NON-INVASIVE SEMI-QUANTITATIVE AND QUANTITATIVE ULTRASOUND IMAGING FOR DIAGNOSTICS OF KNEE OSTEOARTHRITIS

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Abstract

Osteoarthritis (OA) is a common degenerative disease of synovial joints becoming more frequent with age. Pain, stiffness and functional disability caused by OA negatively affect the quality of individuals’ lives. In order to prevent the manifestation of symptoms and further OA progress, early diagnosis is essential. Ultrasonography has the potential to detect various abnormalities in the knee joint, however its place in clinical practice remains uncertain.

The present study aimed to determine the diagnostic performance of the semi-quantitative wide-area ultrasound (US) scanning of knee femoral cartilage degeneration, osteophytes and meniscal extrusion using magnetic resonance imaging as the reference tool. Diagnostic ability of conventional radiography (CR) was also determined and the performances of both modalities compared. Subsequently, the association of structural US findings with knee pain and function was investigated. Finally, quantitative US image analysis focusing on detection and evaluation of subchondral bone integrity in early OA was developed. The US quantitative outcomes were compared with CR and arthroscopy.

Tibio-femoral osteophytes, medial meniscal extrusion and medial femoral cartilage morphological degeneration were identified by US with better or at least comparable accuracy than by CR, in which joint space narrowing was used as a composite measure of cartilage damage and meniscal extrusion. The global femoral cartilage grade associated strongly with increased pain and disability. Site-specifically, especially medial cartilage degeneration and femoral lateral osteophytes were associated with increased pain and disability. Regarding the quantitative outcomes, significant increase in US intensity in the femoral subchondral bone depth 0.35–0.7 mm and decrease in intensity slope up to 0.7 mm depth were observed during radiographic or arthroscopic OA progression.

Novel wide-area US scanning provides relevant additional diagnostic information on tissue-specific OA pathology not depicted by CR. US-detected changes of femoral cartilage and osteophytes are also associated with clinical symptoms. Consequently, the use of US as a complementary imaging tool along with CR may enable more accurate diagnostics of knee OA. Furthermore, developed quantitative US analysis is a promising tool for detection of femoral subchondral bone changes in knee OA.

Keywords: articular cartilage, knee joint, knee pain, meniscus, osteoarthritis, osteophytes, subchondral bone, ultrasonography
Podlipská, Jana, Semi-kvantitatiivinen ja kvantitatiivinen ultraäänikuvaus polven nivelrikon diagnostiikassa.
Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta; Infotech Oulu; Medical Research Center Oulu; Oulun yliopistollinen sairaala; Itä-Suomen yliopisto; Kuopion yliopistollinen sairaala; Mikkelin keskussairaala
Oulun yliopisto, PL 8000, 90014 Oulun yliopisto

Tiivistelmä
Nivelrikko on erittäin yleinen nivelten rappumissairaus, joka aiheutta kipua, jäykkyyttä sekä liikkumisvaikeutta. Nivelrikon nykyistä varhaisempi diagnosointi olisi äärimmäisen tärkeää, jotta voitaisiin vähentää oireiden esiintymistä ja hidastaa sairauden etenemistä. Ultraäänikuvaus on lupaava menetelmä nivelrikon varhaisdiagnostiikkaan, mutta sitä ei kuitenkaan ole vielä yleisesti hyväksytty rutinonimaiseen kliiniseen käyttöön.


Asiasanat: luupiikki, nivelkierukka, nivelrikko, nivelrusto, polvikipu, polvinivel, rustonalainen luu, ultraäänikuva
To all my dearest and in memory of my grandfather
Jaroslav
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Oulu, 24.10.2016 Jana Podlipská
Abbreviations and symbols

- **n**: Number of subjects
- **p**: Statistical significance level
- **r**: Correlation coefficient
- **x**: Image width
- **xy**: Two-dimensional coordinate system
- **y**: Image depth
- **κ**: Weighted Cohen’s kappa coefficient
- **2-D**: Two-dimensional
- **3-D**: Three-dimensional
- **ACL**: Anterior cruciate ligament
- **ACR**: American College of Rheumatology
- **AUC**: Area under the receiver-operating characteristic curve
- **BLOKS**: Boston Leeds Osteoarthritis Knee Score
- **BML**: Bone marrow lesion
- **CI**: Confidence interval
- **CR**: Conventional radiography
- **CT**: Computed tomography
- **DESS**: Dual-echo steady-state
- **DMOAD**: Disease-modifying osteoarthritis drug
- **ECM**: Extracellular matrix
- **EULAR**: European League Against Rheumatism
- **FAS1**: Femoral arthroscopic score
- **FAS2**: Femoral arthroscopic score
- **ICC**: Intra-class correlation coefficient
- **IRR**: Incidence rate ratio
- **JSN**: Joint space narrowing
- **KL**: Kellgren-Lawrence grading
- **KOOS**: Knee Injury and Osteoarthritis Outcome Score
- **KOSS**: Knee Osteoarthritis Scoring System
- **MD**: Medial meniscus displacement
- **MOAKS**: MRI Osteoarthritis Knee Score
- **MRI**: Magnetic resonance imaging
- **NPV**: Negative predictive value
- **NSAID**: Non-steroidal anti-inflammatory drug
- **OA**: Osteoarthritis
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<tr>
<td>OARSI</td>
<td>Osteoarthritis Research Society International</td>
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<td>OKOA</td>
<td>Oulu Knee Osteoarthritis</td>
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<tr>
<td>PCA</td>
<td>Percentage close agreement</td>
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<tr>
<td>PD</td>
<td>Proton-density</td>
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<tr>
<td>PEA</td>
<td>Percentage exact agreement</td>
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<td>PJS</td>
<td>Peripheral joint space</td>
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<td>PPV</td>
<td>Positive predictive value</td>
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<td>ROC</td>
<td>Receiver-operating characteristic receiver-operating characteristic</td>
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<td>ROI</td>
<td>Region of interest</td>
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<td>SPACE</td>
<td>Sampling Perfection with Application optimized Contrasts using different flip angle Evolution</td>
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<tr>
<td>T$_1$</td>
<td>Longitudinal relaxation time</td>
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<tr>
<td>TGC</td>
<td>Time gain compensation</td>
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<td>TKA</td>
<td>Total knee arthroplasty</td>
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<td>TSE</td>
<td>Turbo spin-echo</td>
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<td>US</td>
<td>Ultrasound</td>
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<td>VAS</td>
<td>Visual analogue scale</td>
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<td>WOMAC</td>
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<td>WORMS</td>
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Original publications

This thesis is based on the following publications, which are referred to throughout the text by their Roman numerals:


The study contains also unpublished data.
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1 Introduction

Osteoarthritis (OA) is the most common type of musculoskeletal degenerative disease encountered worldwide (Umlauf et al. 2010). Its prevalence rises with increasing age and obesity of the population (Zhang & Jordan 2010). The most commonly OA affects synovial joints of the hand, knee, hip, and spine (Buckwalter & Mankin 1997, Goldring & Goldring 2006). OA aetiology is complex and multifactorial resulting from the interplay of several biological, genetic, biomechanical and joint-specific risk factors (Glyn-Jones et al. 2015, Zhang & Jordan 2010). Pain, stiffness and functional impairment are typical OA signs negatively affecting the quality of individuals’ lives (Bijlsma et al. 2011). Moreover, OA leads to significant healthcare and society costs by means of loss of working ability, treatment expenses and reduction of a life span (Buckwalter & Mankin 1997, Palazzo et al. 2016).

As reported by large epidemiological studies, the prevalence of radiographic OA in adults aged 45 and above ranges from 19% to 28% and symptomatic knee OA from 7% to 17%, depending on the study population and design (Johnson & Hunter 2014, Neogi 2013). Post-traumatic knee OA accounts for approximately 10% of OA cases in adults over 60 years old (Thomas et al. 2016). Obesity is a strong predisposing factor for incident knee OA (Grotle et al. 2008). Current statistical models predict that by the year 2030, 20% of adults worldwide will be obese and 38% overweight (Smith & Smith 2016), moreover, from year 2013 to 2050 the population aged over 60 years is estimated to rise from 841 million to 2 billion (Dawson & Dennison 2016). In this sense, the growing incidence of OA will further increase its socio-economic burden in the near future.

Nowadays, OA is considered as a whole organ joint disease with several phenotypes (Glyn-Jones et al. 2015), which are, however, still poorly understood (Bijlsma et al. 2011). OA is characterized by progressive degeneration of articular cartilage, thickening and remodelling of subchondral bone, formation of osteophytes, meniscus and ligament abnormalities as well as inflammation of the synovium (Loeser et al. 2012).

Knee OA is currently diagnosed by clinical examination and patient interview, supplemented with conventional radiography (CR) if clinical findings are inconclusive (Zhang et al. 2009). Despite its frequent use, CR is often criticized by the scientific community due to significant drawbacks, such as low sensitivity and specificity to visualize soft tissue articular pathologies as well as poor response to early OA changes (Favero et al. 2015, Hayashi et al. 2016, Iagnocco 2010, Roemer et al. 2014). Moreover, the relationship between the CR findings and symptoms is
known to be discordant (Jungmann et al. 2014, Wenham & Conaghan 2009), which may also be confounded by several person-level factors, such as sociodemographic, genetic, psychological, and physiological (Neogi et al. 2009). All joint structures can be three-dimensionally (3-D) depicted and assessed by magnetic resonance imaging (MRI), which, thereby, represents the most accurate imaging technique in clinical knee OA research. Nevertheless, for practical and cost reasons, low availability, contraindications, as well as limited clinical consequences when no disease-modifying osteoarthritis drug (DMOAD) is available, MRI cannot be routinely used for the initial examination and/or follow-up of OA patients (Guermazi et al. 2011, Iagnocco 2010, Roemer et al. 2014).

Development of a sensitive diagnostic method, which enables identification of OA already in an early pre-radiographic stage, is essential for initiation of a treatment and prevention of the disease progression.

Pain is the hallmark symptom of knee OA (Hunter et al. 2013). The aetiology of pain is heterogeneous and its mechanisms are not entirely understood (Neogi 2013, Wenham & Conaghan 2009). The insight on pain-structure association using imaging methods has been increasingly important and further research has been encouraged in order to establish an accurate definition and recognition of OA phenotypes, to advance targeting OA therapies, and enable mechanism-based pain management (Hunter et al. 2013, Neogi 2013, Wenham & Conaghan 2009).

In the last few years, the role of ultrasonography has become of great interest in the OA research (Favero et al. 2015, Finucci et al. 2015, Iagnocco 2010). Generally, US is a rapid and cost-efficient bedside procedure with minimal patient discomfort. It permits direct depiction of articular cartilage, subchondral bone, meniscus, synovium, ligaments, tendons and muscles of the knee joint. The latest progressive technological development of the high-end ultrasound (US) devices and high-resolution transducers provides very detailed musculoskeletal images with improved sensitivity to reveal a wide range of early OA changes. Furthermore, dynamic probe sweeping gives the opportunity of multi-planar investigation of intra- and peri-articular joint structures (Chao & Kalunian 2008, Finucci et al. 2015, Iagnocco 2010). All of those advantages of modern US can be utilized for development of a more sophisticated semi-quantitative assessment and quantitative US image analysis for detection of OA abnormalities from early to advanced stages of the disease within the knee joint.

OA includes several tissue-level changes before the articular cartilage begins to actually wear away. OA alteration of the articular cartilage and subchondral bone has been successfully observed and quantitatively analysed with US in laboratory
conditions *in vitro* (Leicht & Raum 2008, Saarakkala *et al.*, 2006, Saïed *et al.* 1997, Virén *et al.*, 2009). In addition, studies introducing semi-quantitative US knee OA grading *in vivo* (Koski *et al.*, 2015, Lee *et al.*, 2008, Riecke *et al.*, 2014, Saarakkala *et al.*, 2012) have achieved promising results, highlighting the need for further clinical investigations. While there are a few proposed US grading systems for knee OA (Aisen *et al.*, 1984, Bruyn *et al.*, 2015, Lee *et al.*, 2008, Riecke *et al.*, 2014, Saarakkala *et al.*, 2012), their sensitivity and specificity in comparison to MRI and/or CR has not been studied before. The potential of quantitative US imaging as a new method for OA diagnostics has been also indicated in several other studies (Laasanen *et al.*, 2006, Saarakkala *et al.*, 2006, Saarakkala *et al.*, 2012). However, the structural and compositional changes of knee OA has never been quantitatively studied from non-invasive US images *in vivo*. Therefore, it is important to develop new semi-quantitative and/or quantitative site-specific US knee OA assessment methods, which would provide novel diagnostic information, and would lead to introduction of a validated US grading system for knee articular cartilage, osteophytes, and meniscus, which is still lacking in the clinical practice (Chao & Kalunian 2008).

With regard to the above-described gaps in the current scientific evidence, the present doctoral study attempted to determine the diagnostic performance of knee ultrasonography and its role among other OA imaging modalities (Study I). Subsequently, the association of US-defined features with knee pain and function was examined (Study II), and finally, the potential of US quantitative analysis of subchondral bone to detect the tissue-specific OA changes was explored *in vivo* (Study III).
2 Knee joint

The knee is the largest and the most complex synovial joint in the human body (Miller & Thomson 2014). It consists of femoral, tibial, patellar and fibular bone structures, ligaments, tendons, muscles, synovial capsule and tissue, Hoffa’s fat pad, articular cartilage and meniscus (Fig. 1) (Buckwalter & Mankin 1998, Clockaerts et al. 2010, Hirschmann & Müller 2015). In the following sections the structure and composition of the individual anatomical parts of the knee joint are described, primarily focusing on articular cartilage, subchondral bone, meniscus and synovium.

![Anatomical illustration of the knee joint](image)

Fig. 1. Anatomical illustration of the knee joint. Ligaments and tendons are not labelled in the figure, but they are distinguishable by light stripes. Synovial capsule and muscles are omitted here.

2.1 Articular cartilage

Articular cartilage is a highly specialized connective tissue covering the bone ends in synovial joints (Fig. 1). Histologically, it is classified as a hyaline type (Umlauf...
The Greek name *hyalos*, meaning glass, originates from the smooth, translucent appearance of normal cartilage tissue (Jeffrey & Watt 2003). Its exceptional properties of high resistance to deformation and load-bearing ability and efficient load-distribution enable efficient protection of the subchondral bone from local peak stresses. Together with the synovial fluid cartilage provides stable low-friction joint movement (Buckwalter & Mankin 1998, Huber et al. 2000).

