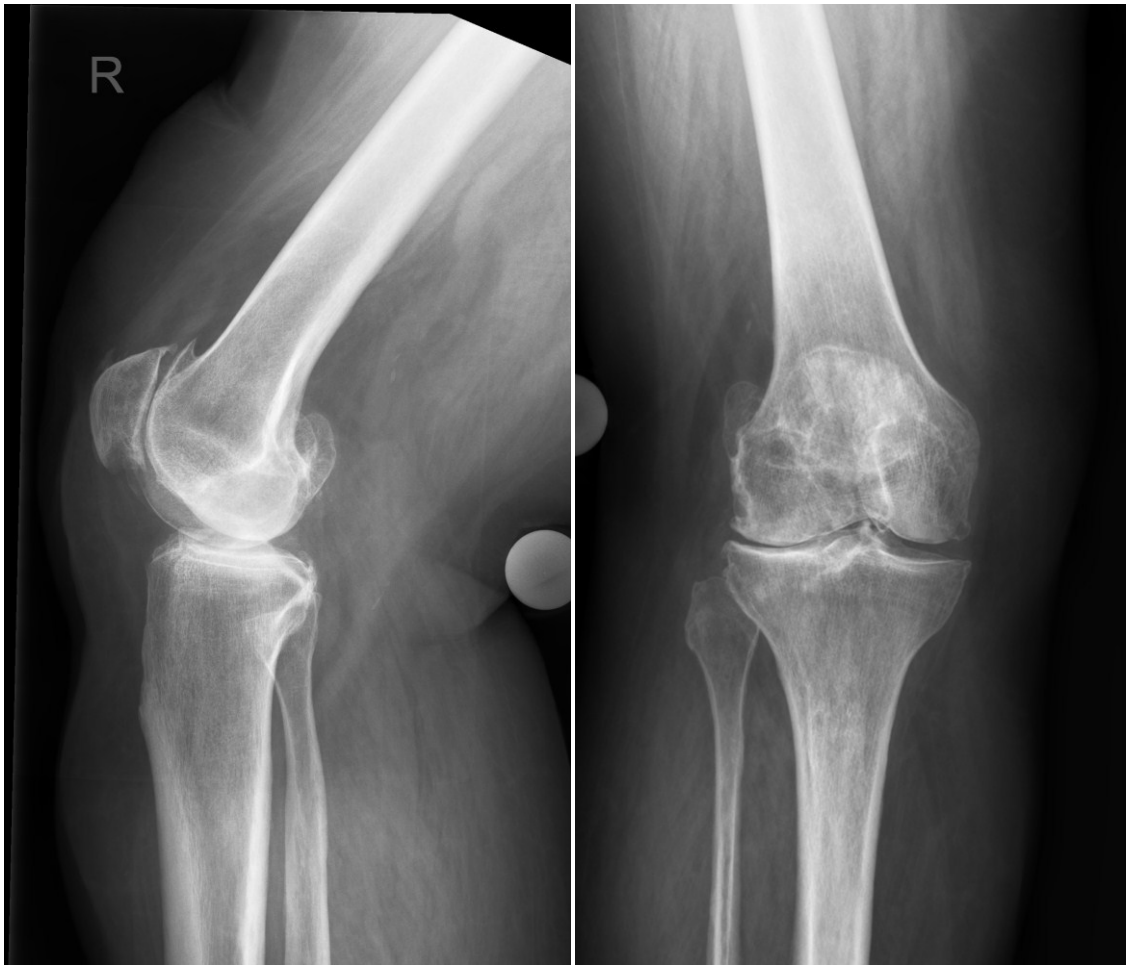
6.1.4. Imaging

6.1.4.1. Conventional X-rays

X-rays are cheap, provide a permanent record and are easily available. Therefore conventional X-ray is the golden standard imaging modality for diagnosis of OA. Progression of the disease, however, cannot be measured well by X-rays. X-rays of the affected joint should be done in anterior posterior (a.p.) and lateral (lat.) planes. The following changes can be seen on plain radiographs [Figure 1] [19, 20, 21]:

Joint space narrowing (JSN) (indirect surrogate of cartilage thickness)
Osteophytes
Bone cysts
Subchondral sclerosis

Figure 1. X-rays of the right knee joint of a 74-year-old female patient with OA (Grade 4 according to K&L classification) in lateral (left) and anterior-posterior (right) plane.



The severity of OA can be estimated using semiquantitative scoring systems. The most widely used system is the Kellgren and Lawrence (K&L) classification [22]:

Grade 0	no radiographic features of OA are present
Grade I	doubtful joint space narrowing (JSN) and possible osteophytic lipping
Grade II	definite osteophytes, definite JSN
Grade III	moderate multiple osteophytes, definite JSN, some sclerosis and possible deformity of bone contour
Grade IV	large osteophytes, marked JSN, severe sclerosis and definite deformity of bone contour

6.1.4.2. Magnetic resonance imaging (MRI)

MRI has no place as routine clinical assessment of OA. However, it offers a number of advantages for OA imaging. MRI offers a tomographic viewing perspective. Therefore cross-sectional images of the anatomy, free of projectional limitations of conventional radiographs are provided. MRI allows evaluation of all components of a joint including the articular cartilage, menisci, intra-articular ligaments, synovium, effusion, bone attrition, bone marrow lesions, subchondral cysts and intra- and periarticular cystic lesions. Therefore, in MRI, the joint can be seen as a whole organ. MRI also allows to detect OA at preradiographic stage and therefore at an earlier time point. A variety of methods exist in combination with MRI exist that allow OA staging and monitoring of progression, assessment of biochemical properties and cartilage morphology [23, 24].
The severity of OA can be estimated using MRI based semiquantitative scoring systems. A widely used system is the Yuslih classification [Yulish Radiology 1987].

Grade 0	normal contour and signal
Grade 1	normal contour with abnormal signal
Grade 2	<50% reduction of cartilage thickness
Grade 3	>50% reduction of cartilage thickness
Grade 4	full thickness or nearly full thickness cartilage defect

6.1.4.3. Other imaging techniques

Computed tomography (CT) has little advantage over plain X-rays unless an axial plane is required in the limbs. CT has an established role in assessing facet joint OA of the spine. Furthermore cortical bone and soft tissue calcifications can be better assessed by CT than MRI [5].
In bone scans radiopharmaceuticals are used to visualize skeletal metabolism. This allows to localize the disease and to assess the severity of pathologic changes in OA. However, bone scans are considered an inadaequate method for investigating OA [5].
Ultrasound is a method that enables multiplanar and real-time imaging at a relatively low cost without radiation exposure. It is allows to image soft tissue, synovial

pathology and assessment of cartilage integrity and destruction. Ultrasound is limited by physical properties. In most weight bearing and deep articular joint however is not easily accessible [5].

6.1.5. Management of OA

The management of patients with OA should be individualised and patient centred. There should be an agreement on the management by both, the patient and the doctor. Non-surgical, non-pharmacological means should be tried first. If patient condition changes the treatment plan should be further adapted.
The management of OA includes patient education, pain control, improvement of function by exercise and physiotherapy, weight loss, use of medical aids, pharmacological intervention and surgery.

6.1.5.1. Non-drug therapy

Education and community support
Formal education of the patient should be the cornerstone and an initial part of the non-pharmacological management of OA. They should be involved in the management of their condition as much as possible. Patients should know about their condition and its progress. Education can be achieved by the use of books or education groups. It has been suggested that education contributes to pain reduction [26].

Exercise programs

Exercise and activity are the most important first line interventions. Inactivity due to the pain of osteoarthritis leads to a reduction of muscle mass surrounding the joints. This leads to a destabilisation of the joints. Physical activity including aerobic, resistance land-based exercises, aquatic exercise, local muscle strengthening (e.g. M. quadriceps femoris) and general fitness is needed to build up muscle strength, improve flexibility and joint range of motion. There are numerous studies that show evidence for the benefit of exercise on OA. Muscular strengthening may improve muscular balance, decrease impact loads and support function. Furthermore it was

reported that it also helps reducing pain in both hip and knee OA. Water-based exercises have been shown to be beneficial in short term pain reduction. Moderate regular running seems to be safe and do not accelerate the development of OA [27].

Weight loss

In obese people, weight loss seems to reduce OA related pain and seems to be beneficial in respect of cartilage thickness in weight bearing areas. Weight loss also positively affects the proteoglycan content in the cartilage [28, 29].

Mechanical aids and Orthotic devices

Orthotic devices including shock absorbing footwear, insoles, knee bracing and canes are recommended for patient with hip and/or knee OA. In knee OA shock absorbing footwear reduces the impact of load on the knee joint. Heel wedging improves proprioception and reduces pain in knee OA. Medially-wedged insoles can be used for patients with lateral compartment knee OA and vice versa. Walking aids such as canes can be used to support patients' mobility. For most of these interventions however there is no evidence [5].

Alternative therapies

Acupuncture and transcutaneous electrical stimulation have been reported to be beneficial for short-term pain relief in OA [29, 30].

6.1.5.2. Drug therapy

In pharmacological therapy, we differentiate between (1) symptomatic drugs that aim to reduce symptoms (pain) and (2) slow-acting symptomatic drugs.

Symptomatic drugs - Oral analgesics

The first-line therapeutic option for mild to moderate pain is Acetaminophen (Paracetamol). It is available at low costs and is effective and safe. It can be used to alleviate pain and as long-term oral analgesic [29, 30].
Non-steroidal anti-inflammatory drugs (NSAIDs) are generally recommended for patients who are unresponsive to acetaminophen. These should be described at the lowest dose and for the shortest possible duration. For patients with gastrointestinal bleeding within the past year, the use of COX-2 inhibitor in combination with proton-pump inhibitor is recommended. COX-2 inhibitors were shown to be as effective as NSAIDs with fewer gastrointestinal bleeding, however, with an increased potential for cardiovascular risk [29, 30, 31].
Opioids are an option for patients who either have contraindications or intolerance to NSAIDs or who failed to respond to acetaminophen and NSAIDs [29, 30, 31].

Symptomatic drugs - Topical treatments

Topical therapies can be used to decrease the consumption of analgesics. Topical NSAIDs seem to be as effective as oral NSAIDs. However, there is a lower risk of gastrointestinal bleeding. Their primary adverse effect is local skin reactions. Generally they are recommended as adjuvant or alternative therapy [29, 30, 31].

Symptomatic drugs - Injectible therapies

Intra-articular injection of a long-acting corticosteroid can be applied for relief of pain from OA flares. Especially when they are accompanied by effusion and when NSAIDs are ineffective. Intra-articular corticosteroid injections are recommended for the initial management of hip and knee OA. Pain reduction can be achieved, however no functional benefits are seen [29, 30, 31].
Viscosupplementation is recommended for knee OA. It shows a delayed, but prolonged beneficial symptomatic effect. In hip OA viscosupplementation is as effective as placebo [29, 30, 31, 32].

Symptomatic slow-acting drugs for osteoarthritis

Disease-modifying agents slow the progression of OA in addition to pain reduction. Chondroitin and glucosamine-sulphate have been widely used in OA for pain relief. Their use is safe and has possible structure-modifying effects. Glucosamine sulphate is a naturally occurring amino monosaccharide and chondroitin sulphate belongs to the group of glycosaminoglycans and is a major component of articular cartilage [29, 30, 31, 33].

6.1.5.3. Surgery

Primum non nocere ("First, do not harm") is a statement that can also be considered for the management of OA. The first choice of treatment should always be non-surgical. However, when pain persists under adequate analgesics and functional loss progresses, surgical intervention should be considered to decrease disease morbidity. Surgical options for knee OA include arthroscopy with lavage and debridement (the value of which is discussed intensively and also controversially), cartilage repair surgery (bone marrow stimulating techniques, transplantation of osteochondral grafts, matrix-induced autologous chondrocyte implantation (MACI)), osteotomy with limb axis correction and arthroplasty (either unicondylar knee arthroplasty (UKA) or total joint replacement (TJR)). Arthroscopic interventions are discussed controversially as their benefit is primarily short-term and the intervention does not lead to a delay of OA progression. Minimal invasive arthroscopic techniques include chondral surface debridement, lavage of articular joints, removal of loose bodies or torn meniscal fragments, but also repair of meniscal structures.
Arthroscopy should be chosen for selected patients with persisting clinically symptomatic meniscal tears that prior have undergone non-surgical interventions such as physiotherapy. In these patients degenerative meniscal fragments can be resected and mechanical symptoms reduced. Bone marrow stimulation through the use of a microfracture technique induces subchondral bone trauma and subsequently bleeding that might cause chondrogenesis by mesenchymal stem cells in the defect area. However, its efficacy is uncertain. There is no good evidence that supports this technique's use [34].
Patients with OA in one compartment (either medial or lateral) in case of malignment of the knee can be treated with corrective osteotomy. It allows a load bearing shift to

the normal compartment and therefore reduces pain and also delays the need for TJR. In neutral alignment and OA of one compartment UKA can be performed if anterior and posterior ligaments are intact. UKA seems to be safe and functionally even better than total joint replacement. Long-term survival rates with UKA are reported to be around 90% at 10-year follow-up. This is inferior to TJR (up to 98% at 15 years follow-up) [34, 35].
Total knee arthroplasty (TKA) is a successful method for end-stage symptomatic OA of the knee [Figure 2]. The main complications are persisting pain, periprosthetic infection, aseptic loosening and reduced range of motion.

The same is true for the hip, where total hip arthroplasty (THA) poses a successful intervention for end-stage symptomatic OA of the hip [Figure 3]. Prior to that corrective osteotomies can be performed in cases of malalignment of the hip. However this is predominantly done in younger patients [29, 31, 34].
Arthrodeses might be applied in advanced stage of OA. This technique is mainly applied in OA of the spine, hand, foot or ankle [36, 37].

Figure 2. Lateral (left) and a.p. (right) X-rays of the right knee joint of a 77-year-old male patient showing a Total Knee Arthroplasty.

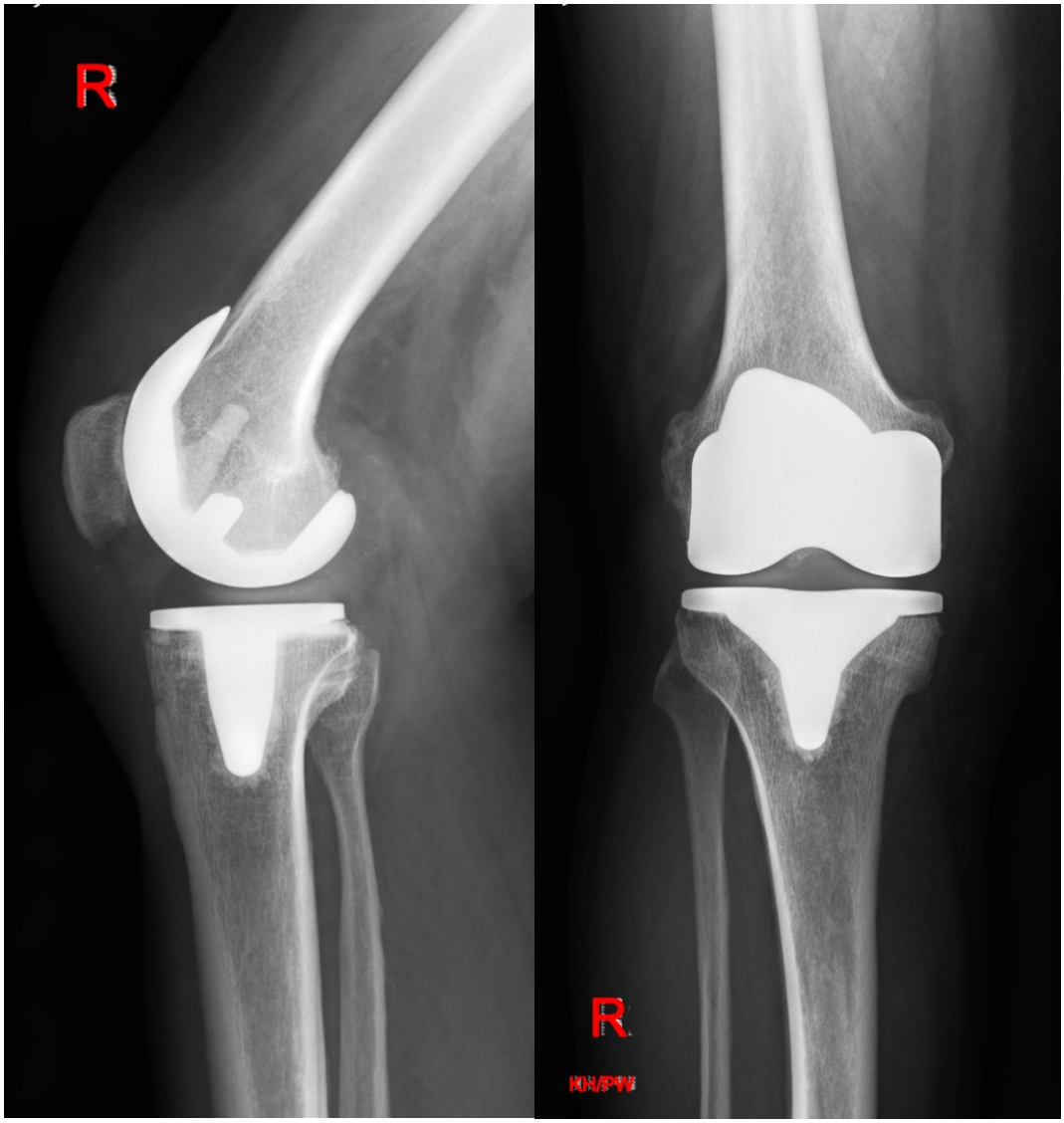
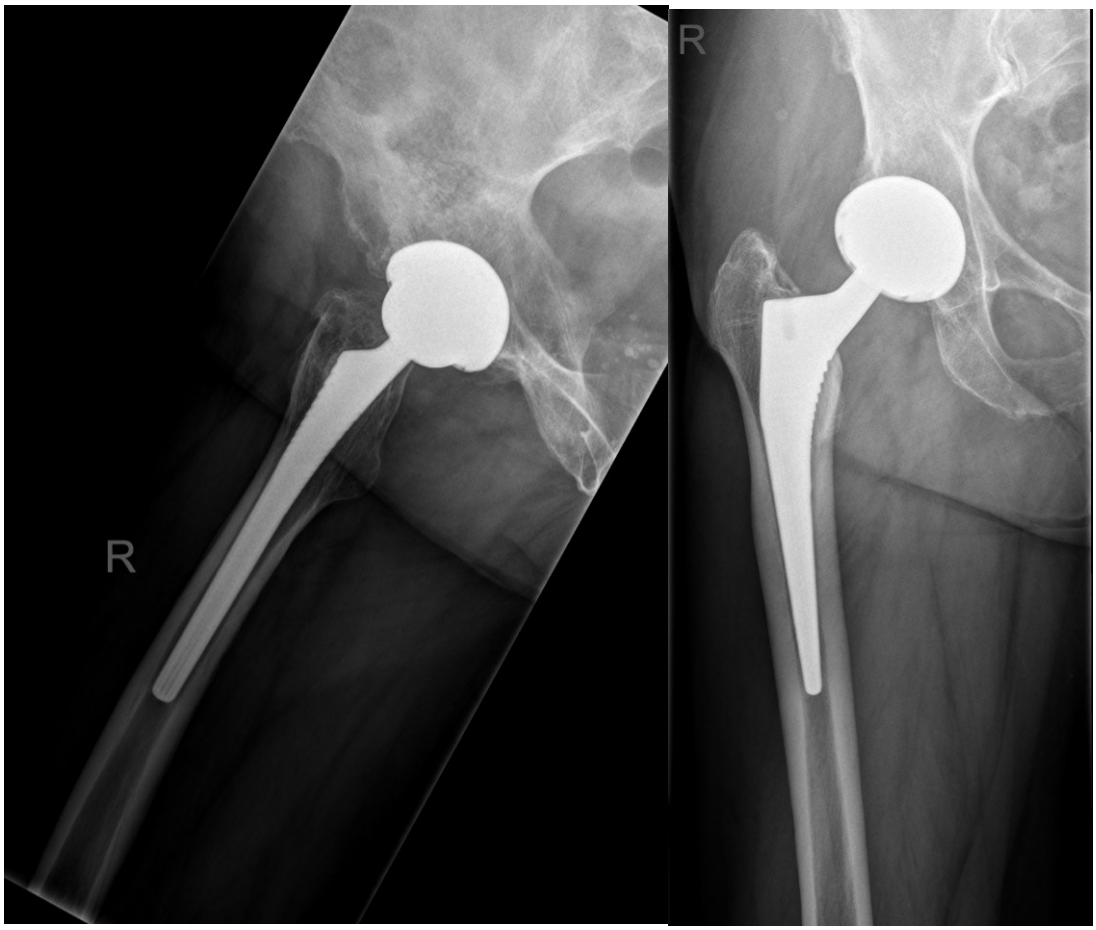