Articular cartilage is composed of specialized cells, *i.e.* chondrocytes, and extracellular matrix (ECM). It is avascular, aneural, alymphatic and in comparison to other soft tissues relatively hypocellular. Only approximately 1% of the total cartilage volume is represented by chondrocytes which produce the highly organized ECM providing the cartilage tissue with its characteristic physical properties, stiffness and resilience (Buckwalter & Mankin 1998, Huber et al. 2000). The main components of the ECM are water and macromolecules, such as collagens, proteoglycans and non-collagenous proteins (Buckwalter & Mankin 1998, Huber et al. 2000). Water content corresponds to up to 60–80% of cartilage wet weight, whereas the structural macromolecules contribute the remaining 20–40% (Buckwalter & Mankin 1998). The collagen fibril network, primarily consisting of collagen type II (80–95% of the collagen content) and other minor types of collagen (III, VI, IX, X, XI, XII, XIV), creates the principal endoskeletal structure of the healthy cartilage (Buckwalter & Mankin 1998, Huber et al. 2000, Umlauf et al. 2010). The collagen distribution greatly varies within the articular cartilage. First, the collagen content is high at the superficial zone, then it decreases at the middle zone and increases towards the tidemark where the content is the highest (Oinas et al. 2016, Saarakkala et al. 2010. The negatively charged proteoglycans are able to constitute larger aggregates and bind water molecules from the surrounding tissue causing swelling of the cartilage. The collagen network, on the other hand, resists the swelling creating osmotic pressure inside the cartilage tissue, which helps to distribute loads and increase cartilage durability (Huber et al. 2000).

Articular cartilage can be structurally divided into four horizontal zones: superficial, transitional, deep and calcified zones (Fig. 2). The zones vary in organization of the collagen network, type and concentration of the proteoglycans and water content, which decreases with depth (82% in the superficial zone and 76% in the deep zone) (Huber et al. 2000, Jeffrey & Watt 2003). Furthermore, chondrocytes differ in number, size, shape and metabolic activity throughout the cartilage zones (Buckwalter & Mankin 1998). The thinnest superficial zone consists of densely organised thin collagen fibres with embedded flattened
chondrocytes both arranged parallel to the cartilage surface. Low concentration of proteoglycans allows good interstitial fluid flow within the zone providing cartilage with the “spongy shock absorber” function and ability to distribute the impact load (Boettcher et al. 2016, Buckwalter & Mankin 1998, Jeffrey & Watt 2003). In general, the organization and composition of the superficial zone primarily determines the mechanical and protective properties of the articular cartilage (Buckwalter & Mankin 1998, Huber et al. 2000). The transitional (middle) zone is characterized by larger randomly arranged collagen fibrils, round-shaped chondrocytes and increased concentration of proteoglycans. In the deep zone, chondrocytes are gathered into columns and collagen fibrils are aligned perpendicular toward the articular surface. Here, the proteoglycan content is at its highest. The deep and calcified zone is separated by a “tidemark”, an irregular line anchoring the fibrils from the non-calcified layer. The calcified zone lacks proteoglycans and the chondrocytes are of small volume and have very low metabolic activity. The collagen fibres are the largest and run perpendicularly to the surface. The osteochondral junction or so called “cement line” separates the calcified zone from a thin layer of subchondral bone plate followed by a trabecular bone beneath (Buckwalter & Mankin 1998, Burr 2004b, Huber et al. 2000, Madry et al. 2010).

2.2 Subchondral bone

Several definitions of the term “subchondral” have been used in the earlier scientific literature (Madry et al. 2010). The definition used in this thesis was given by Burr et al. (2004a) who described subchondral bone as two distinct regions: subchondral bone plate and subchondral trabecular bone (Fig 2). The structure, composition and physical properties of normal subchondral bone vary significantly (Madry et al. 2010).

The subchondral bone plate is 1–3 mm thick, corticalized tissue with occasional vascular channels invading the osteochondral junction (Burr & Gallant 2012, Burr 2004b, Madry et al. 2010). Besides blood vessels, which may nourish the calcified cartilage at the location of the interface perforation, vascular channels are often accompanied by nerves (Hoemann et al. 2012, Madry et al. 2010). The distribution of the blood vessels as well as physical properties, such as plate thickness, density and stiffness, are assumed to increase with higher stresses acting on the surface (Madry et al. 2010).
The subchondral trabecular bone is comprised of supporting trabeculae which are derived from the subchondral plate being perpendicularly crossed by finer trabeculae creating interconnected spaces (Madry et al. 2010). Subchondral trabecular bone differs from the subchondral plate by its structural and mechanical anisotropy. Trabecular bone is inhomogeneous, more porous and richer in blood vessels and sensory nerves. Additionally, it has lower volume, stiffness and density (Li et al. 2013, Sharma et al. 2013).

The subchondral bone has a shock absorbing function and it contributes to load attenuation. The subchondral plate is deformable to some extent and allows thus to create a maximal load-bearing surface and further transmit the load to the trabecular bone (Huber et al. 2000, Madry et al. 2010).

Bone ECM is composed of 50–70% of mineral, 20–40% of organic matrix, 5–10% of water and less than 3% of lipid. ECM corresponds to more than 90% of total bone tissue and the remaining content are cells, cell processes or extensions and blood vessels. Bone tissue is a composite material which comprises an organic and inorganic component. The organic component includes 85–90% of collagen, especially type I, with small amounts of types III, V and XII, and the 10% is represented by non-collagenous proteins and bone-specific proteoglycans (Buckwalter et al. 1996, Clarke 2008). In the subchondral bone plate the collagen fibrils are densely and parallelly lamellated into sheets advancing into lamellae of trabecular bone (Madry et al. 2010). The main element of the inorganic or mineral matrix is hydroxyapatite \([\text{Ca}_10(\text{PO}_4)_6(\text{OH})_2]\) and trace amounts of carbonate, magnesium and acid phosphate (Clarke 2008). The mineral component provides bone with its stiffness and load-bearing strength, and the organic component gives elasticity and flexibility (Buckwalter et al. 1996, Clarke 2008).
Bone cells include osteoblasts, osteoclasts, osteocytes and bone-lining cells (Buckwalter et al. 1996). Osteoblasts mediate synthesis of a new bone, whereas osteoclasts mediate bone resorption, therefore playing an important role during bone modelling and remodelling also during OA (Buckwalter et al. 1996, Goldring & Goldring 2010a). Osteoblasts may become osteocytes, while trapped within lacunae of newly formed bone matrix or stay on the surface of the bone and become bone-lining cells. Osteocytes coordinate the exchange of mineral between the bone fluid in the lacunae and blood, and formation and resorption of bone. Bone-lining cells regulate the flow of mineral ions into and out of the bone extracellular fluid, and furthermore they initiate bone resorption (Buckwalter et al. 1996, Clarke 2008).

2.3 Meniscus

The knee joint meniscus consists of a pair of medial and lateral semi-lunar wedge-shaped structures located between the femoral condyle and tibial plateau (Fig. 1)
Peripheral presence of blood vessels and nerves (10–25% of the tissue) divides the meniscus into an outer, vascular/neural region, and an inner, avascular/aneural region (Makris et al. 2011). Menisci are firmly attached between the femoral and tibial articular surfaces by stabilizing ligaments: medial collateral ligament, transverse ligament, meniscofemoral ligaments and insertional ligaments attaching the anterior and posterior horns. The meniscofemoral ligaments, called Humphrey and Wrisberg ligaments, attach the posterior horn of the lateral meniscus to the medial femoral condyle (Makris et al. 2011, Messner & Gao 1998). In most people only one of these ligaments is present (Gupte et al. 2003). The lateral meniscus has smaller dimensions than the medial meniscus, however it covers 75–93% of the corresponding tibial plateau, whereas medially the meniscus covers 51–74% of the surface (Makris et al. 2011).

Meniscus is glossy-white, complex tissue composed of 72% of water and 28% of organic phase including mainly cells and ECM. Seventy-five percent of the organic phase is made up of collagen, followed by 17% of glycosaminoglycans, 2% of DNA, less than 1% of adhesion glycoproteins and less than 1% of elastin (Makris et al. 2011). The outer region is composed predominantly of collagen type I (90%), whereas in the inner zone the collagen composition accounts for 60% of type II and 40% of type I. The collagen fibres have predominantly circumferential orientation. Tied together by radially oriented fibres they are prevented from splitting (Messner & Gao 1998). Similarly as in articular cartilage, meniscal proteoglycans allow water retention and thus adapt to local stresses (Makris et al. 2011).

Meniscus cells are located both in the outer and inner regions. The outer region contains fibroblast-like cells having long extensions which allow them to communicate with other cells and the ECM. On the contrary, the inner zone contains chondrocyte-like cells embedded in the ECM with a great amount of collagen type II and proteoglycan aggregans. By its composition the inner zone resembles articular cartilage. The third type of flattened, fusiform cells is found in the superficial layer, aligned parallel to the surface (Makris et al. 2011, Messner & Gao 1998).

The physiological function of the meniscus includes load bearing, load transmission, shock absorption, joint stabilization and lubrication and nutrition of the articular cartilage (Makris et al. 2011, Messner & Gao 1998).
2.4 Synovial capsule

The knee joint is surrounded and sealed by the innervated synovial capsule, which consists of an outer fibrous membrane and inner richly vascularized synovial membrane or synovium (Haywood & Walsh 2001, Ralphs & Benjamin 1994). Synovial membrane cells produce important components of the synovial fluid, lubricin and hyaluronic acid. They provide nutrition and lubrication to the articular cartilage and are, therefore, essential for normal joint function (Haywood & Walsh 2001, Scanzello & Goldring 2012).
3 Osteoarthritis

In this section, the current definition and understanding of OA pathogenesis will be briefly reviewed. The text generally applies to all synovial joints, but with regard to the scope of this thesis, in many points the author refers primarily to knee OA.

3.1 Definition

Historically, OA has been classified as a primary (idiopathic, of unknown cause) and secondary (triggered) condition (Herrero-Beaumont et al. 2009). However, based on the recent evidence OA is almost always considered to be driven by some predisposing factor or well-recognized condition causing abnormal mechanics of the joint, and therefore should no longer be considered as primary (Felson 2013).

Knee OA definitions are based on pathological, radiographic or clinical evidence (Johnson & Hunter 2014). The OA pathologies may involve any tissue of the joint, but predominantly the changes include articular cartilage degeneration, subchondral bone thickening, formation of osteophytes, inflammation of synovium, degeneration of ligaments and menisci, and hypertrophy of the joint capsule (Loeser et al. 2012). The radiographic knee or hip OA is traditionally assessed from conventional radiographs by the Kellgren-Lawrence (KL) grading system (described specifically in Chapter 6.3) (Kellgren & Lawrence 1957), which is the most widely used and standardized diagnostic approach in OA research (Johnson & Hunter 2014, Litwic et al. 2013). Grade 2, i.e. at least definite osteophytes, or higher defines the presence of the disease (Kellgren & Lawrence 1957). Clinical OA is determined by patient history and physical examination (Litwic et al. 2013, Palazzo et al. 2016). The most recognized and standardized classification criteria of knee OA has been developed by the American College of Rheumatology (ACR) (Altman et al. 1986). The clinical OA criteria are fulfilled when the patient has knee pain and three out of six of the following clinical signs: age above 50, morning stiffness less than 30 min, crepitus, bone tenderness, bone enlargement and/or no palpable warmth (Altman et al. 1986). Regarding recent research advancements and increasing evidence on OA aetiology and disease mechanisms, the above-described classifications are rather out-dated (Herrero-Beaumont et al. 2009, Kraus et al. 2015, Peat et al. 2006).

In 2015, the most current OA definition was proposed by the Osteoarthritis Research Society International (OARSI) (Kraus et al. 2015): "Osteoarthritis is a disorder involving movable joints characterized by cell stress and extracellular
matrix degradation initiated by micro- and macro-injury that activates maladaptive repair responses including pro-inflammatory pathways of innate immunity. The disease manifests first as a molecular derangement (abnormal joint tissue metabolism) followed by anatomic, and/or physiologic derangements (characterized by cartilage degradation, bone remodelling, osteophyte formation, joint inflammation and loss of normal joint function), that can culminate in illness.” As it can be clearly seen from the definition, OA is no more considered as the disease of cartilage “wear-and-tear”, but as a highly heterogeneous disorder of the entire joint with several phenotypes (Kraus et al. 2015, Loeser et al. 2012).

Risk factors increasing the probability of OA development can be divided into person-level and joint-level ones. The most common person-level factors include age, gender (female), genetic predispositions, obesity, demographic characteristics, and increased bone mineral density and bone mass. Joint-level factors include abnormal bone/joint shape, injury, abnormal joint loading and malalignment, muscle weakness, leg length inequality, occupation, as well as highly repetitive, intense and high-impact physical activity (Allen & Golightly 2015, Glyn-Jones et al. 2015, Johnson & Hunter 2014, Palazzo et al. 2016).

3.2 Pathogenesis and progression

A pathogenesis is very complex with several pathways in which principally articular cartilage, subchondral bone and synovium play the key roles (Glyn-Jones et al. 2015). The early articular cartilage involvement is characterized by activation of chondrocytes, as a response to abnormal changes in the chemical or mechanical environment (Glyn-Jones et al. 2015). In attempt to maintain joint homeostasis, chondrocytes form clusters, proliferate and synthesize matrix proteins more actively, however, with subsequent failure to compensate the tissue degradation (Bijlsma et al. 2011, Loeser et al. 2012, Sharma et al. 2013). Production of matrix-degrading proteases, inflammatory cytokines and chemokines increases, followed by depletion and loss of proteoglycans. Due to increased water content, the collagen matrix becomes more susceptible to mechanical damage leading to irreversible and progressive cartilage degradation, including fibrillation, formation of clefts and eventually complete wear of the cartilage (Bijlsma et al. 2011, Buckwalter & Mankin 1997, Glyn-Jones et al. 2015, Loeser et al. 2012, Sharma et al. 2013).

Scientific discussions regarding whether OA is initiated first in the cartilage tissue leading to changes in the subchondral bone or vice versa, or whether the abnormal events in cartilage and bone are concomitant, has been on-going for
decades (Aisen et al. 1984, Buckwalter & Mankin 1997, Dieppe & Lohmander 2005, Glyn-Jones et al. 2015, Radin & Rose 1986). However, the latest research suggests that bone-first OA development exists as an individual phenotype (Funck-Brentano & Cohen-Solal 2015) and cartilage loss is the common endpoint to all OA phenotypes (Glyn-Jones et al. 2015). The typical subchondral bone changes in OA include bone remodelling, thickening and formation of peri-articular osteophytes. Osteoarthritic subchondral bone carries information reflecting its loading history due to active bone remodelling. The acting of load is translated into enhanced thickness and apparent bone density (Burr 2004a, Loeser et al. 2012). The bone remodelling process is initiated by microcracks which appear at the site of repetitive joint overloading. The microcracks are also likely to be the cause of bone marrow lesions (BMLs) (Taljanovic et al. 2008), which are often co-localized with severe cartilage damage (Kijowski et al. 2006). In comparison to articular cartilage whose reparative capacity is relatively low, subchondral bone has the ability of rapid alteration of its structure (Li et al. 2013, Loeser et al. 2012). The high-rate bone turnover leads to bone hypomineralization, i.e. low mineral content. Conversely, low-rate bone turnover increases the resistance to deformation, which adversely affects the overlying cartilage (Loeser et al. 2012). However, the direct cross-talk between chondrocytes and bone cell-expressed mediators is allowed through the microdisruptions and vascular channels at the cartilage-bone interface further inducing the endochondral ossification, cartilage vascular invasion and advancement of the calcified cartilage layer into the deep zone causing local cartilage thinning (Glyn-Jones et al. 2015, Loeser et al. 2012). Furthermore, it has been suggested that chondrocytes at the cartilage-bone junction can transdifferentiate into osteoblasts and contribute directly to the subchondral plate thickening by new bone formation (Funck-Brentano & Cohen-Solal 2015, Zhou et al. 2014). It has been suggested that neovascularization of cartilage, meniscus and osteophytes accompanied by sensory nerve growth may be the potential source of pain (Ashraf & Walsh 2008, Mapp & Walsh 2012), although the origin of OA pain is still not understood.

Synovitis appears mostly secondary to interruption of cartilage homeostasis and release of inflammatory mediators. Changes in synovium include cell proliferation, expression of inflammatory mediators and degradative enzymes, tissue hypertrophy and enhanced vascularization. The presence of synovitis predicts incidence and progression of OA symptoms (Glyn-Jones et al. 2015).

OA can also develop secondarily as a long-term post-traumatic consequence of anterior cruciate ligament (ACL) or meniscus tear. General estimation is that 10 to
20 years after diagnosis of ACL or meniscus tear, more than 50% of the injured subjects will end up with symptomatic OA (Lohmander et al. 2007). Besides the meniscus tear, meniscal degeneration in OA appears as fissuring, fragmentation, maceration or complete destruction (Man & Mologhianu 2014). Furthermore, calcification of meniscus and cartilage is often observed in older OA patients together with presence of crystals (Loeser et al. 2012), and is also associated with subchondral bone attrition (Abhishek et al. 2016). Meniscal extrusion is highly common in already established OA. It results in abnormal joint load distribution, joint instability and higher susceptibility of subchondral bone to damage. Furthermore, meniscal extrusion contributes to joint space narrowing (JSN) and cartilage thinning (Martel-Pelletier & Pelletier 2010).

At the late stage of OA, various tissues are involved in the disease process resulting in advanced joint destruction, instability and ultimate functional failure of the knee joint (Castañeda et al. 2014, Loeser et al. 2012).

3.3 Phenotypes

The general definition for phenotype, as defined by the Oxford English Dictionary (Oxford 2005), is as follows:

“The sum total of the observable characteristics of an individual, regarded as the consequence of the interaction of the individual’s genotype with the environment; a variety of an organism distinguished by observable characteristics rather than underlying genetic features.”

Regarding OA, phenotype is further specified as a feature that is observable by the naked eye, by images or by blood tests. However, genotype is often a source of observable phenotypic variations (Felson 2010).

In knee OA, various phenotype classifications have been proposed based on anatomical, etiological, genetic, and clinical and/or radiological factors. The phenotypes continuously progress and lead to clinical manifestation depending on their principal underlying pathways and involved tissue (Castañeda et al. 2014).

Several clinical OA phenotype classifications have been proposed (Table 1) (Bijlsma et al. 2011, Felson 2010, Knoop et al. 2011, van der Esch et al. 2015). Radiographically, rare forms of OA have been identified as atrophic and hypertrophic (Table 1). The atrophic phenotype is characterized by definite JSN and absence of concomitant osteophyte formation. Contrarily the hypertrophic phenotype features large osteophytes and little JSN (Roemer et al. 2012, Roemer
et al. 2015). With the help of MRI, anatomical phenotype classification has been developed, defined by the primarily discerned structural site of OA origin (McGonagle et al. 2010) (Table 1).

With deeper knowledge of predominant underlying pathophysiological mechanisms several sub-phenotypes have been defined. For instance, by further recognition of bone involvement in OA, osteoporotic, bone forming and erosive sub-phenotypes have been determined (Geusens & van den Bergh 2016). Psychological factors, comorbidities and joint sensitivity, have been identified as principal characteristics playing important roles in pain sub-phenotype definitions (Kittelson et al. 2016).

Differentiation of clinical OA phenotypes is particularly needed for development of effective personalized disease-modifying therapies and OA prevention (Felson 2010). However, the final OA phenotype classification is still under scientific discussion (Castañeda et al. 2014).

Table 1. Osteoarthritis phenotype classifications.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Author</th>
<th>Phenotype</th>
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<tbody>
<tr>
<td>Clinical</td>
<td>Felson 2010</td>
<td>Generalized vs. Joint-specific</td>
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<tr>
<td></td>
<td></td>
<td>Incident vs. Progressive</td>
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<tr>
<td></td>
<td></td>
<td>Painful vs. Non-painful</td>
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<tr>
<td></td>
<td></td>
<td>Knee malalignment</td>
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<tr>
<td></td>
<td>Bijlsma et al. 2011</td>
<td>Post-traumatic (acute or repetitive)</td>
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<td></td>
<td></td>
<td>Metabolic</td>
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<td>Aging</td>
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<td>Genetic</td>
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<td></td>
<td></td>
<td>Pain</td>
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<tr>
<td></td>
<td>Knoop et al. 2011, van der Esch et al. 2015</td>
<td>Minimal joint disease</td>
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<td>Strong muscle strength</td>
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<td>Severe radiographic OA</td>
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<td></td>
<td></td>
<td>Obese</td>
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<td></td>
<td></td>
<td>Depressive mood</td>
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<tr>
<td>Radiographic</td>
<td>Roemer et al. 2012, Roemer et al. 2015</td>
<td>Atrophic</td>
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<tr>
<td></td>
<td></td>
<td>Hypertrophic</td>
</tr>
<tr>
<td>Anatomical</td>
<td>McGonagle et al. 2010</td>
<td>Cartilage</td>
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<td>Bone</td>
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<td>Meniscal</td>
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<td>Ligament</td>
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<td>Synovial</td>
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<td></td>
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<td>Multifocal</td>
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</tbody>
</table>
3.4  Clinical diagnostic methods

Knee OA is conventionally diagnosed based on patient history, self-reported symptoms, physical examination and medical imaging. Besides pain, which is predominantly leading the individual to seek medical help, morning stiffness and loss of function and mobility are often involved (Bijlsma et al. 2011, Michael et al. 2010). A list of recommendations has been proposed as a guideline for clinical diagnosis of knee OA in primary care (Zhang et al. 2009).

3.4.1 Self-administrated questionnaires

In research settings, not so commonly in daily clinical practice, OA symptoms and signs are often assessed with the help of self-administrated questionnaires. The most popular questionnaire is the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) including three subscales for knee pain, stiffness and function assessment (Bellamy et al. 1988). The Knee Injury and Osteoarthritis Outcome Score (KOOS) has been developed for evaluation of ACL injury, meniscus injury or post-traumatic OA in young and middle-aged subjects with intention for application in follow-up. KOOS consists of five subscales: pain, other symptoms, function in daily living, function in sport and recreation and knee-related quality of life (Roos & Lohmander 2003, Roos & Toksvig-Larsen 2003).

3.4.2 Imaging methods

Despite the recent technological advances and developments in the field of OA imaging-based diagnostics, CR is still considered to be the gold standard (Hayashi et al. 2016). CR can reveal JSN, marginal osteophytes, subchondral bone cysts and sclerosis (Bijlsma et al. 2011, Glyn-Jones et al. 2015). Over half a century, the KL grading system (Kellgren & Lawrence 1957) has been widely used to determine the presence and severity of knee OA. Next to KL grading, which is a composite score of bone abnormalities and JSN, a compartmental feature-specific OARSI score has been developed (Altman & Gold 2007). JSN from CR has been used mainly as an indirect indicator of articular cartilage thinning, but it may also be a sign of meniscal extrusion or a combination of both (Dendy & Heaton 1999). Furthermore, JSN is unable to detect early localized cartilage degeneration (Guermazi et al. 2012, Javaid et al. 2010). Despite its lack of sensitivity and specificity, JSN remains to be recommended by regulatory agencies, the US Food
and Drug Administration and the European Medicines Agency, as the structural endpoint to prove the efficiency of the DMOADs (Bijlsma et al. 2011, Glyn-Jones et al. 2015). Although CR is a fast, widely available and inexpensive technique, limitations, such as lack of standardization of the image acquisition, failure to directly depict the articular cartilage, menisci, synovium and other soft tissues, as well as inability of 3-D joint assessment, make this diagnostic technique insensitive in detection of early OA tissue involvement as well as disease progression (Bijlsma et al. 2011, Favero et al. 2015, Hayashi et al. 2016, Roemer et al. 2014, Teichtahl et al. 2008).

Superficial articular cartilage and meniscal damage can be directly visualized and palpated by arthroscopy (Chu et al. 2012, Favero et al. 2015). Several cartilage degeneration assessment scales have been developed; the most known are Outerbridge (Outerbridge 1961) or Noyes’ grading (Noyes & Stabler 1989). However, invasiveness restricts the diagnostic use of arthroscopy on a daily basis (Blackburn et al. 1996, Iagnocco 2010).

MRI is the most sensitive imaging modality for assessment of OA (Favero et al. 2015, Iagnocco 2010). MRI permits multi-planar, high-resolution depiction and evaluation of the entire joint (Hayashi et al. 2016). Early structural changes can be identified even before they become evident radiographically (Guermazi et al. 2012, Javaid et al. 2010). Three well-established semi-quantitative scoring systems for OA changes have been applied in several clinical trials: the Whole Organ Magnetic Resonance Imaging Score (WORMS) (Peterfy et al. 2004), the Knee Osteoarthritis Scoring System (KOSS) (Kornaat et al. 2005), and the Boston Leeds Osteoarthritis Knee Score (BLOKS) (Hunter et al. 2008a). After recognition of positive and negative aspects of WORMS and BLOKS, a new refined tool, the MRI Osteoarthritis Knee Score (MOAKS), was proposed and validated (Hunter et al. 2011). MOAKS comprises sub-regional morphological assessment of articular cartilage, BMLs and cysts, osteophytes, meniscus, synovitis, effusion, ligaments and tendons and other periarticular features (Hunter et al. 2011). Besides evaluation of morphological tissue changes with scoring systems, compositional MRI may allow detection of pre-morphologic biochemical changes of especially cartilage but also meniscus (Hayashi et al. 2016). On the other hand, MRI morphometry helps to quantify and follow, e.g., cartilage thickness and volumetric changes during the OA course (Hayashi et al. 2016, Wang et al. 2015). The routine clinical use of MRI is restricted by its high cost, low availability and time consumption of both image acquisition as well as whole-organ analysis (Bijlsma et al. 2011, Iagnocco 2010).
US imaging is used on a daily basis for diagnostics of OA in the rheumatology outpatient clinics (Iagnocco et al. 2011). Valid and reliable imaging of a large number of inflammatory and structural OA abnormalities progressively increases its deployment in routine practice (Finucci et al. 2015). For this reason, the use of US should be considered also in radiology departments and general practice. The scientific literature on clinical as well as pre-clinical US imaging for knee OA assessment is reviewed in detail in Chapter 4.2.

3.5 Treatment

To date, no efficient cure for OA exists. The main goal of OA treatment is to relieve pain, to maintain and improve function, to slow down disease progression and to increase the health-related quality of life (Bijlsma et al. 2011, Zhang et al. 2016).

The knee OA treatments are divided into non-pharmacological, pharmacological and surgical (Bijlsma et al. 2011, Zhang et al. 2008). Several evidence-based guidelines for the non-pharmacological and pharmacological OA management have been published by different working groups, such as the ACR (Hochberg et al. 2012), the European League Against Rheumatism (EULAR) (Fernandes et al. 2013) or OARSI (McAlindon et al. 2014). The latest recommendations by OARSI are specifically focused on treatment of OA of the knee joint proposing an individualized combination of conventional therapies suiting patient needs and preferences (McAlindon et al. 2014). From the non-pharmacological therapies, e.g. self-management and education programmes, exercise programmes (aerobic and strengthening training), recommendation of weight loss and use of biomechanical aids are included. From the pharmacological therapies, intra-articular corticosteroids are prescribed. Moreover, specific clinical OA sub-phenotypes have been suggested regarding presence of only knee or multiple joint OA, and comorbidities. Appropriate additional treatments, such as balneotherapy, use of a cane, paracetamol, duloxetine, oral non-steroidal anti-inflammatory drugs (NSAIDs), and topical NSAIDs and capsaicin, can be recommended for specific clinical sub-phenotypes (McAlindon et al. 2014). Irrespectively of OARSI recommendations, recent research has reported no clinical efficiency of paracetamol for OA treatment, and diclofenac has been proposed to be the most efficient medication for pain reduction and physical function improvement (da Costa et al. 2016). However, it has also been pointed out that the potential side effects and individual safety should be taken into account when choosing the appropriate drug for the patient (da Costa et al. 2016).
At the later OA stage, however, when the pain and symptom relieving therapies fail, surgical intervention is usually the only help. Osteotomy or unicompartmental knee arthroplasty can be utilized but a majority of the patients still undergo total joint replacement (Zhang et al. 2008).