Figure 3. X-rays in lateral (left) and a.p. (right) view of the right hip joint of a 71-year-old male patient showing a Total Hip Arthroplasty (THA).



6.2. Bone changes in Osteoarthritis

The bone that lies beneath articular joint cartilage is composed of different specific anatomical regions including the (1) subchondral cortical plate (2) subchondral trabecular bone and (3) subarticular bone [18].
Each of these regions is likely to contribute to OA pathology in a distinct manner. Current imaging modalities however do not allow detecting clear borders between these tissue regions. Bone at joint margins is active and is often the origin of osteophyte development in primary OA. The close relationship between subchondral bone and joint cartilage make them a functional unit. Both tissues are mechanically and biologically connected. Therefore subchondral bone is important to physiological joint function. Mechanical or biological changes of subchondral bone will affect cartilage and vice versa [38].

Subchondral sclerosis is a classic sign of the progression of OA. Its role in the progression of OA has been discussed controversially whether it initiates its progression or can be seen as a consequence of the catabolism of the articular cartilage. One definite role of subchondral bone in the articular joint is to distribute forces, prevent stress concentrations and adapt to maintain conformation of the joint [18].
Articular cartilage is designed for loadbearing. Its high water content allows deformation under compressive loads without failure. Its capacity to withstand tension or shear stresses that occur at the edges of joint contact regions is lower. Such high stresses (either shear or tension) predispose to splitting or fibrillation of cartilage. This could be exacerbated in inhomogenously dense and stiff areas where cartilage would deform more in regions overlying less dense parts of subchondral bone than in denser regions. The regions between these stiff and less stiff regions are sites of stress concentration at which cartilage is more likely to fail under tension conditions [18].

6.2.1. Bone remodelling in early-stage OA

In early OA the mineral apposition is increased by threefold-fivefold [39, 40]. There is also an increase of new remodelling sites within subchondral bone. Increased rates

of bone remodelling are associated with transient loss of bone, increased porosity and reduced density in the subchondral area. In contrast to bone sclerosis, all of these processes reduce the thickness of the subchondral bone [41]. Thinning of subchondral bone in early OA have been shown in various animal models by either creating grooves on the femur condyles or transecting the anterior cruciate ligament (ACL). Increased cartilage damage was also associated with thinning of the subchondral bone [42].
Similar results were shown in humans with early-stage OA. Resorption markers (C-terminal and N-terminal peptides, CTx and NTx) were elevated in patients with progressive disease [43, 44]. Bone resorption and cartilage loss seem to be spatially associated within a single joint with a seven-fold increase in the risk of cartilage loss in regions with subchondral attrition [45]. Changes in subchondral bone mineralization and volume occur only beneath the areas of significant cartilage destruction [46]. Increased rates of bone remodelling in early-stage OA may cause alterations in joint shape and load transmission that predispose to progressive cartilage loss.

Causes of increased remodelling

The causes of increased bone remodelling in early OA are no quite known. Various mechanisms have been suggested that may be involved including cellular signalling for microdamage repair, stimulation of vascular invasion by angiogenic factors and bone-cartilage crosstalk via subchondral pores.

Cellular signalling

Various proteins are elevated in deteriorated cartilage including transforming growth factor β (TGF β), insulin-like growth factor (IGF), interleukin 1 (IL1), interleukin 6 (IL-6) and prostaglandin E_2. All of these proteins are products, but also stimulators of bone remodelling. Experimental studies showed that osteoblasts derived from OA joints produce twofold-sixfold more IL-6 and prostaglandin E_2 thank osteoblasts from physiological tissues [47, 48, 49].
It has been hypothesized that normal repetitive loading of joints cause microcracks in subchondral bone that stimulate bone remodelling. These repetitive loading might

also stimulate osteocytes which in turn produce RANKL (receptor activator of nuclear factor κ-B ligand) [47, 48, 49].

Vascular invasion

Vascular invasion is associated with increased bone remodelling. Vessels can invade the deep layers of articular cartilage. Increased vascularity can occur under stimulation of angiogenic factors such as vascular endothelial growth factor (VEGF). Angiogenic factors are elevated in the synovial fluid of patients with OA [50].

6.2.2. Bone remodelling in late-stage OA

Subchondral sclerosis

Radiological and pathological studies of bone in OA clearly demonstrate apparent density and increased bone volume in late-stage disease [51]. In various clinical studies an increase of up to 15% in bone mineral density (BMD) and up to 30% in bone volume could be seen in patients with OA compared with those without [52]. The mechanical consequences of the subchondral sclerosis are not fully understood yet.
Bone density fractionation studies of humans showed greater bone volume, but lower mineralization, in joints of disease free controls [53]. This paradox can be explained by the difference in apparent bone density (bone mass divided by total tissue volume), that is measured from radiological images and dual-energy X-rays (DXA), and material density (bone mass divided by bone volume), that is measured mechanically from biopsy samples or during autopsy. Apparent bone density will increase if fractional bone volume (bone volume divided by tissue volume) increases. However, if the bone is not well mineralized, its material density will decrease and its mechanical stiffness will also be lower compared to well-mineralized bone [18].

Changes in remodelling balance

Late stage-OA involves four processes: reduced bone turnover, subchondral sclerosis, thickening of calcified cartilage and thinning of trabeculae [54]. In advanced

disease there is a decrease in bone resorption without reduced formation. In late-stage disease, although erosion surface decreases in the subchondral region by nearly 65%, bone formation is only reduced by 20%, resulting in a bone imbalance that favours formation [55]. The mechanisms of the changes are not clear yet. The reduced expression of regulators of osteoblast differentiation may be involved [56]. Osteocalcin, a marker of bone formation, does not normally increase with age. It even might decrease. In patients with OA however there is a significant elevation of osteocalcin [57].

Experimentally-induced subchondral bone sclerosis

Subchondral sclerosis can be induced by the insertion of a metal cylinder into the subchodral bone [58]. This intervention stiffens the bone. It induces stress concentrations at the edges of the metal cylinder, at the junction of the very hard metal and the more compliant bone. A finite element analysis (FEA) of this intervention demonstrated a 50% increase in stresses in the deep layers of cartilage. If subchondral sclerosis and associated stress concentrations alone, were the driving forces in the progression of OA, than such a model would induce cartilage fibrillation. This, however, could not be proven in an experimental model. And it supports the hypotheses that increased remodelling and vascularity are necessary steps in OA progession.

Progression of OA

As there is no clear data considering the progression of OA, Burr proposed a hypothesis of its progression [18]:

"Repetitive loading causes an initial increase in bone remodelling. This might be an adaptation process to repair the damage caused by loading. The increased remodelling is associated with increased vascularity and vascular invasion of the deep layers of cartilage. This allows chondrolytic enzymes to access the cartilage. This process has several effects that include secondary synovial thickening. The loss of cartilage integrity caused by the loss of aggrecan, that usually maintains cartilage matrix compressive stiffness, will increase the overload of joint, feeding back to an

increased bone formation as the joint tries to adapt to greater loads. Finally, this positive feedback loop will promote the continuous loss cartilage integrity allowing progression of deterioration resulting in a clinical evident OA [18]."

6.2.3. Potential disease-modifying osteoarthritis drugs (DMOADs)

A disease-modifying agent slows the disease's progression and improves symptoms and/or function [Castaneda]. This group of drugs includes agents that are commonly used for the treatment of osteoporosis such as antiresorptive drugs (estrogens, SERMs and bisphosphonates), bone-forming/anabolic drugs (such as parathormone/teriparatide) or drugs with a dual mechanism (strontium ranelate) [59, 60, 61].

Calcitonin

In a study in humans, bone resorption and cartilage degradation markers were significantly lower in the oral salmon calcitonin group compared with placebo [62]. In a two-year phase 2 trial of knee OA, oral calcitonin modified symptoms and increased cartilage volume, but did not have an effect on joint space width (JSW) [63].

Bisphosphonates

In animal models a beneficial effect of bisphosphonates in OA could be identified. This effect is thought through their impact on subchondral bone that includes inhibition of remodelling and osteophyte formation along with decreased vascular invasion of calcified cartilage [64].
A phase 2 study of risedronate showed promising results of inhibition of JSN in knee OA. However, this could not be proven in a subsequent phase 3 study. Neither JSN nor clinical symptoms improved significantly [65, 66].
Zolendronic acid has shown chrondroprotective effects in animal models of OA considering its impact on subchondral bone [67]. A one-year randomized placebo controlled trial of zolendronic acid demonstrated reduction of bone marrow edema

and knee pain in patients with knee OA [68]. Therefore zolendronic acid is considered as a prospective DMOAD as it improved clinical parameters as well as structural ones.

Strontium ranelate

The use of strontium ranelate in humans with osteoporosis showed benefical effects in cartilage degradation markers and clinical as well as radiological features in spinal OA patients [69]. A three-year double-blind, placebo controlled study in patients with knee OA the use of strontium ranelate demonstrated a chondroprotective effect and symptomatic improvement measured by the WOMAC (Western Ontario and McMaster Universities) score [70].

Estrogens and SERMs (selective estrogen receptor modulator)

The two estrogen receptors α and β have been found in healthy and OA cartilage [71]. There is strong evidence for beneficial effects of estrogen on articular cartilage, subchondral bone and synovium [72]. Estrogen may decrease remodelling of subchondral bone [72]. In animal model the induction of cartilage damnage could be shown by ovariectomy or estrogen deprivation. Clinical studies showed beneficial effects of estrogen on the progression of some forms of OA [73]. Results of the Women's Health Initiative study showed that estrogen decreased OA severity [74]. SERMs as tamoxifen or raloxifen showed similar effects to estrogen on subchondral bone and its remodelling [72].

Parathormone (PTH)

The beneficial effects of PTH on cartilage and / or subchondral bone seem to be a paradox as PTH's mechanism suggests an induction of increased subchondral bone thickness and stiffness and therefore increased load and mechanical stress on overlying cartilage.
In a rat model the use of PTH (1-34) reduced the differentiation of human articular chondrocytes in vitro and reduced the progression of cartilage damage [75.

In a model of ovariectomy combined with menisectomy plus anterior cruciate ligament (ACL) sectioning showed a decrease of cartilage damage by the use of intermittent administratin of PTH [76]. The effects were explained by alterations of subchondral bone microstructure [76].

OPG/RANK/RANKL system

The OPG/RANK/RANKL system is a well-studied regulatory system for bone remodelling and homeostasis [77]. These regulatory proteins are also produced and expressed by healthy and OA chondrocytes. High levels of synovial fluid OPG and increased serum RANKL/OPG ratio have been correlated with disease severity in patients with primary knee OA [78].
In a mouse model, medial meniscectomy induced secondary OA, the administration of OPG inhibited cartilage degradation in vivo. The effect was attributed to improvement of subchondral bone quality [79]. OPG increased bone volume and reduced trabecular separation in the subchondral bone [79]. In a rabbit model of OA, increased subchondral bone remodelling was related to decreased OPG expression and increased RANKL expression led to a increased OPG/RANKL ratio [80].

6.3. Micro CT and assessment of Bone Microstructure

The use of high-resolution micro-computed tomography (µCT) imaging allows to evaluate trabecular and cortical bone morphology [81]. Its use has grown immensely. Meanwhile there are several commercially available µCT systems that are used worldwide.

6.3.1. Overview of µCT and its use in bone microstructure analysis

Before the arrival of µCT scanning techniques quantitative histologic techniques were the standard methods for the assessment of trabecular and cortical bone morphology and architecture [82]. Histologic analyses give information on cellular and dynamic indices of bone remodelling [82]. In assessing bone microarchitecture they are limited, however, as structural parameters are derived from a few 2D sections. In

contrast, three-dimensional imaging techniques like µCT directly measure bone microarchitecture. So they do not rely on stereologic models.
Since its introduction in the late 1980s by Feldkamp et al. [83] µCT has become the gold standard method for the evaluation of bone morphology and microarchitecture in small animals. µCT uses X-ray attenuation data acquired at multiple viewing angles. This allows the three-dimensional reconstruction of a specimen that characterizes the spatial distribution of material density. Currenty µCT scanners achieve an isotropic voxel size of as low as a few micrometers which is sufficient for investigating structures like trabeculae [81, 83].
µCTs have an excellent reproducibility and accuracy of bone morphology measurements. The accuracy of µCT morphology measurements has been evaluated by comparing with conventional 2 D histomorphometry.
There are a number of advantages when using µCT for the assessment of bone morphology and microarchitecture:

(1) The direct 3D measurement of trabecular morphology such as trabecular thickness and separation
(2) Larger VOIs/ROIs can be analysed
(3) Measurements can be performed much faster
(4) Assessment of bone morphology by µCT is nondesctructive
(5) Might also allow to estimate bone tissue mineralization by comparing X-ray attenuation in the specimen to that of hydroxyapatite standards
(6) Allow further investigation of mechanical behaviour by creating micro-finite element (µFE) models

µCT has been used the first time in 1988 to quantify bone morphology in subchondral bone in a guinea pig model of osteoarthritis [84]. Since its introduction, µCT has been used for a large number of various of studies of bone microarchitecture and morphology [84].

6.3.2. Image Acquisition

The first step in generating a reliable bone morphometry data by μCT is depended on image acquisition. The method section should offer information on scan medium, X-ray tube potential, voxel size and size and location of region of interest (ROI).

Sample preparation

A key concept is to orient the specimen consistently within the sample holder and scanner. Specimen can be aligned with the vertical or horizontal axis of the scanner. Low-density foam or other non-attenuating materials are useful to position the specimen in the sample holder in order to reduce movement during the scan [81].

Scanning medium

It is possible to scan specimen in a variety of media including saline, ethanol, neutral-buffered formalin as well as with no medium like air. Scanning in air affords the highest contrast between the specimen and the surrounding medium. The scan medium significantly affects the X-ray attenuation with measurements in air being different from those with the use of a medium. Therefore when assessing quantitative measurements of tissue mineral density the scans should be done in a liquid medium. Furthermore, the same scanning medium and volume for all specimens should be used within an experiment to allow comparison [85].

X-ray energy

An X-ray is an electromagnetic waveform. The energy of a X-ray photon is inversely proportional to its wavelength meaning that X-ray photons with longer wavelengths have lower energy and vice versa. The energy of an X-ray photon is produced by the acceleration of electrons striking the X-ray tube target. It is expressed as units of electonvolts (eV) [81].