Current research has concentrated on development of potential pharmacological treatments targeting specific OA processes in cartilage, bone and synovium. The regenerative field has been focused on advancements in cell-based and tissue engineering therapies (Zhang et al. 2016). Nevertheless, in order to treat the disease efficiently and/or prevent its progression, early detection and diagnosis of the knee joint abnormalities together with specification of OA phenotypes are required (Bijlsma et al. 2011, Glyn-Jones et al. 2015).
4 Ultrasonography

4.1 Ultrasound image generation

US images are formed in three steps: generation and transmission of the US wave, receiving of the echo, and its interpretation. The first two steps are accomplished with the use of a specifically designed US transducer. The transducer contains piezoelectric crystals exhibiting the piezoelectric effect in which mechanical energy is transformed into electrical energy and *vice versa*. Therefore, a single transducer can be used simultaneously as a transmitter and a receiver of the US. This feature enables employment of the pulse-echo imaging method generally used in medical US (Connolly 2008, Dendy & Heaton 1999).

Modern US probes contain an array of transducer elements driven with a slight delay starting from the outer elements so that the individual beams constructively interfere in the desired depth. Thus, one complete focused beam is generated. The propagating US wave is proportionally reflected back to the transducer depending on tissue acoustic impedance mismatch along the scan line (beam axis). Receiving of the echo by the transducer is based on the same principle as its transmission in order to image a single scan line. A linear array probe is usually used when visualizing large structures close to the surface. It has a narrow beam pattern and provides a rectangular image. In this thesis, knee ultrasonography was performed using the linear array probe (Connolly 2008, Dendy & Heaton 1999, Kossoff 2000, Wodnicki et al. 2009).

4.1.1 B-mode imaging

The B-mode US image representation is the most common in medical US imaging. B-mode images are generated using the following pulse-echo technique. The time of flight between the transmitted pulse and the received echo is measured by sensitive electronics within the US device. By assuming the constant speed of sound for human soft tissues (approximately 1540 m/s) the depth of each returned echo is calculated. The echo amplitude determined by acoustic impedance discontinuities is displayed as a relevant brightness value in \(xy\) location of the US image (\(y = \) echo depth, \(x = \) determined by width of the transducer). Thus, a conventional two-dimensional (2-D) cross-sectional image is obtained (Fig. 3) (Dendy & Heaton 1999, Hendee & Ritenour 2003, Kossoff 2000).
4.1.2 Ultrasound signal and image processing in commercial devices

Penetration of the US wave determines the maximum image depth, thereby the field of view. Penetration depends on the wave frequency, achievable sensitivity at the maximum depth and pulse repetition frequency. Lower US wave frequency allows deeper penetration at the expense of spatial resolution, conversely higher frequency decreases penetration but increases spatial resolution. Sensitivity is the ability of the US scanner to distinguish the weakest scatterer or reflector from the noise. At any depth, sensitivity is determined by setting the output power, the focus position, and overall gain controls. Dynamic range is a ratio of the largest and the smallest echo processed by the US device (Dendy & Heaton 1999). Dynamic range is decreased within the US system due to signal processing, such as time-gain compensation (TGC) and rejection, eliminating large and small signals (Hendee & Ritenour 2003).

Spatial resolution is the ability of the US device to distinguish and visualize two close reflecting or scattering structures as separate. Axial resolution is a
resolution along the direction of a travelling wave or scan line. Lateral resolution is the resolution along the direction perpendicular to the scan line. Both are affected by the US beam frequency. Axial resolution is also determined by the pulse length typically consisting of two wave cycles. With higher frequency, the pulse length is shorter yielding better resolution. Lateral resolution is approximately equal to the beam width, which is inversely proportional to the frequency. Thus, also lateral resolution improves with higher frequency. The wave may be concentrated into a so-called focal zone at the region of the greatest clinical interest. This can be accomplished by, e.g., inserting a lens in front of an unfocused transducer. In the focal zone, the beam width is the narrowest, the intensity is greater, and thereby, enhanced lateral resolution is achieved. Focal depth is equal to distance between the probe and the focal zone, and it can be freely modified by the operator (Dendy & Heaton 1999).

The main goal of the US imaging system is to faithfully convert the reflected US echoes into brightness values without loss of information. Therefore, the signal pre- and post-processing is important. One of the undesired factors limiting the visualization is attenuation. It can be compensated for by pre-processing of the signal utilizing the TGC. The TGC circuit amplifies the receiving echoes by the gain factor increasing with time so that strong echoes coming from close locations (returning at shorter times) receive less amplification and the weak echoes arising from deeper structures are amplified more (Fig. 4). The TGC must be adjusted for each patient and examined body location individually due to specific body/tissue attenuation (acoustic impedance). The required amplification may be set by the operator either using three-knob TGC control or linear TGC potentiometers (Dendy & Heaton 1999, Hendee & Ritenour 2003).

The consequent steps of signal pre-processing are rejection, rectification, enveloping and classification. During rejection, signals that are too large or too small, and thus do not provide any diagnostic information, are eliminated. All remaining signals are rectified and enveloped. The enveloping process refers to low-pass filtering during which the higher frequencies are removed, so the resulting processed signal appears as an outline or envelope of the original, more complex signal. The echo intensity is then classified according to one of the three following criteria: 1) the height of the peak value, 2) the area under the peak, or 3) the maximum rate of rise or slope of the echo (Hendee & Ritenour 2003).

The digitization is the final step of pre-processing. A number defining the intensity signal value (level of brightness, e.g. grayscale value between 0 and 255) is assigned to the amplitude of the received echo and stored in the computer
memory. The stored image values may be further post-processed to enhance the quality of the image. The obtained echo signal is always accompanied by undesired random electrical noise. Elimination of this noise may be achieved by implementation of different post-processing filtering methods such as pixel region averaging or frame averaging further described in the literature (Dendy & Heaton 1999, Hendee & Ritenour 2003).

![Image of TGC before and after amplification]

**Fig. 4.** The US echoes returning from increasingly greater depths are attenuated due to the tissue attenuation effect. This is compensated by amplification of the echoes by progressively increasing gain provided by the TGC circuit.

### 4.2 Ultrasonography in knee osteoarthritis assessment

With evolving technological advances, high-resolution US has been receiving more and more interest in OA research and clinical practice. In general, non-invasive ultrasonography is a diagnostic method with multiple benefits: it provides cheap, fast, real-time, multi-planar and repeatable dynamic imaging, which is widely-


Pain, stiffness and functional discomfort in knee OA have a multifactorial origin (Hunter et al. 2013). Next to CR and MRI, the tissue level imaging biomarkers have been studied as a potential source of OA symptoms also with the help of knee US (Artul et al. 2014, Chan et al. 2014, Chen et al. 2015, D’Agostino et al. 2015, de Miguel Mendieta et al. 2006, Esen et al. 2013, Iagnocco et al. 2010, Kazam et al. 2011, Malas et al. 2014, Mermerci et al. 2011, Naredo et al. 2005, Razek & El-Basyouni 2015, Wu et al. 2012). A study investigating the role of US in association with knee pain severity suggests that neither CR nor US can clearly better explain its cause but they rather complement each other (Chan et al. 2014). Likewise, Bevers and colleagues did not observe a connection between soft tissue changes in knee OA evaluated by US and clinical symptoms (Bevers et al. 2014). On the other hand, there are studies reporting sometimes even strong associations between structural deteriorations of several tissues, such as cartilage, cortical bone or meniscus with OA symptoms (Chen et al. 2015, Malas et al. 2014, Razek & El-Basyouni 2015). Although it is known that inflammatory changes in the knee may substantially contribute to pain and functional impairment, also a strong link between inflammatory and structural abnormalities with US has been shown (D’Agostino et al. 2015, Sarmanova et al. 2016). Furthermore, inflammation is not only a primary phenomenon but can be secondarily induced by released mediators from damaged cartilage and/or bone (Berenbaum 2013, Bijlsma et al. 2011).
Degenerative changes of knee articular cartilage and subchondral bone can be effectively observed and quantitatively analysed with US in the laboratory conditions (Leicht & Raum 2008, Saarikkala et al. 2006, Saïed et al. 1997, Virén et al. 2009). Quantitatively, an increase of the US reflection from the cartilage-bone interface and of the cartilage surface roughness (Saarikkala et al. 2006), decrease in reflection from the cartilage surface (Saarikkala et al. 2004) and increase of inner echogenicity of both tissues (Saïed et al. 1997) has been observed with OA progression in vitro.

Scientific evidence supporting the idea of deploying US as one of the initial diagnostic imaging modalities for detection of structural abnormalities in knee OA is increasing. However, systematic feature- and site-specific cross-comparison of US, CR and MRI is still missing in the current literature. Also, the potential of quantitative US imaging as a promising method for OA diagnostics has been indicated in several other studies (Han et al. 2015, Laasanen et al. 2006, Saarikkala et al. 2012). However, no in vivo study non-invasively investigating the role of quantitative US image analysis for detecting structural and compositional changes of knee OA has been performed.

From the technical point of view, the 5–13 MHz linear transducer is used in standardized scanning of the knee and a 15 MHz frequency is applied for the most superficial structures (Vlad & Iagnocco 2012). Already in 1999, a linear transducer with a frequency of 13 MHz was proposed to be used to obtain a detailed morphological image of the articular cartilage up to the depth of 3 cm (Grassi et al. 1999). Nowadays, even higher frequencies may be employed in imaging of the knee structures providing images with a resolution up to 0.1 mm (Abraham et al. 2011, Schmidt 2014).

The present and the following sections 4.2.1 and 4.2.2 are primarily focused on a literature review of US studies investigating OA structural and compositional changes of knee femoral articular cartilage, osteophytes and menisci, which are the main focus of the present doctoral thesis.

### 4.2.1 Semi-quantitative assessment

Ultrasonographic appearance of normal articular cartilage has been described in earlier studies as a homogenous anechoic band with a sharply defined anterior, i.e. cartilage-soft tissue or cartilage-synovial space, and posterior, i.e. cartilage-bone, interfaces (Aisen et al. 1984, Grassi et al. 1999). The principle features of osteoarthritic cartilage were defined by Grassi et al. (1999) as: 1) loss of the normal sharpness of synovial space-cartilage interface, 2) loss of clarity of the cartilaginous
layer, 3) narrowing of the cartilage, and 4) increased intensity of the posterior bone-cartilage interface. In order to increase reliability, Grassi et al. (1999) suggested to combine assessments of all four features.

To the author’s knowledge, the first semi-quantitative US assessment of knee articular cartilage was introduced by Aisen et al. in 1984. In this study, difference in cartilage quality in femoral superior and inferior medial and lateral condyle and intercondylar notch (sulcus) between groups of symptomatic and asymptomatic subjects was shown by separate evaluation of cartilage clarity (i.e. relative lack of echoes) and sharpness of cartilage-soft tissue interface ranging from 0 to 6 (Aisen et al. 1984). Later, using the same grading system, correlation of US assessment with surgical findings and MRI in patients undergoing total knee arthroplasty (TKA) was reported (McCune et al. 1990). Based on the suggestion given by Grassi et al. (1999) (described above), Lee et al. (2008) proposed a seven-step grading system for cartilage changes and compared that with earlier-validated in vitro US assessment of the specimens and histology (Tsai et al. 2007). Although only weak to moderate agreements with histology and in vitro US assessment were reached (Lee et al. 2008), later the established grading of cartilage degeneration has shown association with clinical and functional outcomes (Chen et al. 2015). Another assessment of cartilage clarity and cartilage grade, defining severity of cartilage lesion (adapted from International Cartilage Repair Society), was able to differentiate asymptomatic subjects from subjects with knee pain (Kazam et al. 2011). Already in 1990, US was proposed as a suitable method for evaluation of intercondylar cartilage damage due to its high sensitivity and positive predictive value (PPV) to predict the surgical or arthroscopic results (Hanneschlager et al. 1990). A recent study has reported a similar conclusion even though with a different definition of semi-quantitative femoral cartilage degeneration grading combining both cartilage echogenicity and cartilage loss (Saarakkala et al. 2012). Moreover, this study has introduced the novel US scanning technique for evaluation of wide area of medial and lateral condyle and sulcus cartilage integrity (Saarakkala et al. 2012). Ragab et al. (2012) established a three-step grading evaluating cartilage sharpness, homogeneity and thinning. Especially an advanced OA stage with thinned cartilage was associated with meniscal degeneration and extrusion (Ragab et al. 2012). The latest established four-step grading system defines the irregularities or loss of sharpness of the cartilage margins without thinning as grade 1. Grade 2 is interpreted as partial or complete loss of thickness of the cartilage in one trochlear condyle, and grade 3 is partial or complete loss of thickness of the cartilage in both trochlear condyles (Bruyn et al. 2015). This cartilage assessment
reaches, however, only fair to moderate inter- and intra-observer agreement, respectively.

Osteophyte appearance in US is defined as a cortical protrusion at the joint margin seen in two planes (Keen & Conaghan 2009), or more accurately as a step-up of bony prominence at the end of a normal bone contour or at the margin of the joint visible from two perpendicular planes with or without an acoustic shadow (Bruyn et al. 2015). Initially, semi-quantitative US grading systems for osteophytes were introduced and validated for assessment of hip (Qvistgaard et al. 2006) and hand (Keen et al. 2008, Mathiessen et al. 2013). In the knee, for many years only dichotomous or quantitative methods were applied in the OA research (see references below and in the next section). Assessment scale, dividing the measured osteophyte size in mm into five degrees of severity, was proposed by Wu and colleagues to record the osteophytes in suprapatellar, medial and lateral knee recess (Wu et al. 2012). In 2015, Koski et al. validated the semi-quantitative US grading system for presence and size of knee tibiofemoral osteophytes which was shown to be a more sensitive method for detection of osteophytes than CR (Koski et al. 2015). Another grading with a similar subjective osteophyte size definition was defined by consensus of 13 expert rheumatologists in musculoskeletal US (Bruyn et al. 2015).

Initially, visualization of menisci with US seemed to be rather difficult (Dragonat & Claussen 1980), however with use of higher-resolution equipment (i.e. already with a 5 MHz linear probe!) uniform depiction of normal menisci and also detection of meniscal damage was achieved (Selby et al. 1986, Selby et al. 1987). Normal meniscus appears as a hyperechoic triangular structure located between the femur and the tibia with the apex pointing towards the middle of the joint (Bianchi et al. 2007, Selby et al. 1986). Recently, following semi-quantitative grading systems determining the degree of medial meniscal extrusion has been proposed: 1) four-step grading defined by 3 mm, 5 mm and 8 mm pathological meniscal displacement thresholds (Wu et al. 2012); 2) three-step scoring established by thresholding at 2 mm and 4 mm, which has been also validated against MRI with moderate to substantial agreement between the modalities (Nogueira-Barbosa et al. 2015), and 3) three-step assessment agreed in consensus by multiple experts in musculoskeletal US and defining visual appearance of normal (i.e. grade 0), protruded (grade 1) and extruded (grade 2) meniscus (Bruyn et al. 2015).

In several studies dichotomous assessment has been used to determine the presence or absence of cartilage integrity (D’Agostino et al. 2015, Hannesschlager et al. 1990, Hong et al. 2000, Iagnocco et al. 1992, Iagnocco et al. 2010, Kumm et

The reported inter-rater reliability of dichotomous or semi-quantitative assessments of medial meniscal extrusion ranges from moderate (Bevers et al. 2012, Bruyn et al. 2015) to almost perfect (Nogueira-Barbosa et al. 2015, Razek & El-Basyouni 2015), of osteophytes from moderate (Bruyn et al. 2015, Koski et al. 2015, Naredo et al. 2006) to substantial (Koski et al. 2015) and to almost perfect (Abraham et al. 2011, Razek & El-Basyouni 2015), and of cartilage degeneration from fair (Bruyn et al. 2015) to substantial (Iagnocco et al. 2012, Lee et al. 2008) and to almost perfect (Razek & El-Basyouni 2015). Furthermore, the overall agreement between multiple US experts was demonstrated to be 85% for bony cortex abnormalities and 86% for cartilage lesions (Naredo et al. 2006). Nevertheless, based on the sonographers’ experience, the inter-observer reliability may vary between fair and almost perfect agreement in evaluation of structural abnormalities (Iagnocco et al. 2012).

In Table 2, the essential studies, related to the present study, on the non-invasive US semi-quantitative and/or quantitative assessment of knee OA features are listed.
Table 2. List of non-invasive ultrasound *in vivo* studies introducing and/or validating semi-quantitative grading systems and/or quantitative measures for assessment of femoral articular cartilage, osteophytes and/or meniscal extrusion in knee osteoarthritis.

<table>
<thead>
<tr>
<th>Author</th>
<th>Pathology</th>
<th>Grading / Measure unit</th>
<th>Construct validation / Reference</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aisen et al. (1984)</td>
<td>Cartilage clarity 0–6</td>
<td>Control group</td>
<td>Cartilage clarity and sharpness correlates better with clinical outcomes than measure of thickness.</td>
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<tr>
<td></td>
<td>Cartilage sharpness 0–6</td>
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<tr>
<td></td>
<td>Cartilage thickness mm</td>
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<tr>
<td>McCune et al. (1987)</td>
<td>Cartilage clarity 0–6</td>
<td>Surgical pathology</td>
<td>Good correlation with surgical findings; Assessment of cartilage clarity and sharpness more reliable than thickness.</td>
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</tr>
<tr>
<td></td>
<td>Cartilage sharpness 0–6</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Cartilage thickness mm</td>
<td></td>
<td></td>
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<tr>
<td>Hannesschlager et al. (1990)</td>
<td>Cartilage lesion 0–1</td>
<td>Surgical or arthroscopic pathology</td>
<td>High sensitivity and PPV of US for detection of cartilage lesion.</td>
<td></td>
</tr>
<tr>
<td>Iagnocco et al. (1992)</td>
<td>Cartilage thickness mm</td>
<td>Control group</td>
<td>Thinner cartilage in OA subjects in comparison to normal; 83% of OA subjects irregular cartilage surface.</td>
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<tr>
<td></td>
<td>Cartilage surface regularity 0–1</td>
<td></td>
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<tr>
<td>Østergaard et al. (1995)</td>
<td>Cartilage thickness mm</td>
<td>MRI</td>
<td>Cartilage thickness in US strongly correlated with thickness in MRI.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cartilage sharpness 0–6</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Cartilage thickness mm</td>
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<tr>
<td>Verdonk et al. (2004)</td>
<td>Lateral meniscal extrusion mm</td>
<td>MRI</td>
<td>Both equally good parameters to determine lateral meniscal extrusion in normal and transplanted meniscus.</td>
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<tr>
<td></td>
<td>Extrusion cross-sectional area (CSA) mm²</td>
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<tr>
<td>Naredo et al. (2005)</td>
<td>Medial meniscal extrusion 0–1 (≥ 2 mm)</td>
<td>Clinical symptoms, CR</td>
<td>Correlation with medial radiographic JSN andVAS pain.</td>
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</tr>
<tr>
<td></td>
<td>Length of osteophytes mm</td>
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</tr>
<tr>
<td>Lee et al. (2008)</td>
<td>Cartilage integrity 0–6</td>
<td>In vitro US, Histology</td>
<td>Weak to moderate correlations with both reference methods. Measurements from longitudinal US correlated with MRI better than from transversal US. Moderate correlations with JSN in medial side. Reliable method.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cartilage thickness mm</td>
<td>MRI, CR</td>
<td></td>
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<tr>
<td>Yoon et al. (2008)</td>
<td>Cartilage thickness mm</td>
<td>MRI</td>
<td></td>
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</tr>
<tr>
<td>Author</td>
<td>Pathology</td>
<td>Grading / Measure unit</td>
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<td>Results</td>
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<tr>
<td>Kazam et al. (2011)</td>
<td>Cartilage thickness mm</td>
<td>Pain</td>
<td></td>
<td>Cartilage clarity significantly decreased and cartilage grade increased in symptomatic subjects compared to asymptomatic.</td>
</tr>
<tr>
<td>Cartilage clarity 0–4</td>
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<tr>
<td>Cartilage grade 0–4</td>
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<tr>
<td>Cartilage calcification 0–1</td>
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<tr>
<td>Osteophytes 0–1</td>
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<tr>
<td>Subchondral bone irregularity 0–1</td>
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<tr>
<td>Kawaguchi et al. (2012)</td>
<td>Medial meniscal displacement mm</td>
<td>CR, Control group</td>
<td>Arthroscopy</td>
<td>Meniscus displaced during weight-bearing and with OA progression.</td>
</tr>
<tr>
<td>Saarakkala et al. (2012)</td>
<td>Cartilage grade 0–4</td>
<td></td>
<td></td>
<td>Abnormal cartilage finding in US predicted changes seen in arthroscopy but negative finding did not rule out the pathology.</td>
</tr>
<tr>
<td>Wu et al. (2012)</td>
<td>Medial meniscal protrusion 0–3 (&lt; 3 mm, 3 to &lt; 5, 5 to &lt; 8, ≥ 8)</td>
<td>VAS pain, WOMAC, Medial knee pain</td>
<td></td>
<td>No association with clinical symptoms and function.</td>
</tr>
<tr>
<td>Osteophytes 0–3 (&lt; 1 mm, 1 to &lt; 2, 2 to &lt; 5, ≥ 5)</td>
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<tr>
<td>Ragab et al. (2012)</td>
<td>Cartilage grade 0–3</td>
<td></td>
<td>-</td>
<td>Cartilage thinning was strongly related to meniscal extrusion and degeneration.</td>
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<tr>
<td>Meniscal extrusion 0–1 (&gt; 3 mm)</td>
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<tr>
<td>Osteophytes 0–1</td>
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<tr>
<td>Acebes et al. (2013)²</td>
<td>Medial meniscal subluxation distance mm</td>
<td>Control group</td>
<td></td>
<td>Meniscus undergoes subluxation in knee OA and is greater during weight-bearing; Reproducible method.</td>
</tr>
<tr>
<td>Medial meniscal subluxation area mm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yanagisawa et al. (2014)³</td>
<td>Medial meniscus displacement (MD) mm</td>
<td>CR</td>
<td></td>
<td>MD increased and PJS decreased in OA. Combination of US parameters was able to diagnose radiographic OA with sensitivity, specificity, PPV and NPV ranging between 84.0%–97.5%.</td>
</tr>
<tr>
<td>Medial peripheral joint space (PJS) mm</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Osteophytes 0–1 (&gt; 2 mm)</td>
<td></td>
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</tr>
<tr>
<td>Author</td>
<td>Pathology</td>
<td>Grading / Measure unit</td>
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<tr>
<td>Riecke et al. (2014)a, b</td>
<td>Morphological domains including osteophytes and meniscal protrusion</td>
<td>mm CR, KOOS</td>
<td></td>
<td>Morphological changes in medial and lateral knee were strongly correlating with KL grade; weak correlation of lateral morphological domain with KOOS pain and daily activity.</td>
</tr>
<tr>
<td>Nogueira-Barbosa et al. (2015)a, b</td>
<td>Medial meniscal extrusion</td>
<td>mm, 0–2 MRI (&lt; 2 mm, ≥ 2 to &lt; 4, ≥ 4)</td>
<td></td>
<td>Moderate to substantial agreement between semi-quantitative and substantial between quantitative US and MRI. Excellent sensitivity and good specificity of US.</td>
</tr>
<tr>
<td>Koski et al. (2015)a, b</td>
<td>Osteophytes</td>
<td>0–3 CR, Arthroscopy</td>
<td></td>
<td>US more sensitive in detection of osteophytes than CR and at least equally reliable and reproducible as CR.</td>
</tr>
<tr>
<td>Bruyn et al. (2015)a, b</td>
<td>Cartilage damage</td>
<td>0–3 -</td>
<td></td>
<td>Fair to good intra- and inter-observer reliability for cartilage damage, medial meniscal damage and osteophytes.</td>
</tr>
<tr>
<td>d’Agostino et al. (2015)</td>
<td>Cartilage abnormalities Osteophytes</td>
<td>0–1 US inflammatory signs, Pain, Function</td>
<td></td>
<td>Structural abnormalities significantly associated with inflammatory.</td>
</tr>
</tbody>
</table>

US – ultrasonography, CR – conventional radiography, MRI – Magnetic resonance imaging, PPV – positive predictive value, NPV – negative predictive value, KL – Kellgren-Lawrence grade, JSN – joint space narrowing, VAS – visual analogue scale, WOMAC – Western Ontario and McMaster Universities Osteoarthritis Index, KOOS – Knee injury and osteoarthritic outcome score, a Reliability study (inter-rater and/or inter-operator), b Reproducibility study (intra-rater and/or intra-operator).

4.2.2 Quantitative assessment

In vitro

Before in vivo studies, high-frequency quantitative US imaging has been widely studied and validated in vitro with a specific focus on assessment of articular cartilage and subchondral bone. Studied parameters included angular distribution of the scattered acoustic field (Adler et al. 1992) and US roughness index (Saarakkala et al. 2004), both quantifying the cartilage surface roughness. Furthermore, US reflection coefficient (Saarakkala et al. 2004) and signal
amplitude (Hattori et al. 2003) characterized the tissue integrity. Most of these parameters have been associated with superficial collagen degradation and cartilage surface fibrillation (Saarakkala et al. 2004, Saarakkala et al. 2006, Wang et al. 2010). Subchondral bone integrity can be detected qualitatively and quantitatively at the cartilage-bone interface as well (Disler et al. 2000, Laasanen et al. 2006, Leicht & Raum 2008, Saarakkala et al. 2006, Saïed et al. 1997). In several studies, decrease of cartilage surface reflection, increase of cartilage-bone echogenicity and increase of the cartilage surface roughness index has been observed in specimens with artificially induced OA changes (Disler et al. 2000, Laasanen et al. 2006, Nishitani et al. 2014, Niu et al. 2012, Saarakkala et al. 2004, Saarakkala et al. 2006, Saïed et al. 1997, Virén et al. 2009). Moreover, depth-wise increase of acoustic impedance of the articular cartilage and at the cartilage-bone interface in OA has been observed in human specimens (Leicht & Raum 2008).

Han and colleagues have compared the grayscale profiles of the conventional US clinical images of human articular cartilage samples with profiles of two differently stained histological images. They have reported that the US was better correlated to the histological images showing glycosaminoglycan depletion in the superficial cartilage layer (Han et al. 2015). The speed of sound in human cartilage can be determined indirectly from the measured time of flight of the US pulse and cartilage thickness measured from histology or light microscopy. The speed of sound has been shown to differ between healthy and OA cartilage samples (Myers et al. 1995) and to decrease with OA progression (Ohashi et al. 2016).

Measurements of the cartilage thickness from conventional B-mode US in vitro has been suggested to be accurate and reproducible (Myers et al. 1995, Ohashi et al. 2012b, Saïed et al. 1997), although in a study by Ohashi et al. (2012b) objective measurement using real-time spatial compound imaging performed somewhat better in reference to light microscopy (Ohashi et al. 2012b). Myers et al. (1995) has also measured the width of the echogenic fibrillated cartilage band which has been found to be directly proportional to the fibrillation depth determined from histology (Myers et al. 1995).

**Invasive**

About a decade ago, the first quantitative in vivo measurements of articular cartilage acoustic properties, including maximum echo magnitude, superficial cartilage integrity, echo duration, measure related to surface irregularity, and interval between signals (i.e. time of flight), related to cartilage thickness, have been introduced for arthroscopic
assessment of normal and early OA cartilage (Hattori et al. 2004, Hattori et al. 2005, Kuroki et al. 2008). Minimally invasive high-frequency (> 10 MHz) and low-frequency (< 10 MHz) intra-articular US has enabled imaging of the knee intra-articular surfaces and has revealed the early OA compositional and microstructural changes of the articular cartilage and subchondral bone during arthroscopic surgery (Kaleva et al. 2011, Liukkonen et al. 2013, Nieminen et al. 2009). Specifically, articular surface characteristics has been described by quantitative parameters, such as reflection coefficient [%], integrated reflection coefficient [dB], apparent integrated backscatter [dB], US roughness index [µm] or cartilage thickness [mm], calculated from the raw 2-D US signals (Kaleva et al. 2011, Liukkonen et al. 2013, Liukkonen et al. 2014). Recently, clinical feasibility of arthroscopic US imaging to detect osteochondral lesions has been demonstrated (Penttilä et al. 2015). Although arthroscopic US may provide more detailed information on articular cartilage and subchondral bone, it is still an invasive procedure and clinical implications for its use remain to be shown.

Non-invasive

The most common quantitative morphological evaluations applied for examination of knee OA from non-invasive ultrasonography in vivo include measurements of femoral cartilage thickness, dimensions of osteophytes, meniscal extrusion and cross-sectional area, capsular distension, Backer’s cyst, effusion and synovial hypertrophy. The following paragraphs are concentrated on studies quantifying morphological properties of articular cartilage, osteophytes and meniscal extrusion.