Voxel size and image resolution

A *voxel* is the discrete unit of the scan volume which is the result of the tomographic reconstruction. It is the 3D volume that represents two dimensions within the slice and the slice thickness. Generally, voxels from µCT images have all three dimensions equal and therefore they are described as *isotropic voxels*. The smallest *voxel* size (the highest scan resolution) should be used for all scans. High resolution scans, however, require long acquisition times due to the higher number of projections and the generation of large data sets. Differences in voxel size (10 to 20 µm) have small effect on the evaluation of structures with relatively high thickness (100 to 200µm), as in humans. When analysing small structures (e.g. mouse or rat trabeculae), however, voxel size can have a significant effect on the results [86].

Region of interest (ROI)

It is important that a sufficient amount of the sample is scanned to allow reliable and reproducible morphology and density measurements. The ROI should be either defined on the location of the starting point of the scan ore the contoured ROI and the size of the region. The starting point should be defined as an absolute (millimetres) or relative distance (percent) from a reproducible landmark, such as the proximal tibia plateau. The size of the scan region should be defined as the distance (either proximal or distal) from a distinct starting point. Distances should be reported in International System of Units (SI units) (millimetres or micro meters); the number of slices can be easily determined knowing the voxel size [87].
The assessment of trabecular bone requires a suitable ROI. Extension of the ROI too far into the diaphysis will decrease the mean bone volume fraction (BV/TV) relative to ROI that is contained in the metaphyseal region. In order to analyse correctly, a representative ROI should contain at least three to five intertrabecular lengths [87]. When comparing bone specimen of varying size, it is important to pertain the definition of ROI. Generally a ROI should be chosen that is anatomically and biomechanically comparable among the specimen. In varying size, it might be more appropriate to define ROI as a percentage of bone length or in reference to easily identified landmarks rather than constant size [88].

6.3.3. Image Processing

Segmentation

The segmentation process is a critically important step in the µCT analysis and involves the separation of mineralized and nonmineralized structures that are used for quantitative analysis. A mistake in this stage will affect subsequent results.
The core task is the contouring method that defines the area in each slide to be included for segmentation and morphology measurements. The easiest approach is to use a constant circular or rectangular area that captures the bone of interest [89]. Once a region of interest is defined, segmentation is necessary to extract a "physiological and anatomically accurate" representation of bone. The simplest approach is to make use of a global threshold that is efficient and requires setting only one parameter. To allow analysis of differences a fixed CT value or percentage of the CT-value range should be set. This enables that differences between study groups are due to experimental effects rather than image-processing effects. However, no consenus exists yet. Care must be given in studies with inhomogenous bone mineralization e.g. growth or fracture healing [91, 92].

6.3.4. Image Analysis: Bone Morphometry and Tissue Mineral Density

Trabecular Bone morphometry

The standard method of quantitatively describing bone architecture is the calculation of morphometric indices (*quantitative morphometry*). Microarchitectural characteristics of trabecular or cortical bone were studied by examining two-dimensional section of bone in combination with calculations of morphologic parameters derived from stereologic methods [92]. BV/TV or bone-surface-to-volume ration (BS/TV) can be derived from two-dimensional images. Other important parameters including trabecular thickness (Tb.Th.) or trabecular separation (Tb.Sp.) are assessed after assuming a three-dimensional fixed-structure model (e.g. rodlike or platelike structure) [92].
The basic morphometric indices include the measurement of bone volume (BV) and total volume of interest (TV) [Parfitt JBMR 1987]. These factors are derived from

either a simple voxel-counting method or more advanced calculations (*volumetric marching cubes*). The ratio of these two measures is termed *bone fraction volume* (BV/TV) [93].
Bone surface (BS) is another basic measure that is calculated by triangulation of the object surface using a marching-cube algorithm. By dividing the total volume or the bone volume, the bone surface density (BS/TV) and the specific bone surface (BS/BV) can be derived, respectively [94].
Mean trabecular thickness (Tb.Th.), mean trabecular separation (Tb.Sp.) and mean trabecular number (Tb.N.) all are based on three-dimensional calculations [95]. A sphere-fitting method is used where the spheres are fitted to the object for thickness measurement and the spheres are fitted to the background for the separation. The basic approach is to determine the diameter of the largest possible sphere that can be fitted through each voxel that is completely contained with the object (or background) and then to average these diameters. This method provides a reasonable average thickness of the structure or background, whereas the latter reflects the mean trabecular separation. The mean Tb.N. number is computed as the inverse of the mean distance between the mid-axes of the structure that are derived by a distance-transformation method [81].
An advantage of this computed approach to trabecular morphometry is that mean values and variations are calculated.

Cortical Bone morphometry

Some indivces of cortical bone morphometry are derived from two-dimensional area measurements. These include total cross-sectional area (Tt.Ar., mm²) or cortical bone area (Ct.Ar., mm²). In contrast to volume measurements, the use of average cross-sectional geometry measurements allows comparison across studies when different sized volumes of interest are scanned [95].
Other key parameters for cortical bone include cortical bone area fraction (Ct.Ar./Ct.Ar., %) and cortical thickness (Ct.Th., µm). It is recommended that cortical thickness is derived by computed distance-transform methods [95].

Bone and tissue mineral density

µCT has been primarily used to evaluate bone structure. It also allows the estimation of tissue mineral density (TMD). TMD differs from bone mineral density (BMD) as TMD is calculated from the average attenuation value of the bone tissue only and does not include attenuation values from non-bone voxels as it is done for BMD. TMD should be reported in units of grams per cubic centimetre. Units of milligrams of hydroxyapatite (HA) per cubic centimetre should be given when a HA phantom is used [81].

6.3.5. Reporting results

The minimal dataset that should be given for trabecular region analyses should include bone volume fraction (BV/TV), trabecular thickness (Tr.Th.), trabecular separation (Tr.Sp) and trabecular number (Tr.N). Most of these parameters can be found in publications. Furthermore they can be compared to some extent with classic histomorphometric variables [81].
The minimal dataset for cortical bone regions include total cross-sectional area (Tt.Ar), cortical bone area (Ct.Ar), cortical thickness (Ct.Th) and cortical bone fraction (Ct.Ar/Tt.Ar) [81].

7. Citation Analysis of Total Hip and Knee Arthroplasty Research Papers

7.1. Introduction

Joint replacement is an orthopaedic subspecialty that aims to restore function in primary or secondary diseased joints of the musculoskeletal system. These include various anatomical sites including large joints of the hip, knee or shoulder or smaller joints e.g. the joints of the fingers. First attempts to reconstruct joints date back to the late 19th Century [96]. Since the 1950s after the introduction of the first implants for hip or knee replacement much research and advancement has been made in the field [97, 98]. In the meantime joint replacements pose standard procedures in orthopaedic surgery. The results of the research that was done in the past decades can be found in various Orthopaedic or general scientific journals that publish scientific papers related to the advancements in the field.

A citation is a reference or a quotation from published scientific work in books, book chapters or articles [99]. The number of citations of a published scientific work has been used as a parameter to evaluate the level of its influence and importance. The number of citations of published scientific articles may not be the only variable in determining the importance of scientific work in its field, but allows to define "citations classics" that could be used e.g. for educational purposes. Furthermore, the number of citations directly influences the impact factor of a journal, a general accepted factor that determines its quality and importance [99].
Analyses of most cited papers have been performed in various medical specialties or subspecialties including anaesthesiology, gynaecology, urology, plastic surgery, pain management or critical care medicine [100, 101, 102, 103, 104, 105, 106]. In two recent analyses the 100 most cited scientific articles were analysed in the whole field of orthopaedics including all subspecialties [107, 108]. Additionally, in some orthopaedic subspecialties analyses of the most cited papers - "citation classics" - have been carried out [109, 110, 11, 112, 113]. However, no such study has been done in Total Hip and Knee Replacement.

The purpose of the present study is to determine scientific articles in the field of Total Hip and Knee Replacement that have been cited most frequently by other authors and to establish a ranking of the 50 highest cited papers in the field by using the Thomson ISI Web of Science® Database.

7.2. Material and Methods

Search strategy

In December 2012, Thomson ISI Web of Science® was searched for the following search terms "Joint Replacement", "Replacement", "Total Hip Replacement", "Total Knee Replacement", " Hip Replacement", "Knee Replacement", "Shoulder Replacement", "Arthroplasty", "Total Hip Arthroplasty", "Total Knee Arthroplasty", "Hip Arthroplasty", "Knee Arthroplasty", "Reconstruction“, "Implant“ and „Prosthesis“. Secondly, in addition to the first search each top ranked Journal (highest 20% according to the Thomson ISI Journal Citations Reports® Science Edition 2011) from the "Orthopedics" category was searched for the highest cited articles according to the above mentioned search terms. This category includes journals from general to subspecialty-specific journals including clinical or basic science. Furthermore the two recent analyses of the top 100 highest cited papers were studied [107, 108]. The search output was then recorded and ranked according to the number of highest citations. All papers dealing with joint replacement, including its peri- and postoperative management were included in this study. Only papers focusing on material science were excluded. In cases with an identical absolute number of citations, the papers that had a higher citation density (see below) were ranked higher. A list of the 50 highest cited articles was established.

Data analyses

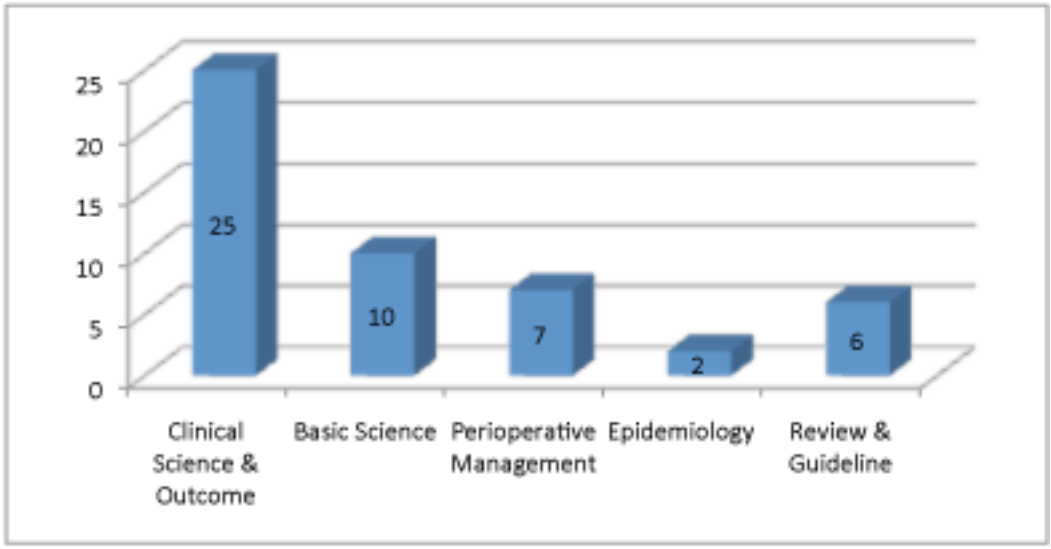
Each of the 50 highest cited articles was reviewed and the following data was extracted: article title, journal title, publication year and origin of corresponding author. Each paper was assigned to a single country in accordance with the corresponding author’ s address because the corresponding author is usually primarily and mainly responsible for the whole study project [114]. To evaluate the

relative impact of a published paper, the citation density ("Number of citations/years since publication") was calculated as described before [110].
Furthermore each article was analysed and an in case of a clinical study a Level of evidence was attributed based on the guidelines for clinical articles by the *Journal of Bone and Joint Surgery American Volume* [115].
Five categories were established including *Clinical Science & Outcome*, *Basic Science*, *Perioperative Management*, *Epidemiology* and *Review & Guideline*. The papers were analysed and attributed to one of these categories.

7.3. Results

The 50 highest cited articles in Orthopaedic Joint Replacement were cited from 347 to 2495 times. The top 10 papers according to absolute numbers were cited at least 580 times. The 50 highest cited papers according to the absolute number of citations can be seen in [Table 1]. The top 10 highest cited papers according to citation density can be seen in [Table 2].
42 papers were attributed to a specific anatomical region. The majority of articles dealt with the hip joint (n=36), whereas six articles could be attributed to the knee.
The majority of articles could be attributed to the *Clinical Science & Outcome* category (n=25). The distribution of the other categories can be seen in Figure 4.

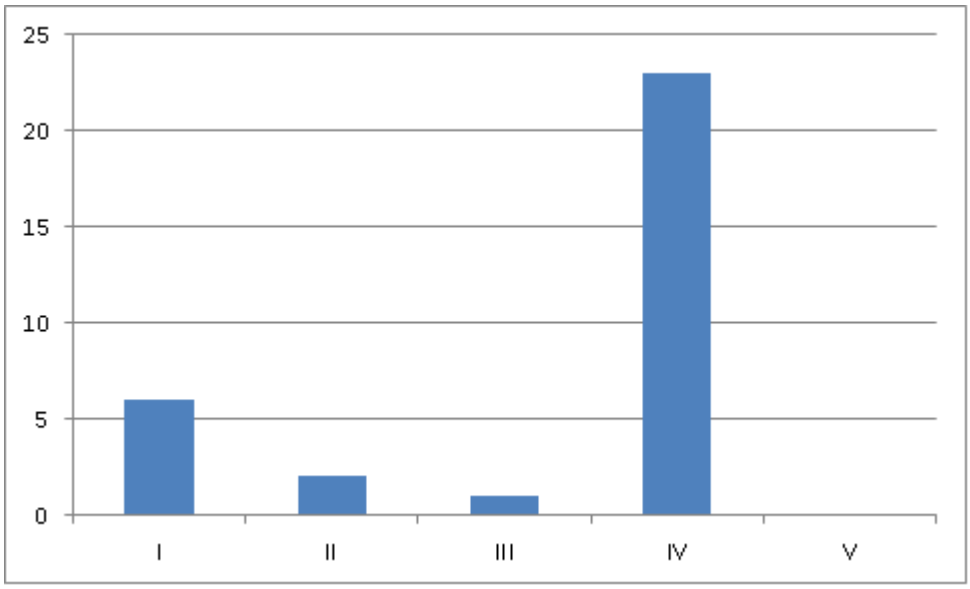
Figure 4. Distribution of highest cited papers according to categories



Level of evidence could be analysed in 32 clinical papers (from the catories Clinical science & Outcome and Perioperative Management). The majority of papers was

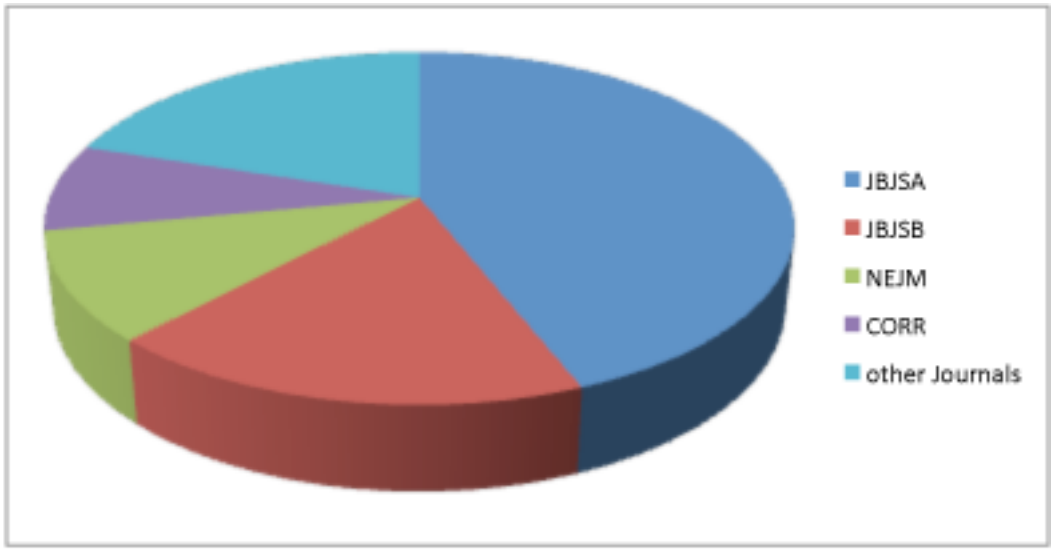
Level of evidence IV (n=23). The Level of evidence of all 32 analysed papers can be seen in Figure 5.

Figure 5. Level of evidence of 32 clinical papers.



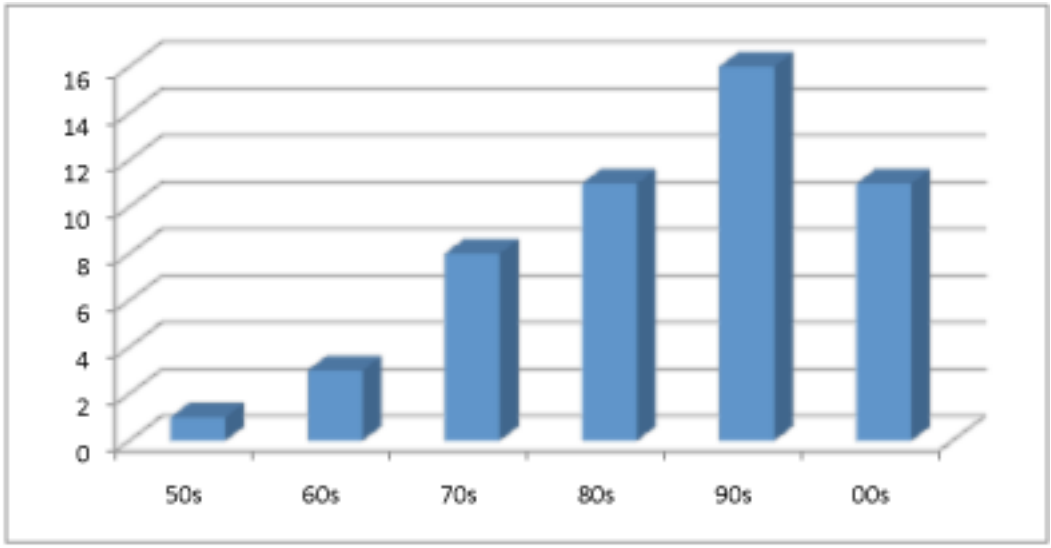
The distribution of the highest cited papers in the various journals can be seen in Figure 6. Most papers (n=22) were published in the *Journal of Bone and Joint Surgery American Volume*. Other Journals include the *Acta Orthopaedica Scandinavica*, *Anesthesiology*, *Archives of Internal Medicine*, *Journal of Biomechanics*, *Journal of Biomedical Materials Research*, *Lancet* and *Osteoarthritis and Cartilage*.