First non-invasive measurement of cartilage thickness in OA patients were performed by Aisen et al. (1984) who concluded that the absolute thickness measure may not be the optimal assessment parameter, partially due to the within individual site-specific cartilage thickness variation, and partially due to the low reliability of the measurement in OA subjects with indistinct cartilage interfaces (Aisen et al. 1984). Martino et al. (1993), however, later reported that the measurement of cartilage thickness from the US images is a sensitive and reproducible method when assessed from the same location, and suggested its use for diagnosis as well as monitoring of OA (Martino et al. 1993a, Martino et al. 1993b). The suprapatellar transverse plane just above the patella with the knee at 90° or maximal flexion was recommended as the standard location for cartilage thickness determination (Backhaus et al. 2001, Friedman et al. 2001, Schmidt et al. 2004). Other studies have shown that cartilage thickness measured from longitudinal US scans correlates with measurements from MRI in controls as well
as in OA subjects (Østergaard et al. 1995, Tarhan & Unlu 2003, Yoon et al. 2008). Ohashi and colleagues developed a mechanical arm with attached US transducer allowing image acquisition of the medial femoral surface from several transversally oriented planes (Ohashi et al. 2012a). In this study, the determination of the cartilage thickness from the generated 3-D cartilage model in healthy volunteers and OA patients has been reported to be reproducible and strongly correlating with MRI (Ohashi et al. 2012a). Regarding the impact of quantitative cartilage loss on symptoms, no association was found (Chan et al. 2014).

Standard reference cartilage thickness values have been investigated from the femoral intercondylar notch (sulcus) in 204 healthy adults’ knees. The mean thickness in women was 2.7 mm (1.5–4.1; 2 SD, 1.3) and 3.5 mm (2.0–4.9; 2 SD 1.4) in men (Schmidt et al. 2004). However, it is important to note that the diurnal cartilage thickness varies up to 10.6% between morning and evening (Kilic et al. 2015).

Measurement of osteophyte dimensions, primarily its distance from the cortical bone margin, has been proposed in mm scale (Chan et al. 2014, Jung et al. 2006) and examined in several studies demonstrating elevated levels of biochemical markers of cartilage, synovium or bone degenerative processes in subjects with longer medial osteophytes (Jung et al. 2006, Kim et al. 2016, Zivanovic et al. 2009). Furthermore, medial osteophyte length correlated with scintigraphic uptake in medial femur and tibia reflecting increased blood flow and bone metabolism (Kim et al. 2008).

Medial meniscal extrusion has been defined by Naredo et al. (2005) as a distance from the peripheral edge of the meniscus to the border of the tibial plateau. However, variable definitions have been applied in other studies, measuring the distance either from the anterior or posterior meniscal horn, or the outmost edge of meniscus to either the joint line, midline, line connecting the femoral and tibial bone margins, or tangent to the external edge of the tibial plateau (Acebes et al. 2013, de Miguel Mendieta et al. 2006, Kawaguchi et al. 2012, Nogueira-Barbosa et al. 2015, Yanagisawa et al. 2014). Also, with regard to the presence of tibial osteophytes, the reference point has been determined inconsistently either as the medial margin of the tibia (Kawaguchi et al. 2012, Ko et al. 2007, Yanagisawa et al. 2014) or the outer margin of the osteophyte (Acebes et al. 2013). Meniscal extrusion is imaged longitudinally from fully extended knee when the patient is either supine (relaxed) (Acebes et al. 2013, Kawaguchi et al. 2012, Yanagisawa et al. 2014) or unipodal (Acebes et al. 2013) and bipodal standing (weight-bearing) (Kawaguchi et al. 2012, Ko et al. 2007, Yanagisawa et al. 2014). Recently, both semi-quantitative and quantitative
methods to assess the medial meniscal extrusion has been validated using MRI as a reference standard (Nogueira-Barbosa et al. 2015). In that study, the following grading system has been used: grade 0 for extrusion < 2 mm, grade 1 for extrusion ≥ 2 mm and < 4 mm and grade 2 for extrusion ≥ 4 mm (Nogueira-Barbosa et al. 2015). Additionally, the extrusion cross-sectional area (Acebes et al. 2013) and peripheral joint space (Yanagisawa et al. 2014) in the medial compartment can be quantified. Lateral meniscal extrusion has been examined only by a few studies (Rowland et al. 2016, Verdonk et al. 2004). Distance and cross-sectional area have been shown as reproducible and equally good measures to determine normal and transplanted lateral meniscal extrusion (Verdonk et al. 2004). Rowland and colleagues experimentally measured the extrusion of intact, sectioned and repaired lateral meniscus with and without physiological loading (Rowland et al. 2016).

In 2000, a descriptive study suggested that meniscal extrusion may indicate radiographic JSN (Grobbelaar & Bouffard 2000). US was able to confirm this interconnection especially in the medial side (Kawaguchi et al. 2012, Malas et al. 2014, Naredo et al. 2005). Furthermore, medial capsular distension, which may be caused by meniscus dislocation, was shown to correlate with elevated biochemical markers of the cartilage and synovium degenerative processes in more severe OA patients (Jung et al. 2006) as well as with increased scintigraphic uptake in medial femur and tibia (Kim et al. 2008).

Reported inter-rater reliabilities for quantitative assessment of meniscal extrusion ranged from good (Bevers et al. 2012) to almost perfect agreement (Nogueira-Barbosa et al. 2015) and of cartilage thickness from moderate (Abraham et al. 2011, Bevers et al. 2012) to good (Abraham et al. 2011, Bevers et al. 2012) to almost perfect agreement (Yoon et al. 2008).
5 Aims of the study

Early detection of knee OA is crucial for the advancement of understanding of the disease mechanisms, initiation of early intervention and development of DMOADs. The current gold standard imaging modality, i.e. CR, reveals the knee joint changes only in the late stages of the disease. Moreover, the relationships between CR and symptoms are discrepant. Therefore, the principal hypothesis of the present doctoral study was that the non-invasive semi-quantitative and quantitative knee ultrasonography may bring more detailed information on the structural abnormalities of femoral articular cartilage, osteophytes, meniscus and subchondral bone, and furthermore, it may help to explain the structure-pain association in knee OA. To answer these general hypotheses, the following specific aims were defined:

1. To systematically determine the site-specific diagnostic performance of the semi-quantitative grading of the US scanned femoral articular cartilage and osteophytes, as well as quantitative measurement of meniscal extrusion, using MRI as a reference tool, and to compare the diagnostic performance of knee ultrasonography with radiographic assessment of JSN and osteophytes.

2. To determine the prevalence of US-defined knee OA structural features in group of symptomatic and asymptomatic subjects, and to determine the association of the structural features and their severity with pain and functional impairment.

3. To examine the potential of quantitative non-invasive knee US for detection of OA changes in the femoral subchondral bone using radiographic KL grade and arthroscopic Noyes’ grade as a reference.
6 Materials and methods

6.1 Study design and populations

The present thesis is a compilation of three article-based observational cross-sectional studies with two distinct subject populations and it contains also unpublished data. Studies I and II include a population of symptomatic and asymptomatic subjects from the Oulu Knee Osteoarthritis (OKOA) study carried out at the Oulu University Hospital between October 2012 and April 2014. Study III includes data from a population of symptomatic knee OA subjects collected in the Mikkeli Central Hospital between years 2008 and 2010.

6.1.1 The Oulu Knee Osteoarthritis (OKOA) study population (I, II)

The OKOA study involved 160 subjects from which 80 were symptomatic and 80 asymptomatic. Written informed consent was obtained from each subject. The study was carried out in accordance with the Declaration of Helsinki and approved by the Ethical Committee of the Northern Ostrobothnia Hospital District, Oulu University Hospital (number 108/2010).

Symptomatic subjects

Symptomatic subjects were selected from patients with an age range from 30 to 70 years (after initial examinations the range was narrowed to 45 to 70 years) who were referred to either knee CR due to non-specific knee pain, or referred to total knee arthroplasty (with radiographs available) at the Oulu University Hospital and Oulu municipality health centres. The knee radiographs were assessed by KL grading, which was used as a criterion for symptomatic subject recruitment. The inclusion and exclusion criteria are listed in Table 3 and the recruitment process described in detail can be found in Figure 1 of Study I.

Asymptomatic subjects

The asymptomatic subjects were recruited from the research unit employees, their family members and friends or by advertisement in the local newspaper. The aim was to age- and gender-match the symptomatic group. The inclusion and exclusion
criteria are listed in Table 3. The detailed description of the recruitment process can be found in Figure 2 of Study I.

### Table 3. Inclusion and exclusion criteria of the OKOA study population.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inclusion</strong></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>30–70 years</td>
<td>S</td>
</tr>
<tr>
<td>20–70 years</td>
<td>A</td>
</tr>
<tr>
<td>45–70 years(^1)</td>
<td>S, A</td>
</tr>
<tr>
<td>X-ray not older than six months</td>
<td>S</td>
</tr>
<tr>
<td>15–20 subjects per each KL grade (1–4)(^2)</td>
<td>S</td>
</tr>
<tr>
<td>60% of subjects being women</td>
<td>S, A</td>
</tr>
<tr>
<td><strong>Exclusion(^3)</strong></td>
<td></td>
</tr>
<tr>
<td>Significant knee joint trauma or surgery</td>
<td>S, A</td>
</tr>
<tr>
<td>Inflammatory disease or other medical condition affecting the knee joint</td>
<td>S, A</td>
</tr>
<tr>
<td>Repetitive or long-term knee pain (more than 2 weeks without interruption)</td>
<td>A</td>
</tr>
<tr>
<td>BMI &gt; 35</td>
<td>A</td>
</tr>
</tbody>
</table>

\(^{1}\)After initial examinations, the age range for selection of both groups was narrowed to easier select the true osteoarthritic subjects and to age-match the groups, \(^{2}\)This criterion was specified after initial examinations, during which two subjects with KL grade 0 were already included, \(^{3}\)Osteoarthritis was not an exclusion criteria as initial clinical examination and radiography were not performed for the asymptomatic subjects.

### 6.1.2 Subjects for Study III

The subjects for Study III were originally recruited for the study by Saarakkala et al. (2012) and in this thesis the already acquired images were quantitatively analysed. In the study by Saarakkala et al. 2012, 40 non-rheumatoid patients (15 women and 25 men) were randomly selected from patients referred to knee arthroscopy because of knee pain complaints. Written informed consent was obtained from all patients and the study was approved by the Ethical Committee of the Mikkeli Central Hospital (number 9118/66/2008).

### 6.2 Ultrasonography

In Study I and II, wide-area US scanning was performed by a trained doctoral student, the author of the present thesis, using a commercially available US device
(LOGIQ E9, GE Healthcare, Milwaukee, WI, USA) with a 15 MHz linear transducer ML6-15. Except for the image depths and focus, which were always set to depict the region of interest with the best resolution, all B-mode imaging settings were kept constant throughout data collection.

In Study III, the imaging was performed by an experienced rheumatologist, the author’s supervisor, Juhani Koski, using a clinical US device (Esaote Technos 2000, Esaote Biomedica, Via Siffredi 58, 16153, Genova, Italy) with a 13 MHz linear transducer (LA424). Most of the imaging settings were kept constant throughout the examinations, however image depth, focus length and gain values were altered in some subjects in order to achieve the best representative images.

During the examination, the symptomatic knee was imaged in patients and knee of the dominant hand side in asymptomatic subjects. First, the subject was positioned supine with his/her knee in full flexion. Medial, lateral and sulcus sites of femoral articular cartilage were depicted by continuous proximal-distal sweeping of the transversally placed transducer over the supra-patellar region as originally described by Saarakkala et al. (2012). Subsequently, the subject was asked to fully extend the knee and the anterior-posterior longitudinal scans from medial and lateral proximal sides were obtained. In Study I and II, two video files were saved for each site. In Study III, the most representative image from each location, i.e. the one which best represented the overall visual subjective impression of the areal US imaging (Saarakkala et al. 2012), was saved for analysis.

6.2.1 Semi-quantitative and quantitative assessment (I, II)

In Study I and II, the osteoarthritic features were site-specifically evaluated from the saved video files using semi-quantitative US grading by a rheumatologist with 25 years of experience in musculoskeletal US. He was blinded to subject grouping, clinical and other imaging findings. The grading systems used for the assessment of site-specific femoral cartilage degeneration (Saarakkala et al. 2012), and presence and size of tibio-femoral marginal osteophytes (Koski et al. 2015) are presented in Table 4. Meniscal extrusion was measured as a perpendicular distance (mm) between the outmost meniscal edge and a line connecting the femoral and tibial bone margins. The line was leading below osteophytes if present (Fig. 5). Additionally, randomized US videos of 25 asymptomatic and 26 symptomatic arbitrarily selected subjects were once more assessed by the same evaluator three months after the first evaluation.
Table 4. Ultrasound grading system for assessment of articular cartilage degeneration (Saarakkala et al. 2012) and presence and size of osteophytes (Koski et al. 2015).

<table>
<thead>
<tr>
<th>Ultrasound grade</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Articular cartilage</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Monotonous anechoic band with sharp hyperechoic anterior and posterior interfaces</td>
</tr>
<tr>
<td>1</td>
<td>Loss of the normal sharpness of cartilage interfaces and/or increased echogenicity of the cartilage</td>
</tr>
<tr>
<td>2A</td>
<td>In addition to the above changes, clear local thinning (less than 50%) of the cartilage</td>
</tr>
<tr>
<td>2B</td>
<td>Local thinning of the cartilage more than 50% but less than 100%</td>
</tr>
<tr>
<td>3</td>
<td>100% local loss of the cartilage tissue</td>
</tr>
<tr>
<td>Osteophyte</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>No osteophyte</td>
</tr>
<tr>
<td>1</td>
<td>Small osteophyte</td>
</tr>
<tr>
<td>2</td>
<td>Medium osteophyte</td>
</tr>
<tr>
<td>3</td>
<td>Large osteophyte</td>
</tr>
</tbody>
</table>

Fig. 5. Medial (a) and lateral (b) meniscal extrusion was measured as a perpendicular distance between a line connecting femoral (F) and tibial (T) bone margins and the outer meniscal edge (dash lines). The femoral and tibial osteophytes are indicated by white arrows.