Figure 6. Distribution of the highest cited papers according to the published journal



Eight countries contributed to the Top 50 list. The United States had the most contributions with 30, followed by the UK with 8, Germany, France and Sweden with 3 each. Canada, Denmark and Switzerland had one paper each.
The number of highest cited papers according to the decade of publication can be found in Figure 7. The majority of papers were published since 1990 (n=27), whereas there is just one in the 1950s. Nine of the top 10 papers according to the citation density were published in the 2000s.

Figure 7. Number of highest cited papers according to decade of publication



7.4. Discussion

Thomson ISI Web of Science® was searched to analyse the highest cited papers in orthopaedic joint reconstruction and to define a list of "citation classics" in this subspecialty.
Articles on orthopaedic joint replacement were cited up to 2495 times. The top ten papers according to absolute numbers were cited at least 580 times. The number of citations is much higher than in other subspecialties. Top ranked articles on arthroscopic surgery or trauma reached about 500 citations [111, 112]. In orthopaedic paediatrics just four papers reached 100 citations [113]. This fact may show that orthopaedics is an industry driven specialty [116]. Furthermore, this points towards the neglect of other subspecialties including paediatrics or musculoskeletal oncology.

The majority of articles could be attributed to the hip joint (86%). Publications on the hip joint may be more frequent than other anatomical regions because hip joint replacement was introduced first and may also serve as a model for replacements in other joints [97, 98]. Furthermore this underlines the magnitude of hip replacements. Epidemiological data from the last decades shows that the highest number of replacements have been performed in the hip joint [117].

Papers focusing on clinical outcome were most frequent. Nine articles were published in the categories *Perioperative Management* and *Epidemiology*. However, four of these articles were among the highest cited journals in relative numbers (citation density). This reflects the frequency of citations in these fields and subsequently the high impact factors of journals in these specific categories [117].

When looking at journals, most articles on orthopaedic joint reconstruction were published in the *Journal of Bone and Joint Surgery American Volume*. This reflects the high reputation of this journal in the community of orthopaedic surgeons and its world-wide readership. The predominance of the *Journal of Bone and Joint Surgery American Volume* could also be seen in analyses of most cited orthopaedic papers in general [107, 108]. However, in other subspecialties including hand surgery, specialty journals dominated [109].

In total, eight countries contributed to the Top 50 list of highest cited papers in orthopaedic joint reconstruction. All countries are highly industrialized and are ranked among the top in both economical and health-care expenditure. The ranking is led by the United States with 30 papers. This predominance is in accordance with other analysis where the US had the highest number of most cited papers [107, 108, 109, 110, 111, 112, 113]. This reflects the high level of orthopaedic surgery in the United States, especially in a field with highly advanced technical methods and highly sophisticated implants. Furthermore, it demonstrates the level of research and scientific output in this country.

All papers were published in English. This clearly demonstrates the predominance of the English language in publications on orthopaedic surgery in general and orthopaedic joint reconstruction in particular [107, 108].

Interestingly, the majority of papers were published since 1990 (n=27), whereas there is just one from the 1950s. Although, joint replacement was introduced quite early many significant advancements have been made in recent years. Resulting

from this trend further developments in the field are to be expected e.g. in different anatomical regions including smaller joints.
The majority of articles were Level IV according to the guidelines of the *Journal of Bone and Joint Surgery American Volume.* Evidence Based Medicine has been introduced just recently. Therefore the majority of articles might have been attributed to Level IV. This points to the fact that there is a need of clinical studies with a higher quality of design in hip and knee replacement. However, six articles were Level of evidence I. All of these were from the category *Perioperative Management* either dealing with thromboprophylaxis or analgesia.
This study has limitations. Identifying the 50 highest cited papers, although using well defined criteria, still remain a selection. So, important and influential papers with lower citation frequency might have been missed. In some cases the value of contribution to the field cannot be quantified by the number of citations. However in the respect of the aim of this study a bottom line is drawn. This approach seems to be more objective as any other selection based on the importance of papers would depend on personal favour.
The absolute or relative number of citations of articles can be influenced be several factors and does not necessarily reflect the importance of research that has been performed or is published [110].
The search was performed in the Thomson ISI Web of Science®. Therefore citations of articles of other sources, like textbooks, lectures or digital media could not be considered.
Another weakness might be the cross-sectional study design and researching at a single time point with focus of absolute number of citations.

A list of 50 highest cited papers in orthopaedic joint replacement was generated, which covers a broad range of categories and decades. Studies focusing on the clinical outcome of hip replacement mainly dominate the literature on orthopaedic joint replacement in respect to absolute citations numbers. In the last decade however, a row of papers have been published that deal with perioperative management and show a high citation frequency.

Table 1. 50 Highest Cited Papers

Rank	Article	Absolute Number of Citations	Level of Evidence
1	Harris WH. Traumatic Arthritis of Hip after Dislocation and Acetabular Fractures - Treatment by Mold Arthroplasty - An End-Result Study Using a new Method of Result Evaluation. J Bone Joint Surg Am 1969; 51:737	2495	IV
2	Brooker AF, Bowerman JW, Robinson RA, Riley LH. Ectopic Ossification following Total Hip-Replacement - Incidence and a Method of Classification. J Bone Joint Surg Am 1973; 55:1629	1475	IV
3	Gruen TA, Mcneice GM, Amstutz HC. Modes of failure of cemented stem-type femoral components – radiographic analysis of loosening. Clin Orthop Relat Res 1979; 141:17	1443	IV
4	Delee JG, Charnley J. Radiological demarcation of cemented sockets in total hip-replacement. Clin Orthop Relat Res 1976; 121:20	1205	IV
5	Engh CA, Bobyn JD, Glassman AH. Porous-coated hip-replacement - the factors governing bone ingrowth, stress shielding, and clinical-results. J Bone Joint Surg Br 1987; 69:45	753	IV
6	Goldring SR, Schiller AL, Roelke M, Rourke CM, Oneill DA, Harris WH. The synovial-like membrane at the bone-cement interface in loose total hip replacements and its proposed role in bone lysis. J Bone Joint Surg Am 1983; 65:575	701	N/A
7	Schmalzried TP, Jasty M, Harris WH. Periprosthetic bone loss in total hip-arthroplasty - polyethylene wear debris and the concept of the effective joint space. J Bone Joint Surg Am 1992; 74:849	699	N/A
8	Ewald FC. The knee-society total knee arthroplasty roentgenographic evaluation and scoring system. Clin Orthop Relat Res 1989; 248:9	646	IV
9	Daubigne RM, Postel M. Functional results of hip arthroplasty with acrylic prosthesis. J Bone Joint Surg Am 1954; 36:451	614	IV
10	Stauffer RN. 10-year follow-up-study of total hip-replacement - with particular reference to roentgenographic loosening of the components. J Bone Joint Surg Am 1982; 64:983	580	IV
11	Bergmann G, Graichen F, Rohlmann A. Hip-joint loading during walking and running, measured in 2 patients. J Biomech 1993;	555	N/A

	26:969		
12	Insall JN, Ranawat CS, Aglietti P, Shine J. Comparison of 4 models of total knee-replacement prostheses. J Bone Joint Surg Am 1976; 58:754	553	III
13	Livermore J, Ilstrup D, Morrey B. Effect of femoral-head size on wear of the polyethylene acetabular component. J Bone Joint Surg Am 1990; 72:518	549	IV
14	Bartel DL, Bicknell VL, Wright TM. The effect of N/Aconformity, thickness, and material on stresses in ultrahigh molecular-weight components for total joint replacement. J Bone Joint Surg Am 1986; 68:1041	546	N/A
15	Kurtz S, Ong K ,Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am 2007; 89:780	527	N/A
16	Sutherland CJ, Wilde AH, Borden lS, Marks KE. A 10-year follow-up of 100 consecutive muller curved-stem total hip-replacement arthroplasties. J Bone Joint Surg Am 1982; 64:970	511	IV
17	Lewinnek GE, Lewis JL, Tarr R, Compere CL, Zimmerman JR. Dislocations after total hip-replacement arthroplasties. J Bone Joint Surg Am 1978; 60:217	508	IV
18	Bergmann G, Deuretzbacher G, Heller M, Graichen F, Rohlmann A, Strauss J, Duda GN. Hip contact forces and gait patterns from routine activities. J Biomech 2001; 34:859	504	N/A
19	Johnston RC, Fitzgerald RH, Harris WH, Poss R, Muller ME, Sledge CB. Clinical and radiographic evaluation of total hip-replacement - a standard system of terminology for reporting results. J Bone Joint Surg Am 1990; 72:161	500	N/A
20	Chandler HP, Reineck FT, Wixson RL, Mccarthy JC. Total hip-replacement in patients younger than 30 years old - a 5-year follow-up-study. J Bone Joint Surg Am 1981; 63:1426	496	IV
21	Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD, Arden N, Bierma-Zeinstra S, Brandt KD, Croft P, Doherty M, Dougados M, Hochberg M, Hunter DJ, Kwoh K, Lohmander LS, Tugwell P. OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines. Osteoarthritis Cartilage 2008; 16:137	491	N/A
22	Willert HG, Semlitsch M. Reactions of articular capsule to wear products of artificial joint prostheses. J Biomed Mater Res 1977; 11:157	483	N/A
23	Harris WH, Mccarthy JC, Oneill DA. Femoral component loosening using contemporary techniques of femoral cement fixation.	478	IV

	J Bone Joint Surg Am 1982; 64:1063		
24	Zimmerli W, Trampuz A, Ochsner PE. Current concepts: Prosthetic-joint infections. N Engl J Med 2004; 351:1645	458	N/A
25	Barrack RL, Mulroy RD, Harris WH. Improved cementing techniques and femoral component loosening in young-patients with hip-arthroplasty - a 12-year radiographic review. J Bone Joint Surg Br 1992; 74:385	439	IV
26	Beckenbaugh RD, Ilstrup DM. Total hip arthroplasty - review of 333 cases with long follow-up. J Bone Joint Surg Am 1978; 60:306	438	IV
27	Eriksson BI, Dahl OE, Rosencher N, Kurth AA, van Dijk CN, Frostick SP, Prins MH, Hettiarachchi R, Hantel S, Schnee J, Bueller HR. Dabigatran etexilate versus enoxaparin for prevention of venous thromboembolism after total hip replacement: a randomised, double-blind, non-inferiority trial. Lancet 2007; 370:949	433	I
28	Woo RYG, Morrey BF. Dislocations after total hip-arthroplasty. J Bone Joint Surg Am 1982; 64:1295	432	IV
29	Malchau H, Herberts P, Ahnfelt L. Prognosis of total hip-replacement in sweden - follow-up of 92,675 operations performed 1978-1990. Acta Orth Scand 1993; 64:497	429	N/A
30	Harris WH. The problem is osteolysis. Clin Orthop Relat Res 1995; 311:46	415	N/A
31	Gie GA, Linder L, Ling RSM, Simon IP, Slooff TJJHN/A, Timperley AJ. Impacted cancellous allografts and cement for revision total hip-arthroplasty. J Bone Joint Surg Br 1993; 75:14	413	IV
32	Darouiche RO. Current concepts - Treatment of infections associated with surgical implants. N Engl J Med 2004; 350:1422	408	N/A
33	Turpie AGG, Bauer KA, Eriksson BL, Lassen MR. Fondaparinux vs enoxaparin for the prevention of venous thromboembolism in major orthopedic surgery - A meta-analysis of 4 randomized double-blind studies. Arch Intern Med 2002; 162:1833	407	I
34	Jasty M, Maloney WJ, Bragdon CR, Oconnor DO, Haire T, Harris WH. The initiation of failure in cemented femoral components of hip arthroplasties. J Bone Joint Surg Br 1991; 73:551	402	N/A
35	Eriksson BI, Borris LC, Friedman RJ, Haas S, Huisman MV, Kakkar AK, Bandel TJ, Beckmann H, Muehlhofer E, Misselwitz F, Geerts, W. Rivaroxaban versus enoxaparin for thromboprophylaxis after hip arthroplasty. N Engl J Med 2008; 358:2765	399	I
36	Mont MA, Hungerford DS. Nontraumatic avascular necrosis of the femoral-head. J Bone Joint Surg Am 1995; 77:459	395	N/A
37	Agins HJ, Alcock NW, Bansal M, Salvati EA, Wilson PD, Pellicci PM, Bullough PG. Metallic wear in failed titanium-alloy total hip	385	N/A

	replacements - a histological and quantitative-analysis. J Bone Joint Surg Am 1988; 70:347		
38	Charnley J. Anchorage of the femoral head prosthesis to the shaft of the femur. J Bone Joint Surg Br 1960; 42:28	385	N/A
39	Lassen MR, Ageno W, Borris LC, Lieberman JR, Rosencher N, Bandel TJ, Misselwitz F, Turpie AGG. Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty. N Engl J Med 2008; 358:2776	384	I
40	Capdevila X, Barthelet Y, Biboulet P, Ryckwaert Y, Rubenovitch J, d'Athis F. Effects of perioperative analgesic technique on the surgical outcome and duration of rehabilitation after major knee surgery. Anesthesiology 1999; 91:8	383	I
41	Bauer KA, Eriksson BI, Lassen MR, Turpie AGG, Group Author. Fondaparinux compared with enoxaparin for the prevention of venous thromboembolism after elective major knee surgery. N Engl J Med 2001; 345:1305	381	I
42	Daniel J, Pynsent PB, McMinn DJW. Metal-on-metal resurfacing of the hip in patients under the age of 55 years with osteoarthritis. J Bone Joint Surg Br 2004; 86:177	365	IV
43	Jiranek WA, Machado M, Jasty M, Jevsevar D, Wolfe HJ, Goldring SR, Goldberg MJ, Harris WH. Production of cytokines around loosened cemented acetabular components - analysis with immunohistochemical techniques and in-situ hybridization. J Bone Joint Surg Am 1993; 75:863	360	N/A
44	Callaghan JJ, Salvati EA, Pellicci PM, Wilson PD, Ranawat CS. Results of revision for mechanical failure after cemented total hip-replacement, 1979 to 1982 - a 2 to 5-year follow-up. J Bone Joint Surg Am 1985; 67:1074	360	IV
45	Dawson J, Fitzpatrick R, Murray D, Carr A. Questionnaire on the perceptions of patients about total knee replacement. J Bone Joint Surg Br 1998; 80:63	355	II
46	Mulroy RD, Harris WH. The effect of improved cementing techniques on component loosening in total hip-replacement - an 11-year radiographic review. J Bone Joint Surg Br 1990; 72:757	355	IV
47	Dawson J, Fitzpatrick R, Carr A, Murray D. Questionnaire on the perceptions of patients about total hip replacement. J Bone Joint Surg Br 1996; 78:185	352	II
48	Crowe JF, Mani VJ, Ranawat CS. Total hip-replacement in congenital dislocation and dysplasia of the hip. J Bone Joint Surg Am 1979; 61:15	348	IV
49	Charnley J. Arthroplasty of hip - a new operation. Lancet 1961; 277:1129	348	IV
50	Planes A, Vochelle N, Darmon JY, Fagola M, Bellaud M, Huet Y. Risk of deep-venous thrombosis after hospital discharge in	347	IV

	patients having undergone total hip replacement: Double-blind randomised comparison of enoxaparin versus placebo. Lancet 1996; 348:224		

Table 2. Top 10 Highest Citation Density Papers

Rank	Article	Citation Density
1	Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD, Arden N, Bierma-Zeinstra S, Brandt KD, Croft P, Doherty M, Dougados M, Hochberg M, Hunter DJ, Kwoh K, Lohmander LS, Tugwell P. OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines. Osteoarthritis Cartilage 2008; 16:137	122,75
2	Kurtz S, Ong K ,Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am 2007; 89:780	104,8
3	Eriksson BI, Borris LC, Friedman RJ, Haas S, Huisman MV, Kakkar AK, Bandel TJ, Beckmann H, Muehlhofer E, Misselwitz F, Geerts, W. Rivaroxaban versus enoxaparin for thromboprophylaxis after hip arthroplasty. N Engl J Med 2008; 358:2765	99,75
4	Lassen MR, Ageno W, Borris LC, Lieberman JR, Rosencher N, Bandel TJ, Misselwitz F, Turpie AGG. Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty. N Engl J Med 2008; 358:2776	96
5	Eriksson BI, Dahl OE, Rosencher N, Kurth AA, van Dijk CN, Frostick SP, Prins MH, Hettiarachchi R, Hantel S, Schnee J, Bueller HR. Dabigatran etexilate versus enoxaparin for prevention of venous thromboembolism after total hip replacement: a randomised, double-blind, non-inferiority trial. Lancet 2007; 370:949	86,6
6	Harris WH. Traumatic Arthritis of Hip after Dislocation and Acetabular Fractures - Treatment by Mold Arthroplasty - An End-Result Study Using a new Method of Result Evaluation. J Bone Joint Surg Am 1969; 51:737	59,4
7	Zimmerli W, Trampuz A, Ochsner PE. Current concepts: Prosthetic-joint infections. N Engl J Med 2004; 351:1645	57,25
8	Darouiche RO. Current concepts - Treatment of infections associated with surgical implants. N Engl J Med 2004; 350:1422	51
9	Bergmann G, Deuretzbacher G, Heller M, Graichen F, Rohlmann A, Strauss J, Duda GN. Hip contact forces and gait patterns from routine activities. J Biomech 2001; 34:859	45,81
10	Daniel J, Pynsent PB, McMinn DJW. Metal-on-metal resurfacing of the hip in patients under the age of 55 years with osteoarthritis. J Bone Joint Surg Br 2004; 86:177	45,62

8. Citation Analysisof Research Papers in Osteoporosis and Related Research

8.1. Introduction

Osteoporosis is a systemic disease of the bone that affects millions of people and causes burdens for both the affected individual and health systems and societies worldwide [118]. Osteoporosis is a multidisciplinary disease and therefore relevant to different medical specialities, e.g. general medicine, internal medicine, endocrinology, gynaecology, orthopaedic surgery and traumatology, but also to pre-clinical and basic disciplines such as physiology, pathology and biomechanics.
Since the 1970's much research has been done in the field of osteoporosis. Research in osteoporosis poses a wide field and includes basic, clinical and translational studies in the above mentioned specialities. Meanwhile several journals have been established that are dedicated to publishing articles related to the disease.
A citation is a quotation or a reference of published scientific work in books, book chapters or articles [99]. The number of citations of published scientific work has been used as a marker to evaluate the level of its influence and importance. However, the number of citations may not be the only factor in determining the importance of scientific work in the field, but allows to define "citations classics" that could be used e.g. for educational purposes. Furthermore, the number of citations directly influences the impact factor of a journal, a generally accepted factor that determines its quality and importance [99].
Analyses of most cited papers have been performed in various medical specialties including anaesthesiology, gynaecology, urology, orthopaedic surgery, plastic surgery, or subspecialties such as pain management, critical care medicine, hand surgery, shoulder surgery or orthopaedic joint replacement [100, 101, 102, 103, 106, 111, 112, 113, 119]. Furthermore, such lists exist for various pathologies or diseases such as pancreatitis, Parkinson's disease, depression, sepsis or epilepsy [120, 121, 122, 123, 124]. However, no such study has been carried out in in osteoporosis and related research.
The purpose of the present study is to determine scientific articles in the field of osteoporosis and related research that have been cited most frequently by other

authors and to establish a ranking of the fifty most cited papers in the field by using the Thomson ISI Web of Science® database.