6.2.2 Quantitative image analysis (III)

The quantitative US image analysis of Study III was conducted with Matlab software (MathWorks Inc., Natick, MA, USA) applying a custom-made script. Regions of interest (ROIs) were semi-automatically segmented from femoral medial, sulcus and lateral subchondral bone. First, a border line was manually
placed onto the cartilage-bone interface, with the intention to select the region perpendicular to the incident US beam, and therefore having the strongest US reflection from the subchondral bone. Consequently, a rectangular ROI was automatically delineated. The width of the rectangular ROI was set to 2 mm (i.e. ~ 29 pixels for images with an image depth 31 mm, and ~ 22 pixels for images with image depth 41 mm) and the initial height was 8 mm. The bone profile vector of mean grayscale intensity values was obtained by averaging the values of each horizontal row in the segmented ROI. In order to compare the intensity values between patients, and consequently minimize the effect of alteration of the imaging parameters, such as gain, the bone profile vector was normalized by dividing all values by the maximum vector value. Subsequently, the first vector values were omitted, so that the vector started with the maximum intensity, i.e. from the place of maximum subchondral bone reflection. Furthermore, the vectors derived from images with a depth of 41 mm were linearly interpolated to correspond to the 31 mm image depth and thus the vectors with the corresponding pixel size of ~ 0.07 mm. Finally, the length of the vector, and thereby the height of the ROI, was reduced to 25 pixels (~ 1.74 mm) and five consecutive uniform bone depth levels were defined. The sectioning into five levels was conducted in order to clarify which bone location is the most sensitive to ultrasonically determined bone changes during OA. The mean of each level and the entire profile mean (level all) were calculated. Additionally, the intensity slope was calculated within the first two levels where the most changes were expected. Eventually, the mean femoral bone profile vector, mean of each depth level and mean intensity slope were calculated from the site-specific data (Fig. 6).
Fig. 6. Ultrasound images of sulcus cartilage-bone interface of healthy knee (a) and knee with severe osteoarthritis (OA) (b). The rectangular subchondral bone profiles were semi-automatically segmented. The plotted grayscale intensity profiles (healthy in black, OA in red) demonstrate the decreasing subchondral bone reflection with depth (c). Five uniform depth levels, entire profile (level all) and slopes calculated for first two levels are indicated.

6.3 Radiography (I, II, III)

Symptomatic subjects from the OKOA population (Studies I and II) underwent bilateral weight-bearing postero-anterior CR within six months prior, and subjects of Study III within four months prior to other examinations. In Study III, the radiographs were available for 31 patients. All radiographs were evaluated using KL grading (Table 5) by the same, however blinded, rheumatologist who was also evaluating US videos and images. Furthermore, the radiographs from Study I and II were semi-quantitatively evaluated for medial and lateral JSN, as well as osteophytes in the medial and lateral femur and tibia by two blinded readers using the revised OARSI atlas (Altman & Gold 2007). The OARSI system grades JSN and osteophytes by the degree of change as follows: grade 0 = normal, grade 1 = mild change, grade 2 = moderate change and grade 3 = severe change (Altman & Gold 2007). If the evaluation differed between the readers, the final grade was agreed in the consensus analysis.
Table 5. Radiographic Kellgren-Lawrence (KL) grading system (Kellgren & Lawrence 1957).

<table>
<thead>
<tr>
<th>KL grade</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No changes referring to OA</td>
</tr>
<tr>
<td>1</td>
<td>Doubtful JSN and possible osteophytic lipping</td>
</tr>
<tr>
<td>2</td>
<td>Definite osteophytes and possible JSN</td>
</tr>
<tr>
<td>3</td>
<td>Multiple osteophytes, definite JSN, some sclerosis and possible deformity of bone ends</td>
</tr>
<tr>
<td>4</td>
<td>Large osteophyte, marked JSN, severe sclerosis and definite deformity of bone ends</td>
</tr>
</tbody>
</table>

OA – osteoarthritis, JSN – joint space narrowing, 'KL grade 2 was determined based on the presence of definite osteophytes without the absolute requirement for JSN presence.

6.4 Magnetic resonance imaging (I, II)

On the same day as US was performed, the subjects’ knees were imaged with a 3 Tesla MRI scanner (Siemens Skyra, Siemens Healthcare, Erlangen, Germany) using a 15-channel transmit/receive knee coil. The following sequences were carried out: sagittal T₂-weighted dual-echo steady-state (DESS), 3-D sagittal proton-density (PD) weighted Sampling Perfection with Application optimized Contrasts using different flip angle Evolution (SPACE) fat-suppressed turbo spin-echo (TSE), coronal PD-weighted TSE and coronal T₁-weighted TSE.

MRI was evaluated by the blinded musculoskeletal radiologist for structural degeneration of cartilage in anterior, central and posterior sub-regions of medial and lateral, femoral and tibial condyles using the MRI Osteoarthritis Knee Score (MOAKS) (Table 6) (Hunter et al. 2011). Furthermore, presence and size of medial and lateral osteophytes in femur and tibia, and extrusion of medial and lateral meniscus were assessed by MOAKS (Table 6) (Hunter et al. 2011).
Table 6. MRI Osteoarthritis Knee Score (MOAKS) for semi-quantitative assessment of articular cartilage, osteophytes and meniscal extrusion (Hunter et al. 2011).

<table>
<thead>
<tr>
<th>MOAKS grade</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cartilage I</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>none</td>
</tr>
<tr>
<td>1</td>
<td>&lt; 10% of sub-regional cartilage surface area</td>
</tr>
<tr>
<td>2</td>
<td>10–75% of sub-regional cartilage surface area</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 75% of sub-regional cartilage surface area</td>
</tr>
<tr>
<td>Cartilage II</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>none</td>
</tr>
<tr>
<td>1</td>
<td>&lt; 10% of sub-regional cartilage surface area</td>
</tr>
<tr>
<td>2</td>
<td>10–75% of sub-regional cartilage surface area</td>
</tr>
<tr>
<td>3</td>
<td>75% of sub-regional cartilage surface area</td>
</tr>
<tr>
<td>Osteophytes</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>none</td>
</tr>
<tr>
<td>1</td>
<td>small</td>
</tr>
<tr>
<td>2</td>
<td>medium</td>
</tr>
<tr>
<td>3</td>
<td>large</td>
</tr>
<tr>
<td>Meniscal extrusion</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>&lt; 2 mm</td>
</tr>
<tr>
<td>1</td>
<td>2–2.9 mm</td>
</tr>
<tr>
<td>2</td>
<td>3–4.9 mm</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 5 mm</td>
</tr>
</tbody>
</table>

1 Size of any cartilage loss (including partial and full-thickness loss), 2 Size of full-thickness cartilage loss.

6.5 Arthroscopy (III)

In Study III, on the same day after the US examination, diagnostic arthroscopy of the patient’s symptomatic knee was performed. Degeneration at the articular cartilage surface in the femoral medial, lateral and sulcus areas was graded by the Noyes’ semi-quantitative grading system (Table 7) (Noyes & Stabler 1989). To simplify further analysis, the grading was converted into numerical form, ranging from 0 to 6, and overall femoral arthroscopic score 1 (FAS1), ranging from 0 to 18, was obtained as a sum of all three site-specific Noyes’ grades. Additionally, femoral arthroscopic score 2 (FAS2) was established by dividing FAS1 into groups as follows: grade 0 = grade 0; grade 1 = grades 1–6; grade 2 = grades 7–12; grade 3 = grades 13–18.
6.6 Clinical symptoms and function (II)

A questionnaire including self-assessment of knee pain at the moment of filling, further referred to as pain, and knee symptoms and function over the past week was administrated to participants of Study II. The visual analogue scale (VAS), ranging from 0–100 mm was used for evaluation of knee pain at the moment, and the standardized and widely used WOMAC questionnaire (with VAS) was used for evaluation of clinical symptoms and disability over the past week (Bellamy et al. 1988). The WOMAC index, as a mean of three subscales, was used in the analysis.

6.7 Statistical analyses

In Study I and II, statistical differences between symptomatic and asymptomatic groups were investigated for categorical descriptors using the chi-square test and for continuous descriptors using the parametric Student’s $t$-test or unequal variances $t$-test, and non-parametric Mann-Whitney U test.

In study I, the US cartilage grades 0 and 1 were combined in order to correspond to the MOAKS of cartilage, which assesses purely structural changes. Furthermore, in order to compare MOAKS and US grade of corresponding anatomical cartilage regions, the following grades were established: a maximum MOAKS grade from anterior and central sub-regions for medial and lateral femoral condyle, and the maximum US grade from the medial and sulcus regions was considered as a medial cartilage grade. We hypothesized that JSN is mainly affected by cartilage loss and/or meniscal extrusion in the weight-bearing region, therefore the maximum MOAKS grade of central femoral and central tibial sub-regions (Femoral-tibial central cartilage I and II) was applied as a reference in each condyle.
The intra-rater reliability of US grading in Study I was determined by calculating the following parameters: linearly weighted Cohen’s kappa coefficient (κw), percentage exact agreement (PEA), percentage close agreement (PCA) and intra-class correlation coefficient (ICC).

To evaluate the feature-specific diagnostic ability of US and CR, area under the receiver-operating characteristic (ROC) curve (AUC), sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) with 95% confidence interval (CI) were calculated. Continuous measure of meniscal extrusion was used in US to calculate the AUC, otherwise the 3 mm threshold was used to determine the meniscal pathology. Dominant JSN of either the medial or lateral compartment causes pseudo-widening of the complementary joint space (Felson 2009, Wirth et al. 2012). Therefore, only knees fulfilling the following criteria were selected when assessing the medial compartment: medial JSN ≥ lateral JSN, and similarly for the lateral compartment: lateral JSN ≥ medial JSN. Statistical differences between US and radiographic ROC curves were examined using the method of DeLong et al. (DeLong et al. 1988).

In Study II, the associations of US-defined features with pain severity, WOMAC index and WOMAC subscales were analysed. Due to positive skewness of the questionnaire outcome variables, negative binary regression modelling was used to estimate the associations (Hilbe 2007). Besides site-specifically assessed US features, also the maximum osteophyte grade in medial and lateral compartment, global, i.e. overall maximum, osteophyte grade, and global femoral cartilage grade were entered separately into the models. Each model was adjusted for age, gender and BMI, and for global osteophyte grade and/or global femoral cartilage grade depending on the analysed US-defined feature. The incidence rate ratios (IRRs) with 95% CI were estimated from the regression models to explain the associations. Furthermore, the co-occurrence of global femoral cartilage degeneration and global osteophytes using Spearman’s rank correlation (r) with 95% CI was determined. Site-specifically, cartilage and osteophyte grade co-occurrence with meniscal extrusion was also examined (unpublished data).

In Study III, the Spearman’s rank correlation with 95% CI was used to correlate the subchondral bone level US intensities and intensity slopes with arthroscopic Noyes’ grading, FAS1 and radiographic KL grading. Student’s t tests or unequal variances t-test were conducted for individual femoral bone levels and intensity slopes to test the statistical difference between KL grades 0 and 1, and FAS2 grades 1 and 2. FAS2 grade 0 could not be applied in the analysis due to insufficient sample size.
The statistical analyses were carried out with IBM SPSS software (ver. 20 or ver. 22, SPSS Inc., Chicago, IL, USA), Microsoft Excel custom-made script and MedCalc software (ver. 15.6, MedCalc Software bvba, Belgium). Statistically significant results were determined by \( p \)-value < 0.05.
Results

7.1 Study populations

In Studies I and II, 80 and 63 asymptomatic subjects, respectively, and 79 symptomatic subjects from the OKOA population were included. In both Studies, one (1%) symptomatic subject was excluded due to a missing US video of sulcus cartilage. In Study II, 17 (21%) asymptomatic subjects were excluded due to identified symptoms they did not report during the recruitment phase. In Study III, 39 subjects were included: one (3%) subject was excluded due to lack of representative US images. The subjects’ demographic characteristics are listed study-wise in Table 8.

Table 8. Demographic and imaging characteristics of distinct study sub-populations.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Study I &amp; II</th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Symptomatic</td>
<td>Asymptomatic</td>
<td>All subjects</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td></td>
<td>(n = 79)</td>
<td>(n = 80)</td>
<td>(n = 159)</td>
<td>(n = 63)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>49 (62.0)</td>
<td>50 (62.5)</td>
<td>99 (62.3)</td>
<td>38 (60.3)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>30 (38.0)</td>
<td>30 (37.5)</td>
<td>60 (37.7)</td>
<td>25 (39.7)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>59.9 ± 7.8</td>
<td>55.6 ± 13.9</td>
<td>57.7 ± 11.4</td>
<td>54.6 ± 14.1</td>
</tr>
<tr>
<td>Age range (y)</td>
<td>34–70</td>
<td>24–70</td>
<td>24–70</td>
<td>24–70</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168.9 ± 7.7</td>
<td>168.4 ± 9.3</td>
<td>168.7 ± 8.5</td>
<td>169.1 ± 9.3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83.1 ± 14.4</td>
<td>71.0 ± 12.2</td>
<td>71.3 ± 14.6</td>
<td>71.3 ± 12.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.1 ± 4.3</td>
<td>24.9 ± 3.1</td>
<td>24.8 ± 3.2</td>
<td>27.2 ± 4.4</td>
</tr>
<tr>
<td>Knee flexion (°)</td>
<td>129.2 ± 8.7</td>
<td>138.3 ± 6.8</td>
<td>138.3 ± 6.1</td>
<td>133.8 ± 8.6</td>
</tr>
</tbody>
</table>

KL grade, n (%)

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 (2.5)</td>
<td>21 (26.6)</td>
<td>19 (24.1)</td>
<td>20 (25.3)</td>
<td>17 (21.5)</td>
</tr>
<tr>
<td></td>
<td>2 (2.5)</td>
<td>14 (17.2)</td>
<td>1 (1.3)</td>
<td>2 (2.6)</td>
<td>1 (1.3)</td>
</tr>
</tbody>
</table>

*p < 0.05, *If not indicated otherwise, the values are means ± standard deviations.

7.2 Ultrasound feature prevalence (II)

The prevalence of global US-defined features in symptomatic, asymptomatic and all 142 subjects is presented in Table 9.
Table 9. Prevalence of global knee ultrasound-defined features in 142 subjects with statistical differences between symptomatic and asymptomatic group.