8.2. Material and Methods

Search strategy

In October 2013, Thomson ISI Web of Science® database was searched for the following search terms: "osteoporosis", "fracture", "bone mineral density", "bone density", "bone mass", "BMD", "dual energy x-ray absorptiometry", "DXA", "DEXA", "osteoclast", "osteoblast", "osteocyte", "bone formation", "bone resorption", "hormone replacement therapy", "estrogen replacement therapy", "bisphosphonate", "teriparatide", "denosumab" and "SERMs".

All papers with the main focus of their research in osteoporosis and related basic, clinical and translational research were included in this study. Papers including the above used terms, but focusing on other research areas were excluded. The search output was then recorded and ranked according to the absolute number of highest citations. In cases with an identical absolute number of citations, the papers that had a higher citation density (see below) were ranked higher. A list of the fifty most cited articles was established.

Data analyses

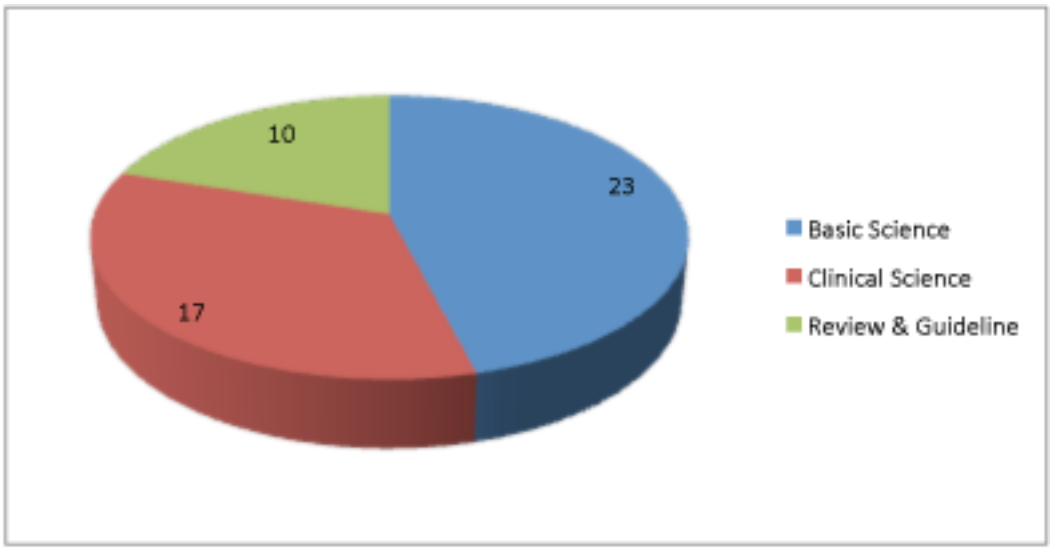
Each of the fifty most cited articles was reviewed and the following data was extracted: number of citations, authors, article title, journal title, publication year and origin of corresponding author. Each paper was assigned to a single country in accordance with the corresponding author's address because the corresponding author is usually primarily and mainly responsible for the whole study project [114]. To evaluate the relative impact of a published paper, the citation density ("number of citations / years since publication") was calculated as described before [119]. Furthermore, each article was analysed and in case of a clinical study a level of evidence was attributed to the paper based on the guidelines for clinical articles by Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence, (Oxford, UK:

www.cebm.net) [125]. Three categories were established: basic science, clinical science, and reviews & guidelines. The papers were analysed and attributed to one of these categories.

8.3. Results

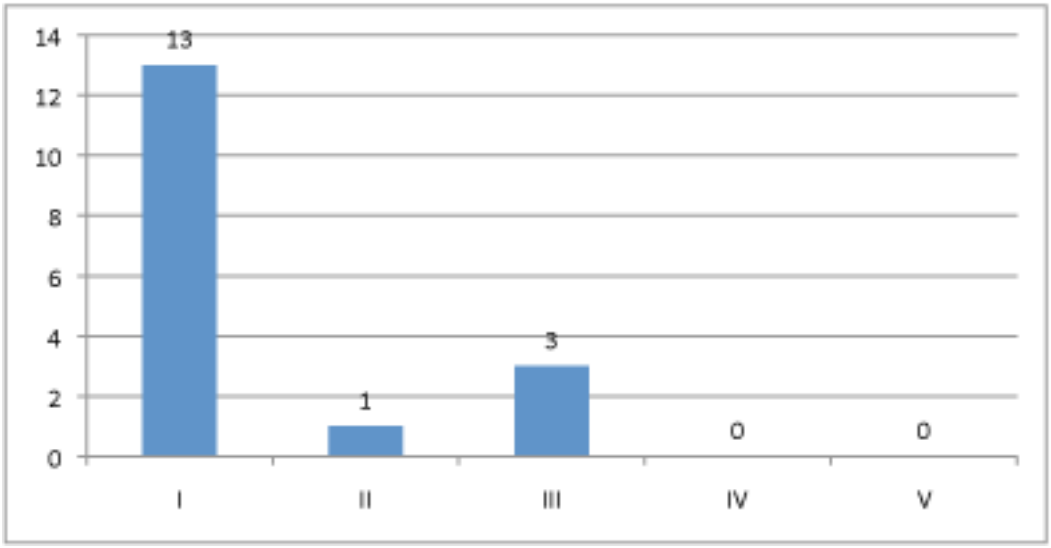
The fifty most cited articles on osteoporosis and related research were cited from 877 to 3056 times (mean 1141 ± 537), the top ten papers at least 1741 times. For the whole list see Table 3. The most frequently cited paper was by Lacey et al. published in 1998 with a mean number of 184,5 citations per year. The top ten papers according to citation density can be seen in Table 4. The majority of articles could be attributed to the *basic science* category (n=23). The distribution of the other categories can be seen in Figure 8.

Figure 8. Distribution of categories.



Level of evidence could be analysed in 17 clinical papers (from the Clinical Science category) and can be seen in Table 3. The majority of papers (n=13) were Level of Evidence I, one paper was Level of Evidence II and three papers Level of Evidence III [Figure 9].

Figure 9. Level of evidence in the clinical papers.



Eleven search terms were found in the title of the papers, altogether these terms were found 70 times (mean: 6.4 ± 5.5 times). For the list of all search terms found see Table 5. Search terms “fracture” (n=18) and “osteoporosis” (n=14) were found most frequently (45.7 % of all terms searched). Other terms searched were found between one and nine times (mean: 4.2 ± 2.9 times). More search terms were found in the abstract and the keywords of the papers.

Table 5. Most frequent search terms (n=11)

Search term	**Number of search terms found**
fracture	18
osteoporosis	14
bone formation	9
osteoclast	8
bone mineral density	5
bone density	5
bone resorption	4
osteoblast	3
bone mass	2
dual energy x-ray absorptiometry	1
estrogen replacement therapy	1

All together 395 authors contributed to the papers of the Top 50 list. A single paper had between one and 62 authors (mean: 10.02 ± 9.9 times). 12 authors (3.04%) contributed to between seven and four papers, 13 authors (3.3%) to three, and 30

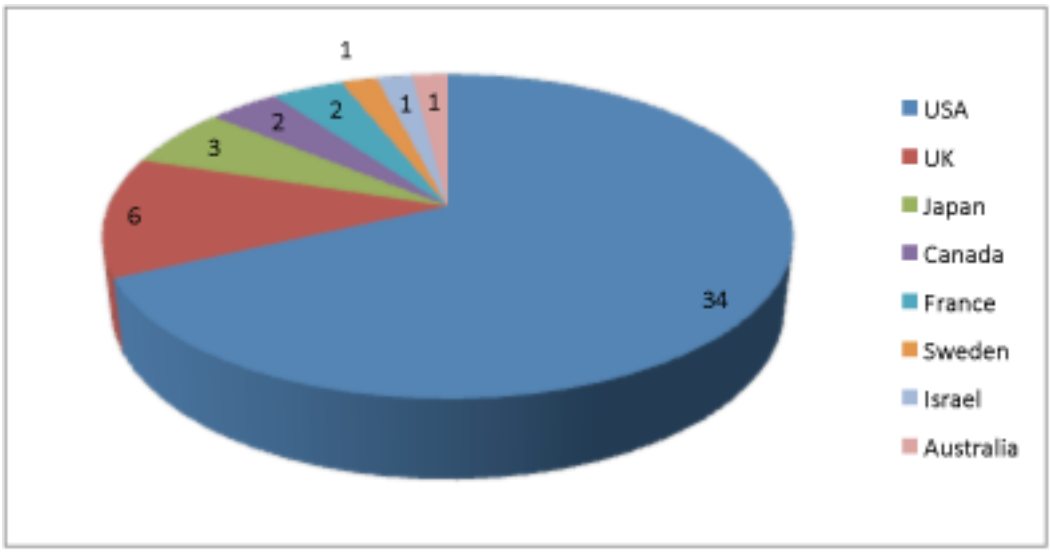
twice (7.6%). 340 authors (86.1%) were named at least once. The top 12 authors are presented in Table 6.

Table 6: Top 12 authors who contributed to the Top 50 list

Author's name	Number of papers by author
Cummings SR	7
Boyle WJ	6
Gennant HK	6
Lacey DL	6
Black DM	5
Dunstan CR	5
Melton LJ	5
Nevitt MC	5
Tan HL	5
Caparelli C	4
Ensrud KE	4
Sarosi I	4

Eight countries contributed to the Top 50 list [see Figure 10].

Figure 10. Distribution of countries.

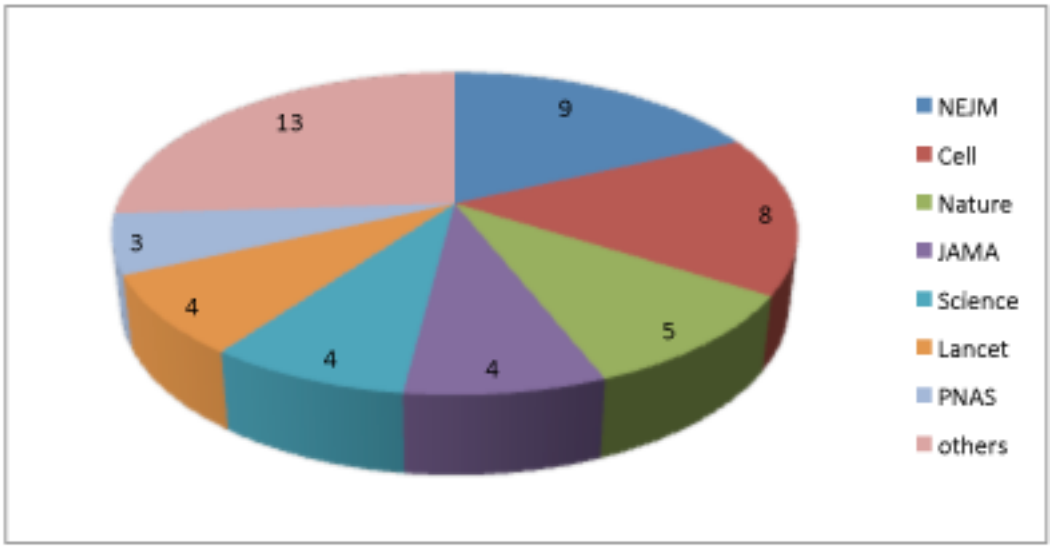


Authors from the United States contributed most frequently as a corresponding author (n= 34, 68%), followed by authors from the United Kingdom (n= 6), Japan (n= 3), Canada and France (2 papers each), and Sweden, Israel and Australia with one paper each.

Papers were published in 18 different journals publishing both basic and clinical research. Most papers (n=9) were published in the *New England Journal of*

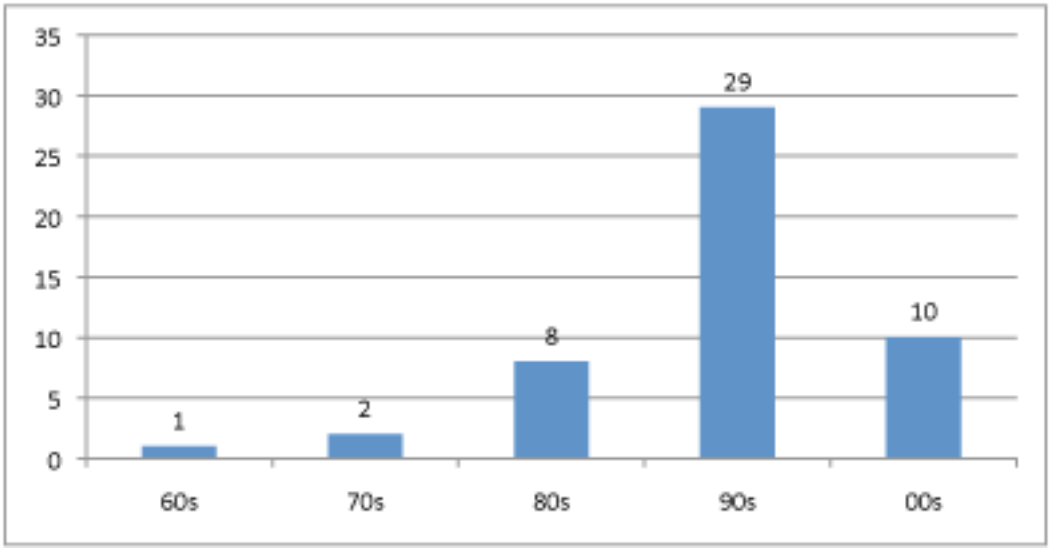
Medicine. Other journals include *Cell* (n=8), *Nature* (n=5), *The Journal of the American Medical* Association, *The Lancet, and Science* (n=4), *Proceedings of the National Academy of Sciences* (n=3), *Journal of Bone and Mineral Research* and *Journal of Clinical Investigation* (two paper each), and *American Journal of Clinical Nutrition, American Journal of Medicine, British Medical Journal, Cell and Tissue Research, Endocrinology, Endocrine Review, Epidemiology Reviews, Genes & Development, Osteoporosis International* one paper each. The distribution of the most cited papers in the various journals can be seen in Figure 11.

Figure 11. Distribution of journals.



The number of most cited papers according to the decade of publication can be found in Figure 5. The majority of papers were published since 1990 (n=39), whereas there is just one in the 1960's. Eight of the top 10 papers according to the citation density were published in the 1990's.

Figure 12. Number of papers published in each decade.



8.4. Discussion

Thomson ISI Web of Science® was searched to analyse the most cited papers on osteoporosis and related research and to define a list of "citation classics" in this field.

In order to identify the most cited papers and cover all aspects and the whole field of osteoporosis and related research not only the term “osteoporosis” per se, but also processes involved in the biology and pathology of bone and bone metabolism as well as risk factors and the consequences of the disease were used. Furthermore, diagnostic and therapeutic options were considered. Altogether 20 search terms were used. The majority of articles could be attributed to “fractures” and "osteoporosis” (45.7% of papers altogether). This underlines the magnitude of osteoporosis and osteoporotic fractures. Eleven out of 20 search terms were found in the title of the papers, others in the abstract and keywords. All keywords were found, but some papers did not reach enough citations to be put onto the list of the Top 50. Articles on osteoporosis and related research were cited up to 3056 times, the top ten papers according to absolute numbers were cited at least 1744 times. This fact shows the importance of osteoporosis as it affects a large number of patients world-wide and being of interest for many different medical specialities. Papers on other conditions such as acute pancreatitis had a maximum of 1281 citations [120], the highest cited papers on septic conditions reached 2932 [121], whereas papers on

epilepsy were cited even more frequently than osteoporosis (3749 citations) [122]. Top ranked articles in other fields such as papers on arthroscopic surgery or trauma reached about 500 citations [111, 112]. In orthopaedic paediatrics just four papers reached 100 citations [113].

The most cited paper in the present list was cited 3056 times. It is the oldest in the list and was published by a single author in 1956. The reason why this paper has been cited so often could be that “bone morphogenetic proteins” were described and for years their existence seemed to be the core of possible solutions for all aspects in bone metabolism [126].

395 authors contributed to the papers of the Top 50 list. This reflects the wide range of osteoporosis and related research. Some authors contributed more than once, one author contributed seven times (Steven C. Cummings).

Regarding corresponding authors, in total, eight countries contributed to the list. Authors from the United States contributed most frequently as corresponding author (n= 34), followed by authors from the United Kingdom (n= 6), Japan (n= 3), Canada and France (2 papers each), and Sweden, Israel and Australia with one paper each. All countries are highly industrialized and are ranked among the top in both economical and health-care expenditure. This is in accordance with previous analyses of other specialties or diseases [100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 119, 120, 121, 122, 123, 124].

The ranking is led by the United States with 34 papers (68%). This predominance is in accordance with other analyses where the US had the highest number of most cited papers [100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 119, 120, 121, 122, 123, 124]. This reflects the high frequency of research and the high scientific output in general and also in osteoporosis in the United States.

All papers were published in English. This clearly demonstrates the predominance of the English language in publications on osteoporosis and related research.

The spectrum of osteoporosis is also expressed in the large number of journals in which results of osteoporosis research is published. Papers were published in 18 different journals. Although there are journals focusing on osteoporosis itself and, more generally, on bone and bone metabolism, studies are also published in journals with a wide spectrum and more general medical background such.

Papers were attributed to three different categories. Their distribution can be seen in Figure 8. Most papers were focusing on basic science.

Evidence Based Medicine has been introduced just recently using Levels of Evidence. Level of evidence could be analysed in 17 clinical papers (from the Clinical Science category). According to the guidelines of the Oxford Centre for Evidence-Based Medicine the majority of articles (n=13) were Level of Evidence I, one paper was Level of Evidence II and three papers Level of Evidence III. This points to the fact that there is a high quality of study design and evidence in clinical osteoporosis research.

Interestingly, the majority of papers were published since 1990 (n=39), whereas there is just one from the 1960's. Although, bone changes were recognized quite early many significant advancements have been made in recent years. Considering this trend further developments in the field are to be expected.

This study has limitations. Identifying the 50 most cited papers they still remain a selection although using well defined criteria. So, important and influential papers with lower citation frequency might have been missed. In some cases the value of contribution to the field cannot be quantified by the number of citations. However, in respect of the aim of this study a bottom line is drawn. This approach seems to be more objective. Any other selection based on the importance of papers would depend on personal favour. The absolute or relative number of citations of articles can be influenced by several factors and does not necessarily reflect the importance of research that has been performed or has been published [Holzer J Arthoplasty 2012], nor does it directly translate into clinical practice changes. The search was performed in the Thomson ISI Web of Science® database. Therefore citations of articles from other sources, such as textbooks, lectures or digital media could not be

considered. Another weakness might be the cross-sectional study design and research at a single point in time with focus on the absolute number of citations.

A list of 50 most cited papers in osteoporosis and related research covers a broad range of medical sub-specialities. This is reflected by the number of journals and decades. Studies focusing on basic and clinical science of osteoporosis mainly dominate the literature in respect to absolute citations numbers. Considering the high number of high impact papers in the last two decades further developments in the field are to be expected.

Table 3. The fifty citation classics in Osteoporosis and related research

Rank	Paper	Absolute Number of Citations	Citation Density	Level of Evidence
1	Urist MR (1965) Bone - formation by autoinduction. Science 150:893-9.	3056	63,6	N/A
2	Lacey DL , Timms E, Tan HL , Kelley MJ , Dunstan CR, Burgess T , Elliott R , Colombero A , Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A , Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ (1998) Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93:165-176.	2768	184,5	N/A
3	Wozney JM, Rosen V, Celeste AJ, Mitsock LM (1988) Novel regulators of bone-formation - molecular clones and activities. Science 242:528-1534.	2662	106,4	N/A
4	Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, RenshawGegg, L , Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ. Cell 1997; 89:309-319.	2629	164,3	N/A
5	Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T.Osteoclast differentiation factor is a ligand for osteoprotegerin osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 1998; 95:3597-3602.	2206	147	N/A
6	Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Sasaki K, Deguchi K, Shimizu Y, Bronson RT, Gao YH, Inada M, Sato M, Okamoto R, Kitamura Y, Yoshiki S, Kishimoto T. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to aturational arrest of osteoblasts. Cell 1998; 89:755-764.	2132	133,2	N/A
7	Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, Cauley JC, Black D, Vogt TM. Risk-factors for hip fracture in white women. N Engl J Med 1995; 332:767-773.	2102	116,7	I
8	Black DM, Cummings SR, Karpf DB, Cauley JA, Thompson DE, Nevitt MC, Bauer DC, Genant HK, Haskell WL,	2067	121,5	I

	Marcus R, Ott SM, Torner JC, Quandt SA, Reiss, TF Ensrud. Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Lancet 1996; 348:1535-1541.			
9	Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, Delmas PD, Meunier PJ. Vitamin-D(3) and calcium to prevent hip-fractures in elderly women. N Engl J Med 1992; 23:1637-1642.	1771	84,3	I
10	Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ, Penninger JM. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 1999; 397:315-323.	1741	124,3	N/A
11	Ettinger B, Black DM, Mitlak BH, Knickerbocker RK, Nickelsen T, Genant HK, Christiansen C, Delmas PD, Zanchetta JR, Stakkestad J, Gluer CC, Krueger K, Cohen FJ, Eckert S, Ensrud KE, Avioli LV, Lips P, Cummings SR. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene. JAMA 1999; 282:637-645.	1732	123,7	I
12	Riggs BL, Melton LJ. Involutional Osteoporosis. N Engl J Med 1986; 314:1676-1686.	1719	63,6	N/A
13	Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. BMJ 1996; 312:1254-1259.	1689	99,3	I
14	Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster JY, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK, Wang OH, Mitlak BH. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. Lancet 2001; 344:1434-1441.	1686	140,5	I
15	Cummings SR, Black DM, Nevitt MC, Browner W, Cauley J, Ensrud K, Genant HK, Palermo L, Scott J, Vogt TM. Bone-density at various sites for prediction of hip-fractures. N Engl J Med 1993; 341:72-75.	1667	83,3	I
16	Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Cell 2003; 423:337-342.	1651	165,1	N/A
17	Soriano P, Montgomery C, Geske R, Bradley A. Targeted disruption of the c-src protooncogene leads to osteopetrosis in mice. Nature 1991; 64:693-702.	1609	73,1	N/A

18	Kanis JA, Melton LJ, Christiansen C, Johnston CC, Khaltaev N. Perspective - the diagnosis of osteoporosis. Cell 1994; 9:1137-1141.	1532	80,6	N/A
19	Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR, Stamp GWH, Beddington RSP, Mundlos S, Olsen BR, Selby PB, Owen MJ. Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. J Bone Miner Res 1997; 89:765-771.	1529	95,5	N/A
20	Liberman UA, Weiss SR, Broll J, Minne HW, Quan H, Bell NH, Rodriguezportales J, Downs RW, Dequeker J, Favus M, Seeman E, Recker RR, Capizzi T, Santora AC, Lombardi A, Shah RV, Hirsch LJ, Karpf DB. Effect of oral alendronate on bone-mineral density and the incidence of fractures in postmenopausal osteoporosis. N Engl J Med 1995; 333:1437-1443.	1484	82,4	I
21	Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA. Prediction of bone-density from vitamin-d receptor alleles. Nature 1994; 367:284-287.	1372	72,2	N/A
22	Teitelbaum SL. Bone resorption by osteoclasts. Science 2000; 289:1504-1508.	1370	105,3	N/A
23	Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu WL, Lacey DL, Boyle WJ, Simonet WS. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 1998; 12:1260-1268.	1292	86,1	N/A
24	Harris ST, Watts NB, Genant HK, McKeever CD, Hangartner T, Keller M, Chesnut CH, Brown J, Eriksen EF, Hoseyni MS, Axelrod DW, Miller PD. Effects of risedronate treatment on vertebral and nonvertebral fractures in women with postmenopausal osteoporosis - a randomized controlled trial. JAMA 1999; 282:1344-1352.	1291	92,2	I
25	Cummings SR, Kelsey JL, Nevitt MC, Odowd KJ. Epidemiology of osteoporosis and osteoporotic fractures. Epidemiol Rev 1985; 7:178-208.	1259	44,9	N/A
26	Klibanski A, Adams-Campbell L, Bassford T, Blair SN, Boden SD, Dickersin K, Gifford DR, Glasse L, Goldring SR, Hruska K, Johnson SR, McCauley LK, Russell WE. Osteoporosis prevention, diagnosis, and therapy. JAMA 2001; 285:785-795.	1254	104,5	N/A
27	Cummings SR, Black DM, Thompson DE, Applegate WB, Barrett-Connor E, Musliner TA, Palermo L, Prineas R,	1245	83	I

	Rubin SM, Scott JC, Vogt T, Wallace R, Yates AJ, LaCroix AZ. Effect of alendronate on risk of fracture in women with low bone density but without vertebral fractures - Results from the fracture intervention trial. JAMA 1998; 280:2077-2082.			
28	Bertolini DR, Nedwin GE, Bringman TS. Stimulation of bone-resorption and inhibition of bone-formation invitro by human-tumor necrosis factors. Nature 1986; 319:516-518.	1176	43,5	N/A
29	DawsonHughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone, density in men and women 65 years of age or older. N Engl J Med 1997; 337:670-676.	1170	73,1	I
30	Delmas PD, Bjarnason NH, Mitlak BH, Ravoux AC, Shah AS, Huster WJ, Draper M, Christiansen C. Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine endometrium in postmenopausal women. N Engl J Med 1997; 337:1641-1647.	1164	72,7	I
31	Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev 1999; 20:345-357.	1162	83	N/A
32	Nakashima K, Zhou X, Kunkel G, Zhang ZP, Deng JM, Behringer RR, de Crombrugghe B. The novel zinc finger-containing transcription factor Osterix is required for osteoblast differentiation and bone formation. Cell 2002; 108:17-29.	1143	103,9	N/A
33	Parfitt AM, Mathews CHE, Villanueva AR, Kleerekoper M, Frame B, Rao DS. Relationships between surface, volume, and thickness of iliac trabecular bone in aging and in osteoporosis - implications for the microanatomic and cellular mechanisms of bone loss. J Clin Invest 1983; 72:1396-1409.	1117	37,2	N/A
34	Cummings SR, Melton LJ. Epidemiology and outcomes of osteoporotic fractures. Proc Natl Acad Sci USA 2002; 359:1761-1767.	1089	99	N/A
35	Wang EA, Rosen V, Dalessandro JS, Bauduy M, Cordes P, Harada T, Israel DI, Hewick RM, Kerns KM, Lapan P, Luxenberg DP, McQuaid D, Moutsatsos IK, Nove J, Wozney JM. Recombinant human bone morphogenetic protein induces bone-formation. Lancet 1990; 87:2220-2224.	1077	46,8	N/A
36	Manolagas SC, Jilka RL. Mechanisms of disease - bone-marrow, cytokines, and bone remodeling - emerging	1047	58,1	N/A

	insights into the pathophysiology of osteoporosis. N Engl J Med 1995; 332:305-311.			
37	Cooper C, Campion G, Melton LJ. Hip-fractures in the elderly - a worldwide projection. Osteoporos Intl 1992; 2:285-289.	1037	49,3	N/A
38	Genant HK, Wu CY, Vankuijk C, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. Science 1993; 8:1137-1148.	1018	50,9	III
39	Gong YQ, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, Wang HW, Cundy T, Glorieux FH, Lev D, Zacharin M, Oexle K, Marcelino J, Suwairi W, Heeger S, Sabatakos G, Apte S, Adkins WN, Allgrove J, Arslan-Kirchner M, Batch JA, Beighton P, Black GCM, Boles RG, Boon LM, Borrone C, Brunner HG, Carle GF, Dallapiccola B, De Paepe A, Floege B, Halfhide ML, Hall B, Hennekam RC, Hirose T, Jans A, Juppner H, Kim CA, Keppler-Noreuil K, Kohlschuetter A, LaCombe D, Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar RS, Romanengo M, Somer H, Steichen-Gersdorf E, Steinmann B, Sullivan B, Superti-Furga A, Swoboda W, van den Boogaard MJ, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BR, Warman ML. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell 2001; 107:513-523.	1009	84	N/A
40	Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas SC. Increased osteoclast development after estrogen loss - mediation by interleukin-6. Science 1992; 257:88-91.	1008	48	N/A
41	Mazess RB, Barden HS, Bisek JP, Hanson J. Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. J Bone Miner Res 1990; 51:1106-1112.	1000	43,4	III
42	Riggs BL, Wahner HW, Dunn WL, Mazess RB, Offord KP, Melton LJ. Differential changes in bone-mineral density of the appendicular and axial skeleton with aging - relationship to spinal osteoporosis. J Clin Invest 1981; 67:328-335.	971	30,3	III
43	Maniatopoulos C, Sodek J, Melcher AH. Bone-formation invitro by stromal cells obtained from bone-marrow of young-adult rats. Cell Tiss Res 1988; 254:317-330.	964	38,5	N/A
44	McClung MR, Geusens P, Miller PD, Zippel H, Bensen WG, Roux C, Adami S, Fogelman I, Diamond T, Eastell R, Meunier PJ, Reginster JY, Wasnich RD, Greenwald M, Kaufman J, Chestnut CH. Effect of risedronate on the risk of hip fracture in elderly women. N Engl J Med 2001; 344:333-340.	954	79,5	I

45	Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, Shen JH, Vinson C, Rueger JM, Karsenty G. Leptin inhibits bone formation through a hypothalamic relay: A central control of bone mass. Cell 2000; 100:197-207.	946	72,7	N/A
46	Lindsay R, Hart DM, Aitken JM, MacDonald EB, Anderson JB, Clarke AC. Long-term prevention of postmenopausal osteoporosis by estrogen – evidence for an increased bone mass after delayed onset of estrogen-treatment. Lancet 1976; 1:1038-1041.	938	25,3	II
47	Hsu HL, Lacey DL, Dunstan CR, Solovyev I, Colombero A, Timms E, Tan HL, Elliott G, Kelley MJ, Sarosi I, Wang L, Xia XZ, Elliott R, Chiu L, Black T, Scully S, Capparelli C, Morony S, Shimamoto G, Bass MB, Boyle WJ. Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. Nature 1999; 96:3540-3545.	904	64,5	N/A
48	Gowen M, Wood DD, Ihrie EJ, McGuire MKB, Russell RGG. An interleukin-1 like factor stimulates bone-resorption invitro. Proc Natl Acad Sci USA 1983; 306:378-380.	903	30,1	N/A
49	Klein DC, Raisz LG. Prostaglandins - stimulation of bone resorption in tissue culture. Endocrinology 1970; 86:1436-40.	880	20,4	N/A
50	Manolagas SC. Birth and death of bone cells: Basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev 2000; 21:115-137.	877	67,4	N/A

Table 4. The top ten papers according to citation density

Rank	Paper	Absolute Number of Citations	Citation Density	Level of Evidence
1	Lacey DL , Timms E, Tan HL , Kelley MJ , Dunstan CR, Burgess T , Elliott R , Colombero A , Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A , Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ (1998) Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93:165-176.	2768	184,5	N/A
2	Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Cell 2003; 423:337-342.	1651	165,1	N/A
3	Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, RenshawGegg, L , Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ. Cell 1997; 89:309-319.	2629	164,3	N/A
4	Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T.Osteoclast differentiation factor is a ligand for osteoprotegerin osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 1998; 95:3597-3602.	2206	147	N/A
5	Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Sasaki K, Deguchi K, Shimizu Y, Bronson RT, Gao YH, Inada M, Sato M, Okamoto R, Kitamura Y, Yoshiki S, Kishimoto T. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to aturational arrest of osteoblasts. Cell 1998; 89:755-764.	2132	133,2	N/A
6	Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ, Penninger JM. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 1999; 397:315-323.	1741	124,3	N/A
7	Ettinger B, Black DM, Mitlak BH, Knickerbocker RK, Nickelsen T, Genant HK, Christiansen C, Delmas PD, Zanchetta JR, Stakkestad J, Gluer CC, Krueger K, Cohen FJ, Eckert S, Ensrud KE, Avioli LV, Lips P, Cummings	1732	123,7	I

	SR. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene. JAMA 1999; 282:637-645.			
8	Black DM, Cummings SR, Karpf DB, Cauley JA, Thompson DE, Nevitt MC, Bauer DC, Genant HK, Haskell WL, Marcus R, Ott SM, Torner JC, Quandt SA, Reiss, TF Ensrud. Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Lancet 1996; 348:1535-1541.	2067	121,5	I
9	Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, Cauley JC, Black D, Vogt TM. Risk-factors for hip fracture in white women. N Engl J Med 1995; 332:767-773.	2102	116,7	I
10	Wozney JM, Rosen V, Celeste AJ, Mitsock LM (1988) Novel regulators of bone-formation - molecular clones and activities. Science 242:528-1534.	2662	106,4	N/A

9. Microstructural Analysis of Subchondral Bone in Knee Osteoarthritis

9.1. Introduction

OA is a long-term chronic disease that is characterized by the deterioration of cartilage in joints causing stiffness, pain, and impaired range of motion [3]. OA is classified in two groups: (1) Primary OA that can occur localized or systematically and (2) Secondary OA that has an underlying cause such as obesity, inflammatory arthritis or trauma. OA most commonly affects the joints in the knee, hands, feet as well as shoulder and hip where it is relatively common [4].

OA of the knee is the most common joint disorder and a leading cause of (walking) disability among the older adults. The prevalence of knee OA is about 27 millions in the USA on the basis of symptoms and physical findings and it is predicted to rise up to 67 millions OA patients in the USA per year 2030 [127, 128]. Radiologically, the prevalence of OA increases with age (mild, moderate to severe). 13.9% of adults age 25 and older have clinical OA of at least one joint, while the age group of 65 years and older 33.6% have OA [128].

The etiology of OA is idiopathic and multifactorial. OA is a disease that is associated with ageing. However, there are also a variety of intrinsic and extrinsic factors that predispose patients for OA [1]. Aging, obesity, genetical aberrations or estrogen loss due to menopause seem to be major risk factors for knee OA [1].

Furthermore, it has been shown that mechanical loading contributes to OA progression by affecting the subchondral bone. It is believed that due to chronic impacts that are associated with microtraumas (microfractures) in the subchondral bone, increased remodeling is initiated. This has been seen in preclinical experimental studies. In late-stage OA, a chronic (microfracture-) healing process results in an imbalance of remodeling in favor of bone formation that leads to sclerosis and thickening of subchondral bone. As a consequence of this process subchondral bone sclerosis can be seen in late-stage OA X-rays [51].

Subchondral bone provides mechanical support and nutritional supply to the overlying articular cartilage [129]. So subchondral bone disturbances play an important role in the pathomechanism of OA and contribute to progression of the disease [18].

In recent years the role of subchondral bone in the progression of OA has been studied intensively. Subchondral bone and cartilage are a functional unit in which structural changes in one tissue will affect the other and vice versa [38].

Data is accumulating that subchondral bone changes may be involved in the progression of OA. Experimental as well as clinical studies showed a correlation of cartilage damage and subchondral bone density [18, 52, 129, 130]. Microstructural analyses have been performed in human specimen of OA of the hip as well as OA of the knee. In both anatomic regions increased subchondral bone density beneath areas of cartilage damage has been found [130].
In an experimental biomechanical study setting, it has been demonstrated that OA is a condition with less potential of shock absorption compared to normal physiological knee cartilage. Furthermore, the loss of a meniscus' integrity by either “meniscus injury” or “total meniscectomy” resulted in an increased impact force of 113% up to 121% in “total meniscectomy”. Therefore, the resulting increased impact in the subchondral bone will result in sclerotic bone after microfracture healing [131].

Knee alignments as well as meniscus’ integrity are structural and geometric parameters that influence mechanical loading in the knee joint. Varus alignment for example leads to higher loading conditions of the medial compartment and vice versa. An intact meniscal structure prevents the cartilage to degenerate [132, 133]. This is also of major interest in clinical practice where resection of meniscus in case of tears and the effect in patients with OA is discussed controversially [134, 135].

Aim of the Study

We aimed to analyse subchondral bone microarchitecture in respect to cartilage damage, meniscus' integrity and knee joint alignment in proximal tibia specimens of patients with late-stage OA. We hypothesized that subchondral bone beneath areas

with a high degree of cartilage damage may have increased bone density compared to areas with lower degrees of cartilage damage.
Furthermore we hypothesized that intact meniscal structures may have reduced subchondral bone density compared to luxated or subluxated ones. Another secondary hypothesis was that knee joint malalignment may cause higher density in the subchondral bone of the affected compartment.

9.2. Patients, Materials and Methods

Patients

Thirty consecutive patients (20 female and 10 male) with a mean age of 70,4 ± 9,9 years who were scheduled for TKA were prospectively included in this study. Inclusion criteria were age between 50 and 90 years, either female or male sex and the presence of primary late-stage OA of the knee (K&L classification Grade 3 or 4). Exclusion criteria were the presence of benign or malignant tumour in the affected joint, local or systemical metabolic bone disorders (except osteoporosis), rheumatoid arthritis, prior trauma or surgery of the joint (except arthroscopy).

Clinical assessment

Patients were evaluated and recruited at our department's knee outpatient clinic and subsequently scheduled for TKA. One day prior to surgery the patients were examined physically and assessed using a standardized knee questionnaire, the Knee Society Score (KSS) at the ward [36].
The KSS was developed by consensus of the Knee Society [36]. The KSS is a widely used functional outcome score for knee arthroplasty. The KSS consists of two parts: The first part, the Knee Score which considers pain, stability and range of motion as the main parameters, with deductions for flexion contractures, extension lag and malalignment. A maximum of 100 points could be obtained for a well-aligned knee with no pain, 125 degrees of motion and negligible anteroposterior or mediolateral instability. The second part, the Function Score, utilizes walking distance and stair climbing as the main parameters, with deduction for the use of a walking aid. The

maximum points for the Function Score were 100, given to an individual who is able to walk an unlimited distance and can ascend and descend stairs normally.

Preoperative all patients had X-rays of the knee joint in anterior posterior and lateral view, axial patella “skyline view” (Merchant’s view), and a full-leg X-ray to analyse the degree of malalignment.
Demographic and medical data was retrieved from records. Patients had routine laboratory blood analyses including bone metabolism parameters one day prior surgery.

Magnetic Resonance Imaging (MRI)

Preoperative, within six weeks before TKA, patients had a MRI scan of the affected knee joint.
MR images were obtained using a 3,0 Tesla MRI with a maximum gradient amplitude of 40mT/m (Siemens Magnetom Trio ®, Siemens AG, Erlangen, Germany). The following protocols were used: a transversal T2-weighted 3D CISS (Constructive Interference in Steady State), a sagittal 3D GRE (Gradient recalled Echo) and a sagittal 3D DESS (Dual Echo Steady State).
The severity of OA was estimated by using MRI scans by a board-certified radiologist using a semiquantitative scoring system [25]. This scoring system includes the following five grades:

- Grade 0: normal contour and signal
- Grade 1: normal contour with abnormal signal
- Grade 2: <50% reduction of cartilage thickness
- Grade 3: >50% reduction of cartilage thickness
- Grade 4: full thickness or nearly full thickness cartilage defect

Furthermore, the integrity of both the medial and lateral menisci was assessed byMRI scanning. Menisci were defined either as “intact meniscus” (degenerative, but normal structure) or “not intact meniscus” (luxated or subluxated).

Specimens

Proximal tibial specimens were retrieved during TKA. The specimen were fixed in formalin (commercially available formaldehyde solution) and stored at -20° Celsius.

Micro-computed Tomography (µCT) imaging and image processing

µCT imaging

The tibial specimens were scanned using a preclinical MicroCT system (Inveon MicroCT ®, Siemens AG, Erlangen, Germany). A scan of 360° was made at a voltage of 80 kVp and a current of 500µA and an exposure time of 1300 ms. The effective pixel size was 43.96µm.
The specimens were consistently oriented in the scanner and aligned with the horizontal axis of the scanner. The specimens were positioned on a soft cotton tissue to reduce movement during the scan.

Image Segmentation and Trabecular bone processing

The segmentation of the three-dimensional (3D) cubic VOIs was performed using an in-house imaging software developed in MATLAB Version 8.2 R2013b (The MathWorks Inc., Natick, MA, USA). In each tibial bone sample five different cubic volumes with an isotropic dimension of 4 mm^3 were defined on the median coronal axis: lateral (submeniscal) (1), lateral compartment – center (2), center of the tibia as a reference (in between the tibial footprint of the ACL and PCL) (3), medial compartment – center (4), medial (submeniscal) (5) VOI [Figure 13]. These compartments were segmented according to the following processing steps. At first a preliminary threshold was used to extract the mineralized bone phase. Each coronal and sagittal slice of the resulting binary data was superimposed onto a single coronal and sagittal plane depicting the outermost contour of the sample. Within the resulting coronal plane the user preselected a rectangular region covering the majority of the cancellous bone starting with the positioning of the VOIs within the coronal plane [Figure 13]. The sides of the oblong were aligned parallel to the image margins. The width of the rectangle in medial-lateral direction was defined at the level of the

median. At this level a gap of 4 mm between the two sides and the outermost medial and lateral bone contour was applied. Subsequent the centroids of the resulting oblong and of the superimposed bone were matched and the five VOIs were automatically placed along the medial-lateral axis intersecting the two centroids. The VOI 3, the center-reference, was placed on top of the centroid. The distance between the sides of the quadratic VOIs 1/5 and the corresponding edges of the rectangle were kept in the order of 1.2 mm. VOIs 2/4 were placed half the distance between VOI 1/5 and VOI 3, respectively. After positioning in the coronal plane the extent along the coronal direction of these VOIs was defined. Using the previously derived sagittal plane the VOIs were manually placed adjacent to the joint surface [Figure 14]. Subsequent the resulting coordinates were translated to the originally tomographic data and automatically extended over the successive coronal slices. However, in case the VOI jutted out over the bone sample the proximal end of the corresponding VOI was placed two slices above the margin of that sample. Finally, these semi-automatically derived volumes of each sample were transferred to a commercially available software for microstructural analysis (see below).

Figure 13. Coronal plane of a representative bone sample. A white rectangle was marked by the user (white box) in the coronal plane wherein the locations of the five VOIs were automatically derived.

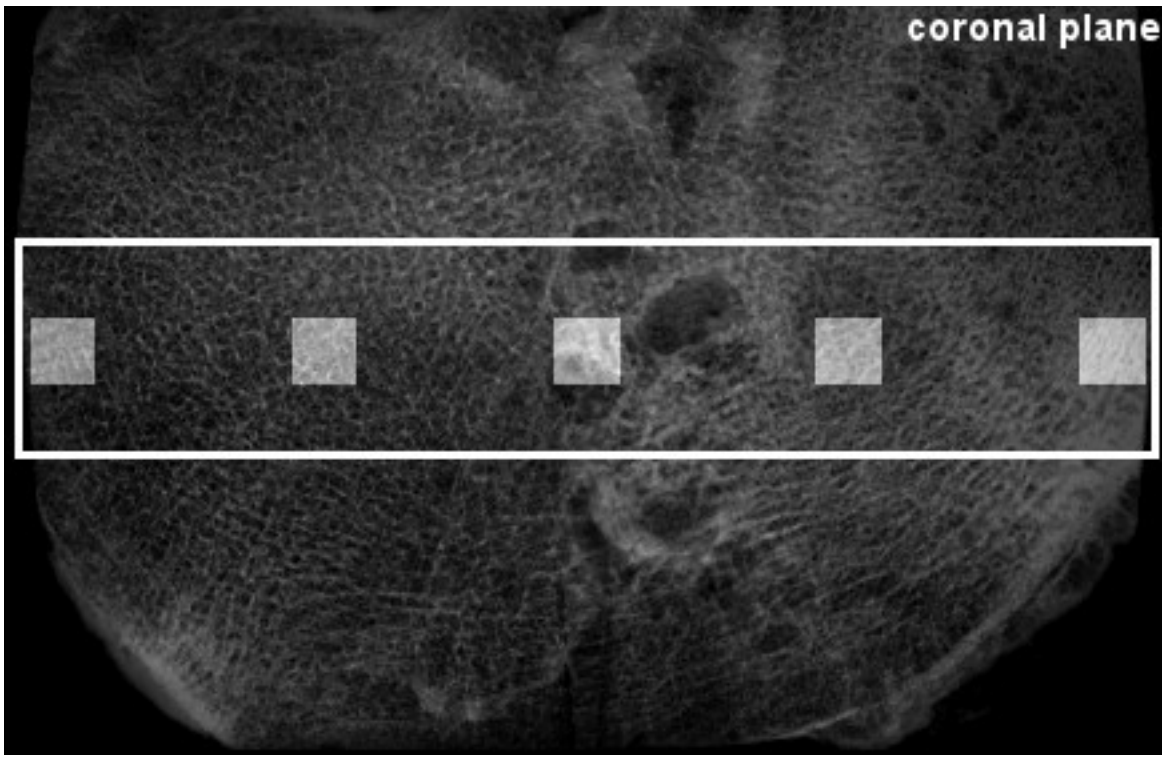
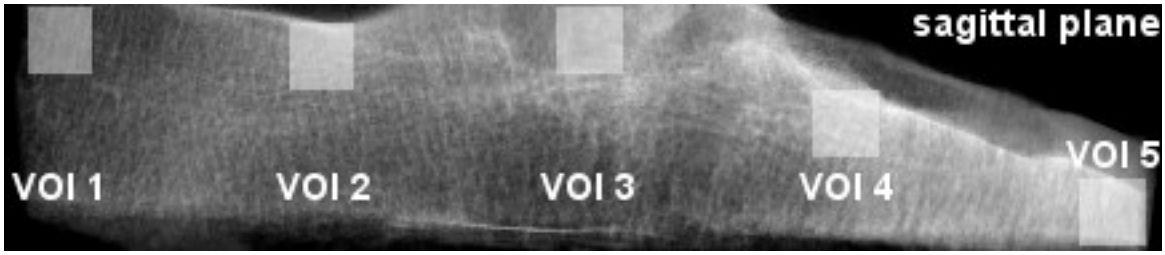


Figure 14. Saggital plane of a representative bone sample. As second step, the first coronal slice of each predefined VOI was manually adjusted in the sagittal plane and automatically extended over the range of 4 mm.



Analysis of bone microstructural parameters

Siemens Inveon Research Workplace 3.0 (Siemens Medical Solutions Inc., Malvern, PA, USA) was used to analyse bone microstructural parameters. The following bone microstructural parameters were analysed: Bone fraction volume (Bone Volume / Total Volume (BV/TV)), Bone Surface Area / Bone Volume (BSA/BV), Trabecular Thickness (Tr.Th.), Trabecular Number (Tr.N.), Trabecular Spacing (Tr.Sp.), and Trabecular Pattern Factor (Tb. Pf.).

Ethics

The conduction of the study was approved by the Institutional Review Board of the Medical University of Graz (EK-Nr.: 25-203 ex 12/13). The representative material and patient demographic data were collected after obtaining written informed consent. In all cases, acquisition of samples complied with the 1975 Declaration of Helsinki.

Statistics

Data are presented as mean and standard deviations. For statistical analysis a two-sided one-sample t-test was used to determine significant differences. When a normal distribution could not be assumed, nonparametric tests were performed. To analyse clinical factors chi square or Fisher's exact test for categorical data and Spearman rank correlation for continuous data were used. Furthermore multivariate and univariate testing was peformed. A p-value less than 0.05 was considered

statistically significant. Data were analysed using the statistical software IBM SPSS Statistics 20.0.0 (SPSS Inc., Chicago, IL, USA).

9.3. Results

Thirty proximal tibial specimens of 30 patients (20 women and 10 men) with late-stage knee OA were included in this study. Patients' demographic data see Table 7.

Table 7. Patients' demographic data

	all patients (n=30)	**Female patients (n=20)**	**Male patients (n=10)**
age (in years)	70,4 ± 9,9	74,3 ± 6,9	62,5 ± 10,6
height (in cm)	166,7 ± 7,7	162, 8 ± 4,9	174,6 ± 6,3
weight (in Kg)	82,0 ± 11,3	79,8 ± 10,4	86,4 ± 12,4
BMI	29,5 ± 3,7	30,1 ± 3,7	28,3 ± 3,7

Knee Society Score

The mean Knee Society Score for all patients was 52,4 was ± 11,6 and the mean Knee Society Function Score was 44,0 ± 12,2. The preoperative KSS values can be seen in Table 8.

Table 8. Preoperative Knee Society Score (Knee Score / Function Score)

KSS	**Knee Score / Function Score all patients: (n=30)**	**Knee Score / Function Score women (n=20)**	**Knee Score / Function Score men (n=10)**
Mean	52,36 / 44,00	51,7 / 43,75	53,7 / 44,5
STD	11,58 / 12,2	12,84 / 14,31	8,99 / 6,85

Cartilage damage assessed by MRI

The mean grade of cartilage damage in the medial compartment was 3,5 ± 0,7 compared to the mean grade of cartilage damage of 2,28 ± 0,8 in the lateral

compartment. The degree of cartilage damage analysed in various locations can be seen in Table 9.

Table 9. Degree of cartilage damage measures MRI

patients	**CD med.**	**CD sm med.**	**CD lat.**	**CD sm lat.**
All (n=30)	3,5 ± 0,67	3,3 ± 0,67	2,28 ± 0,82	1,8 ± 0,63
Female (n=20)	3,44 ± 0,72	3,33 ± 0,75	2,25 ± 0,88	1,66 ± 0,81
Male (n=10)	3,66 ± 0,57	3,28 ± 0,57	2,33 ± 0,81	2 ± 0,51

Legend: CD: Cartilage damage; sm: submeniscal; med: medial; lat: lateral

The results of the µCT analysis can be seen in Table 10.

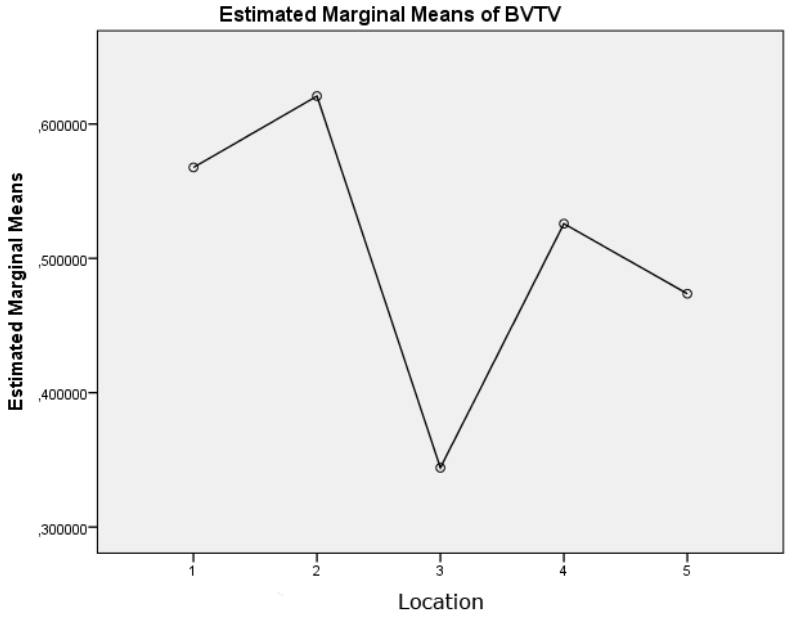
Table 10. Bone parameters for the five locations assessed by µCT. MM: medial Meniscus; MT: medial Tibia plateau; LM: lateral Meniscus; LT: lateral Tibia plateau; ACL: anterior cruciate ligament

	MM	**MT**	**ACL**	**LT**	**LM**
BV/TV (%)	56,76 ± 12,80	62,07 ± 12,53	34,40 ± 07,87	52,57 ± 13,33	47,36 ± 14,97
BSA/BV (1/mm)	6,04 ± 1,96	5,41 ± 1,9	9,01± 2,29	6,29 ± 2,53	7,27 ± 2,15
Tr.Th. (µm)	401,42 ± 215,72	429,27 ± 199,29	222,13 ± 41,83	374,90 ± 231,95	315,58 ± 168,64
Tr.N. (µm)	1,58 ± 0,28	1,58 ± 0,39	1,55 ± 0,23	1,58 ± 0,35	1,59 ± 0,31
Tr. Sp. (1/mm)	272,98 ± 0,6705	245,59 ± 0,7788	435,27 ± 0,9930	304,83 ± 1,0146	340,83 ± 1,2945

The mean bone fraction volume in the medial compartment was significantly higher compared to the mean bone fraction volume in the lateral compartment (62,07 ± 12,53 vs. 52,57 ± 13,33). The differences were statistically significant (p=0,007). The submeniscal mean bone fraction volume in the medial compartment was statistically significantly higher compared to submeniscal mean bone fraction volume in the lateral compartment (56,76 ± 12,8 vs. 47,36 ± 14,97; p=0,015). There was a significantly lower bone fraction volume in the medial submeniscal subchondral bone compared to the subchondral bone fraction volume from the medial tibial plateau compartment (p=0,041). There was a significant difference in the lateral submeniscal subchondral bone fraction volume compared to the subchondral bone fraction volume from the lateral tibial plateau compartment (p=0,024). The bone fraction volume in all weight bearing locations (medial meniscus, medial tibial plateau, lateral

meniscus, lateral tibial plateau) was significantly higher compared to the non weight-bearing reference point below the ACL (p=0,000)]Figure 15].

Figure 15. Mean subchondral bone fraction volume in the five analysed locations: medial meniscus (MT) (1), medial tibial compartment (MT) (2), anterior cruciate ligament (ACL) (3), lateral tibial compartment (LT) (4), lateral meniscus (LM) (5).



Meniscus integrity

Thirteen of the medial menisci were intact, whereas 17 were either subluxated or luxated. On the other hand, 24 of the lateral menisci were intact, whereas 6 were either subluxated or luxated.
In intact menisci, there was a significantly lower subchondral bone fraction volume compared to subluxated or luxated meniscus in the medial (p=0,020) and lateral compartment (p=0,005) [Figure 16 and Figure 17].

Figure 16. Mean subchondral bone fraction volume in the 5 analysed locations in respect to intact (green line) and subluxated or luxated (blue line) medial menisci. Location 1 is submeniscal medial, whereas location 2 is the medial tibial plateau.

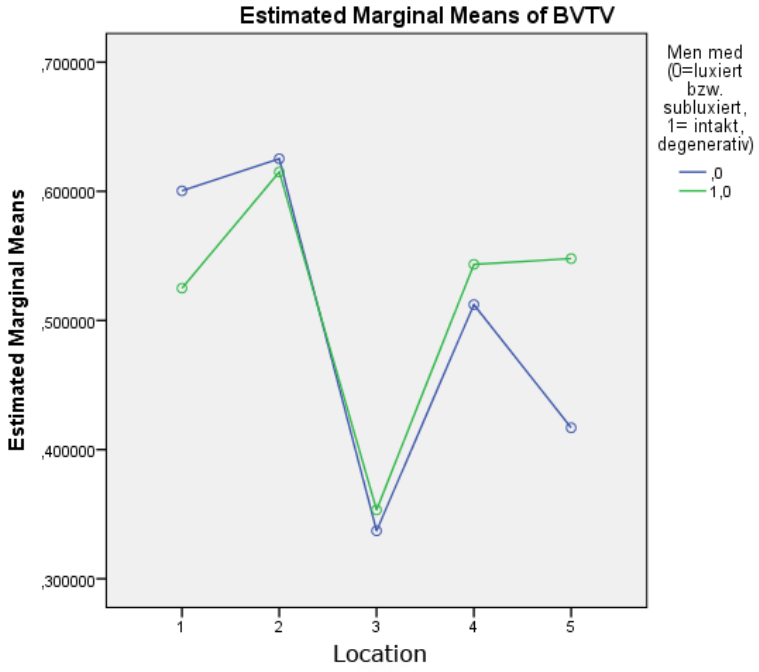
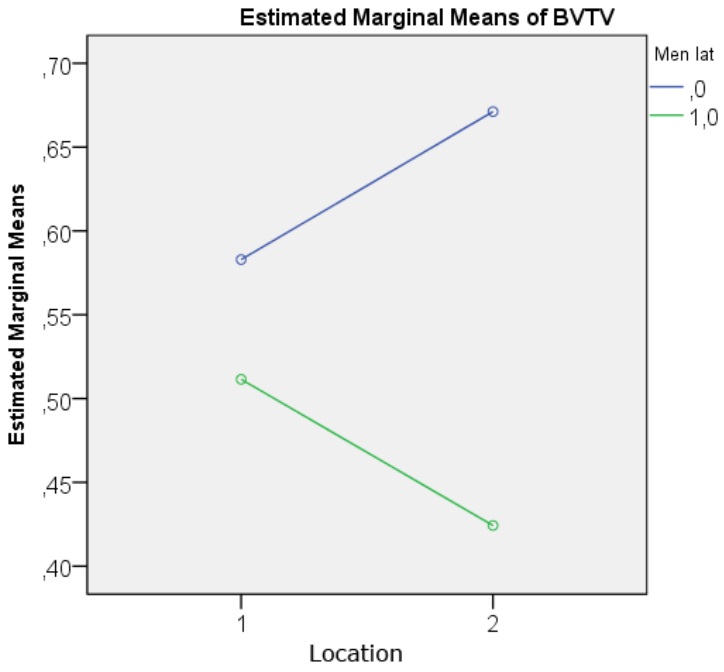


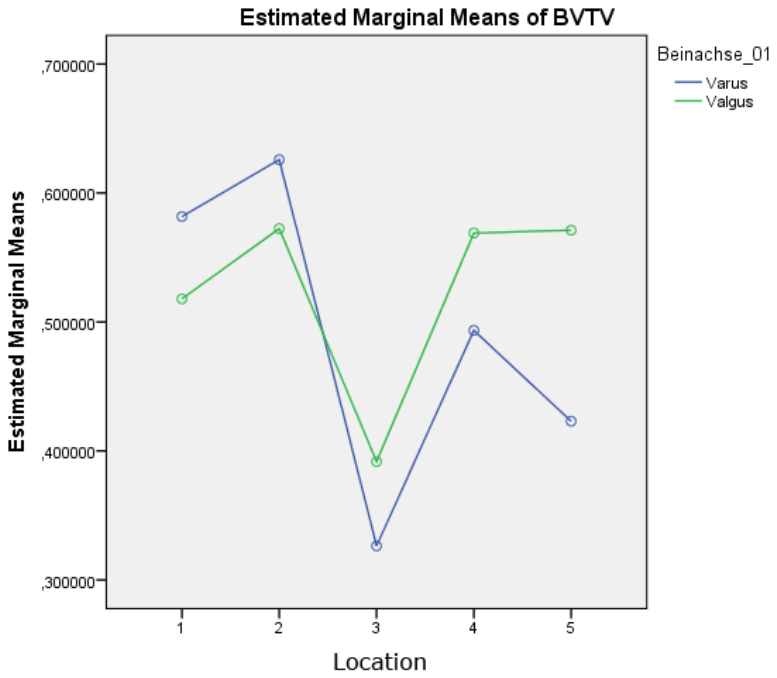
Figure 17. Mean subchondral bone fraction volume in the 5 analysed locations in respect to intact (green line) and subluxated or luxated (blue line) lateral menisci. Location 1 is submeniscal lateral, whereas location 2 is the lateral tibial plateau.



Knee joint alignment

There was a varus alignment in 22 patients with a mean of 6,3° ± 4,5°. Six patients had a valgus alignment with a mean of 7° ± 4,4°. Two patients had an orthograde axis.
Varus alignment had a significantly higher subchondral bone fraction volume in the the medial compartment than valgus, whereas valgus alignment had a significantly higher subchondral bone fraction volume in the lateral compartment (p=0,011) [Figure 18].

Figure 18. Mean subchondral bone fraction volume in 5 analysed locations in respect to knee joint alignment. Varus (blue line) and valgus alignment (green line).



9.4. Discussion

OA is a long-term chronic disease characterized by the deterioration of cartilage in joints resulting in stiffness, pain, and impaired ROM. OA is associated with ageing, but a variety of intrinsic and extrinsic factors predispose patients for OA.

Among other radiological signs, thickening of subchondral bone has been noticed in X-rays of late-stage OA [18, 48, 51] as well as an inverse correlation of BMD and OA

[52]. Therefore subchondral bone shifted into the focus as a possible cause of OA initiation and progession and subsequently as a potential treatment target [59].

A variety of studies in experimental as well as clinical settings showed a possible role of subchondral bone in the progession and initiation of OA [18, 129]. Increased bone remodelling in the subchondral bone can be found in early-stage disease proven in both in experimentally induced OA as well as in clinical settings [41, 42, 43, 44]. In late-stage disease, the rate of remodelling decreases. However, there is an imbalance in favour of bone formation in the subchondral bone leading to bone thickening (increased density) as OA progresses in experimental as well as clinical settings. In tissue specimen of late-stage hip OA with focal cartilage damage increased subchondral bone density was seen in histomorphometry as well as in microstructural analyses by µCT [55]. As a conclusion from literature review, cartilage and bone can be seen as functional unit in which pathological changes in one tissue will affect the other one and vice versa. The tissue of origin, however, has been discussed controversially as it is not known yet whether the changes start in cartilage or bone.

In this study microstructural bone parameters of 30 proximal tibial specimens of 30 patients with late-stage OA who underwent TKA were investigated by µCT. We aimed to analyse the influence of cartilage damage, meniscus' integrity as well as knee joint alignment on subchondral bone density.

Cartilage damage

In a biomechanical setting it was seen that cartilage of OA knees had a lower force attenuation compared to physiological cartilage [131]. Therefore, we hypothesised that in areas with a high degree of cartilage damage subchondral bone fraction volume would be increased compared to areas with lower degree of cartilage damage or the reference point below the ACL. The results of this study showed that the degree of cartilage damage varies within the knee compartments and that these changes are associated with subchondral bone changes. In areas of higher cartilage damage, a significant increased subchondral bone volume fraction could be identified. The highest bone fraction volume could be identified in the medial

subchondral tibial compartment. In this area also the highest degree of cartilage damage could be assessed. This finding is supported by the study of Zhang et al. in which femoral heads of patients with hip OA were studied [127].
These results provide evidence that subchondral bone changes in OA are secondary processes. Still these processes seem to be associated with OA progression.

Meniscus integrity

It was hypothesized that intact meniscal structures reduce the mechanical impacts at the proximal tibia. Therefore intact meniscal structures have been seen to be beneficial for cartilage integrity and to prevent OA progression. In our study, the degree of submeniscal cartilage defect was lower compared to the central weight-bearing tibial compartment. Similar results were seen in the lateral compartment. As a result, we could show that submeniscal subchondral bone had significantly lower bone fraction volume compared to weight-bearing locations centrally at the medial and lateral tibial compartment.
We also looked at meniscal integrity by the use of MRI. Intact versus luxated or subluxated menisci seem to be preventive in in respect to subchondral bone fraction volume.
At the moment the role of partial or complete meniscectomy is discussed controversially [134, 135]. The findings of our study support the non-surgical management of degenerative, but structurally intact menisci. On the other hand our results support the indication of surgical intervention in case of luxation or subluxation of menisci.

Knee joint alignment

Furthermore, we hypothesised that a knee joint malalignment with either varus or valgus deformity causes increased subchondral bone fraction volume as a results of increased loading. It is known that knee joint malalignment poses a risk factor for the initiation and progression of OA [137].
The majority of patients studied had a varus aligment with a mean of 6,3°. The results of our study indicate that that knee joint malalignment significantly influenced the subchondral bone fraction volume. In varus alignment higher subchondral bone

fraction volume was seen in the medial compartment compared to the lateral one. This might support the role of corrective osteotomy to prevent OA progression in both cartilage and subchondral bone.

The major strength of our study is that human knee OA specimens were studied. Most of the previous studies investigating the role of subchondral bone in OA were in an experimental setting in animals [18, 42]. Most studies in humans were performed at the hip. Unlike most experimental studies previously published, knee OA induction and progression was induced by either sectioning of the ACL or PCL or collateral ligaments or complete meniscectomy [18, 42]. So these studies induced a secondary OA of the knee. In our study, patients had a continuous natural progression of disease resulting in a primary OA of the knee.

Another strength of this study is the use of a semi-automatical segmentation software for identifying the ROI/VOI in the tibial scans. This prevents subjective influence on selection of the studies region. Therefore results are comparable on a more objective level. An auto-segmented ROI size of 4mm³ was chosen, which also strengthens the study, compared to manually extracted core cylinders that analysed specimens of up to 10mm longitudinal size. The most dramatic changes of subchondral bone density in OA are described within the first 6mm at a cranial to caudal orientation [138]. So, more realistic values could be reached compared to other studies that used cores to extract cylinders manually. Furthermore, by using an auto-segmentation in a whole scan of a specimen, the risk of artefacts also is reduced as parts close to the cutting edge are not being destroyed compared to manual extraction [139].

A weakness of the study might be the small sample size of 30 specimens. However, this sample number is comparable or even higher than other studies using µCT [18, 130]. Another limiting factor might be the evaluation of cartilage degeneration by MRI using a semiquantitative method as described by Yulish et al. [25]. On the other hand, it has been shown that evaluation of cartilage damage by MRI correlates well with histopathologic analyses [140].

Concluding, we could show significant differences of subchondral bone microstructural parameters in respect to cartilage damage, meniscus' structural

integrity and knee joint alignment. Therefore, subchondral microstructural bone changes seem to be a secondary process in the late-stage OA of knee and the proper management of these changes might prevent or stop the progression of OA.

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11. Curriculum viate

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Date of birth:	Mar. 28th, 1985
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Education

2004-2010	Medical school, Medical University of Vienna, Vienna, Austria, and University of Bonn, Bonn, Germany
2007-2010	Clinical clerkships: Prince of Wales Hospital - The Chinese University of Hong Kong Royal National Orthopaedic Hospital - University College London Ospedale di Cattinara - Università degli Studi di Trieste BG Trauma Center Murnau UniversitätsSpital Zürich
2010	M.D., Medical University of Vienna, Vienna, Austria

Professional experience

2005 – 2010	Research assistant, Department of Orthopaedics, Medical University of Vienna
2010	Intern, Department of Medicine, Evangelisches Krankenhaus Vienna
2011	Intern, Department of Surgery, Deutsch-Ordens-Spital Friesach
2012	Resident, Department of Traumatology Medical University of Vienna
2012 -	Resident, Department of Orthopaedic Surgery Medical University of Graz
2013	Visiting research fellow, Center for Advanced Orthopaedic Studies, Beth Israel Deaconess Medical Center, Harvard Medical School

Editorial activities, Peer-review

2006 – 2010	Student adviser, studentBMJ
2006 – 2009	External editor, McGill Journal of Medicine
2008 - 2009	PR officer, McGill Journal of Medicine
2008 - 2010	Reviewer, The Lancet student
2012 -	Reviewer, International Journal of Clinical Rheumatology
2012 -	Reviewer, ISRN Rheumatology
2013 -	Reviewer, BMC Musculoskeletal Diseases
2014 -	Reviewer, New England Journal of Medicine
2014 -	Reviewer, Acta Orthopaedica
2014 -	Reviewer, Archives of Physical Medicine and Rehabilitation
2014 -	Reviewer, International Orthopaedics
2014 -	Associate editor, Plastic Aesthetic Research
2014 -	Editorial board member, International Journal of Orthopaedics
2015 -	Editorial board, Journal of Rheumatic Disease and Treatment

Merits & Grants

2006 Merit grant, Medical University of Vienna
2007 ASBMR Young Investigator Travel Grant
2007 Research grant, Medical University of Vienna
2007 Merit grant, Medical University of Vienna
2008 shortlisted for BMJ Clegg Scholarship 2008
2008 ERASMUS (European Community Action Scheme for the Mobility of University Students) grant
2009 Dr. Josef Martinz grant
2011 Best Orthopedic Clinical Study of 2011 (co-author)
2013 BA Visiting Scientist Fellowship
2015 OMI-AAF Salzburg Weill Cornell Bone & Joint Surgery Scholarship
2015 Excellent Case Presentation Award, 2015 Salzburg Weill Cornell /HSS Bone & Joint Surgery Seminar
2015 ESCEO El-Lilly 2015 Scholarship