<table>
<thead>
<tr>
<th>Ultrasound feature</th>
<th>All subjects (n = 142)</th>
<th>Symptomatic (n = 79)</th>
<th>Asymptomatic (n = 63)</th>
<th>$\rho$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Cartilage grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global femoral</td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.001$^2$</td>
</tr>
<tr>
<td>0</td>
<td>15 (10.6)</td>
<td>1 (1.3)</td>
<td>14 (22.2)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>35 (24.6)</td>
<td>9 (11.4)</td>
<td>26 (41.3)</td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>45 (31.7)</td>
<td>27 (34.2)</td>
<td>18 (28.6)</td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>29 (20.4)</td>
<td>26 (32.9)</td>
<td>3 (4.8)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>18 (12.7)</td>
<td>16 (20.3)</td>
<td>2 (3.2)</td>
<td></td>
</tr>
<tr>
<td>Osteophyte grade</td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.001$^2$</td>
</tr>
<tr>
<td>Global</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>45 (31.7)</td>
<td>10 (12.7)</td>
<td>35 (55.6)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>46 (32.4)</td>
<td>21 (26.6)</td>
<td>25 (39.7)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>23 (16.2)</td>
<td>21 (26.6)</td>
<td>2 (3.2)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>28 (19.7)</td>
<td>27 (34.2)</td>
<td>1 (1.6)</td>
<td></td>
</tr>
<tr>
<td>Meniscal extrusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial</td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.001$^2$</td>
</tr>
<tr>
<td>&lt; 3 mm</td>
<td>55 (38.7)</td>
<td>17 (21.5)</td>
<td>38 (60.3)</td>
<td></td>
</tr>
<tr>
<td>$\geq$ 3 mm</td>
<td>87 (61.3)</td>
<td>62 (78.5)</td>
<td>25 (39.7)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD), mm</td>
<td>4.06 (2.20)</td>
<td>5.04 (2.44)</td>
<td>2.84 (0.88)</td>
<td></td>
</tr>
<tr>
<td>Median (IQR), mm</td>
<td>3.43 (2.18)</td>
<td>4.54 (2.98)</td>
<td>2.78 (1.48)</td>
<td>&lt; 0.001$^3$</td>
</tr>
<tr>
<td>Lateral</td>
<td></td>
<td></td>
<td></td>
<td>&gt; 0.999$^7$</td>
</tr>
<tr>
<td>&lt; 3 mm</td>
<td>18 (12.7)</td>
<td>10 (12.7)</td>
<td>8 (12.7)</td>
<td></td>
</tr>
<tr>
<td>$\geq$ 3 mm</td>
<td>124 (87.3)</td>
<td>69 (87.3)</td>
<td>55 (87.3)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD), mm</td>
<td>4.67 (1.52)</td>
<td>4.68 (1.65)</td>
<td>4.65 (1.37)</td>
<td></td>
</tr>
<tr>
<td>Median (IQR), mm</td>
<td>4.37 (2.45)</td>
<td>4.29 (2.22)</td>
<td>4.44 (2.13)</td>
<td>0.730$^7$</td>
</tr>
</tbody>
</table>

SD – standard deviation, $^1$For definition see Chapter 6.7 Statistical analyses, $^2$Chi-square test, $^3$Mann-Whitney U test.

In total, any existing cartilage changes were detected in 127 (89%) subjects and any existing osteophytes were detected in 97 (68%) subjects, out of which 49 (39%) and 28 (29%) were asymptomatic, respectively. Site-specifically, medial cartilage degeneration and medial femoral osteophytes were the most prevalent, in 119 (84%) and 69 (49%) subjects, respectively. Eighty-seven (61%) subjects, out of which 25 (29%) were asymptomatic, had pathological meniscal extrusion in the medial site and 124 (87%), out of which 55 (44%) were asymptomatic, in the lateral site.
Except of lateral meniscal extrusion, the symptomatic and asymptomatic groups differed ($p < 0.001$) in presence of all features (Table 9).

The representative US images of the individual articular cartilage degeneration and osteophyte grades detected in the OKOA population using atlases by Saarakkala et al. (2012) and Koski et al. (2015) are shown in the appendix, Fig. 10 and Fig. 11, respectively.

### 7.2.1 Co-occurrence of ultrasound features

Strong positive correlations were found between global femoral cartilage and global osteophyte grades ($r \ [95\% \ CI] = 0.78 \ [0.71–0.84]$), global medial osteophyte grade and medial meniscal extrusion ($0.72 \ [0.63–0.79]$), and medial cartilage grade and medial meniscal extrusion ($0.67 \ [0.56–0.75]$) (unpublished data). The cross-tabulation (Table 10) presents the co-occurrence between femoral cartilage and global osteophyte grades.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Grade</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global femoral cartilage</td>
<td>0</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>grade</td>
<td>1</td>
<td>21</td>
<td>13</td>
<td>1</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>2a</td>
<td>7</td>
<td>27</td>
<td>8</td>
<td>3</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>2</td>
<td>5</td>
<td>11</td>
<td>11</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>14</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>46</td>
<td>23</td>
<td>28</td>
<td>142</td>
<td></td>
</tr>
</tbody>
</table>

### 7.3 Reliability of semi-quantitative knee ultrasonography (I)

The intra-rater reliability of the original semi-quantitative knee US grading ranged from moderate to almost perfect agreement in assessment of cartilage and osteophytes, and good to excellent in measurement of meniscal extrusion (Table 11). The modified (i.e. grouping of grade 0 and 1, and using the maximum from medial and sulcus grades as a medial cartilage grade) medial and lateral cartilage grades used in Study I reached agreement of $\kappa_w \ (95\% \ CI) = 0.67 \ (0.51–0.84)$ and $0.45 \ (0.22–0.69)$, respectively. PEA and PCA were 74.5% and 78.4% for both.
Table 11. Intra-rater reliability of semi-quantitative knee OA ultrasound grading (n = 51).

<table>
<thead>
<tr>
<th>Ultrasound feature</th>
<th>$\kappa_w$ (95% CI)</th>
<th>PEA (%)</th>
<th>PCA (%)</th>
<th>ICC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial cartilage $^1$</td>
<td>0.68 (0.52–0.84)</td>
<td>70.6</td>
<td>96.1</td>
<td></td>
</tr>
<tr>
<td>Sulcus cartilage $^1$</td>
<td>0.52 (0.36–0.68)</td>
<td>52.9</td>
<td>94.1</td>
<td></td>
</tr>
<tr>
<td>Lateral cartilage $^1$</td>
<td>0.51 (0.33–0.69)</td>
<td>60.8</td>
<td>96.1</td>
<td></td>
</tr>
<tr>
<td>Lateral femoral osteophyte</td>
<td>0.82 (0.70–0.93)</td>
<td>82.4</td>
<td>98.0</td>
<td></td>
</tr>
<tr>
<td>Lateral tibial osteophyte</td>
<td>0.72 (0.56–0.88)</td>
<td>82.4</td>
<td>98.0</td>
<td></td>
</tr>
<tr>
<td>Medial femoral osteophyte</td>
<td>0.77 (0.61–0.93)</td>
<td>82.4</td>
<td>98.0</td>
<td></td>
</tr>
<tr>
<td>Medial tibial osteophyte</td>
<td>0.76 (0.61–0.91)</td>
<td>82.4</td>
<td>98.0</td>
<td></td>
</tr>
<tr>
<td>Medial meniscal extrusion</td>
<td>0.91 (0.85–0.95)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral meniscal extrusion</td>
<td>0.72 (0.55–0.83)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$k_w$ – weighted kappa coefficient, CI – confidence interval, PEA – percentage exact agreement, PCA – percentage close agreement, ICC – intra-class correlation coefficient, $^1$Original US cartilage grading.

7.4 Diagnostic performance (I)

The semi-quantitative US reached good to excellent diagnostic performance values in detection of any osteophytes in medial and lateral femur, and medial and lateral tibia (AUC [95% CI] = 0.97 [0.94–1.00]; 0.96 [0.93–1.00]; 0.89 [0.83–0.95]; 0.90 [0.83–0.96], respectively). US was able to identify medial cartilage structural degeneration in comparison to MOAKS femoral cartilage I and II assessments with fair and good accuracy, respectively, however in the lateral femoral condyle the capability was poor and fair, respectively. The diagnostic ability to detect meniscal extrusion in the medial site was excellent and in the lateral site was good. All diagnostic performance values (i.e. AUC, sensitivity, specificity, accuracy, PPV and NPV) of semi-quantitative knee US at cut-off grade 1 in all subject populations are summarized in Table 2 of Study I.

US was superior to CR in detection of medial and lateral femoral osteophytes ($p < 0.001$) and medial meniscal extrusion ($p = 0.003$) with AUC values ranging from good to excellent, while CR reached only fair to good values (Table 12). CR was performing significantly better only in identification of lateral femoral cartilage full thickness loss ($p = 0.035$), otherwise both modalities identified the remaining features with a similar good level of accuracy. All diagnostic performance values of US and radiographic OARSI grading for the symptomatic group are summarized in Table 3 of Study I.
Table 12. The diagnostic performance presented as area under the receiver operating curve (AUC) with 95% confidence intervals (CI) of semi-quantitative ultrasound (SQUS) and radiographic Osteoarthritis Research Society International (OARSI) grading of knee structural features with reference to corresponding Magnetic Resonance Imaging Osteoarthritis Knee Score (MOAKS) in the symptomatic subject group.

<table>
<thead>
<tr>
<th>Feature evaluated by OARSI; SQUS; MOAKS</th>
<th>SQUS (n = 79)</th>
<th>OARSI (n = 70)</th>
<th>MOAKS (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC (95% CI)</td>
<td>AUC (95% CI)</td>
<td>AUC (95% CI)</td>
</tr>
<tr>
<td>Medial compartment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral osteophyte</td>
<td>0.95$^3$</td>
<td>0.80$^3$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(0.90–0.99)</td>
<td>(0.71–0.90)</td>
<td></td>
</tr>
<tr>
<td>Tibial osteophyte</td>
<td>0.87$^3$</td>
<td>0.84$^3$</td>
<td>0.617</td>
</tr>
<tr>
<td></td>
<td>(0.79–0.95)</td>
<td>(0.75–0.93)</td>
<td></td>
</tr>
<tr>
<td>Medial OA (n = 70)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JSN; Cartilage; Femoral cartilage I</td>
<td>0.85$^2$</td>
<td>0.83$^2$</td>
<td>0.680</td>
</tr>
<tr>
<td></td>
<td>(0.76–0.95)</td>
<td>(0.72–0.94)</td>
<td></td>
</tr>
<tr>
<td>JSN; Cartilage; Femoral cartilage II</td>
<td>0.90$^3$</td>
<td>0.85$^3$</td>
<td>0.312</td>
</tr>
<tr>
<td></td>
<td>(0.82–0.97)</td>
<td>(0.76–0.95)</td>
<td></td>
</tr>
<tr>
<td>JSN; Cartilage; Femoral-tibial central cartilage I</td>
<td>0.88$^3$</td>
<td>0.84$^3$</td>
<td>0.655</td>
</tr>
<tr>
<td></td>
<td>(0.78–0.95)</td>
<td>(0.74–0.94)</td>
<td></td>
</tr>
<tr>
<td>JSN; Cartilage; Femoral-tibial central cartilage II</td>
<td>0.88$^3$</td>
<td>0.86$^3$</td>
<td>0.744</td>
</tr>
<tr>
<td></td>
<td>(0.79–0.96)</td>
<td>(0.77–0.96)</td>
<td></td>
</tr>
<tr>
<td>JSN; Meniscal extrusion</td>
<td>0.94$^2$</td>
<td>0.81$^2$</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>(0.89–0.99)</td>
<td>(0.71–0.91)</td>
<td></td>
</tr>
<tr>
<td>Lateral compartment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral osteophyte</td>
<td>0.94$^3$</td>
<td>0.70$^2$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(0.89–0.99)</td>
<td>(0.58–0.81)</td>
<td></td>
</tr>
<tr>
<td>Tibial osteophyte</td>
<td>0.89$^3$</td>
<td>0.87$^3$</td>
<td>0.640</td>
</tr>
<tr>
<td></td>
<td>(0.81–0.97)</td>
<td>(0.79–0.95)</td>
<td></td>
</tr>
<tr>
<td>Lateral OA (n = 28)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JSN; Cartilage; Femoral cartilage I</td>
<td>0.67</td>
<td>0.82$^2$</td>
<td>0.091</td>
</tr>
<tr>
<td></td>
<td>(0.47–0.87)</td>
<td>(0.66–0.98)</td>
<td></td>
</tr>
<tr>
<td>JSN; Cartilage; Femoral cartilage II</td>
<td>0.70</td>
<td>0.86$^2$</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>(0.47–0.93)</td>
<td>(0.65–1.00)</td>
<td></td>
</tr>
<tr>
<td>JSN; Cartilage; Femoral-tibial central cartilage I</td>
<td>0.74$^1$</td>
<td>0.79$^1$</td>
<td>0.634</td>
</tr>
<tr>
<td></td>
<td>(0.55–0.93)</td>
<td>(0.61–0.96)</td>
<td></td>
</tr>
<tr>
<td>JSN; Cartilage; Femoral-tibial central cartilage II</td>
<td>0.79$^1$</td>
<td>0.92$^2$</td>
<td>0.104</td>
</tr>
<tr>
<td></td>
<td>(0.59 – 0.98)</td>
<td>(0.82–1.00)</td>
<td></td>
</tr>
<tr>
<td>JSN; Meniscal extrusion</td>
<td>0.88$^2$</td>
<td>0.94$^3$</td>
<td>0.401</td>
</tr>
<tr>
<td></td>
<td>(0.75–1.00)</td>
<td>(0.84–1.00)</td>
<td></td>
</tr>
</tbody>
</table>

$^1p < 0.05$, $^2p < 0.01$, $^3p < 0.001$
Figure 7 shows an example of the same symptomatic subject’s knee imaged by different diagnostic modalities (US, CR and MRI). Cartilage morphological degeneration and meniscal extrusion can be clearly distinguished in the MRI and US images but not in CR. Figure 8 shows a comparison of the same subject’s knee US and CR images showing the ability of US to detect tibio-femoral osteophytes more sensitively than CR.

Fig. 7. Example images of articular cartilage degeneration and meniscal extrusion as imaged by magnetic resonance imaging (MRI), ultrasound (US) and conventional radiography (CR). Medial femoral condyle cartilage thinning is indicated by white arrows in the sagittal MRI (a) and US transversal B-mode image (b). Medial meniscal extrusion can be seen in MRI (c) (white arrow) and longitudinal B-mode US image (d) (double headed arrow). Anterior-posterior radiograph (e) demonstrates medial joint space narrowing (white arrows) as a surrogate of meniscal and cartilage structural damage.
Fig. 8. Comparison of radiographic (a) and ultrasound (US) (b) images for detection of osteophytes in the medial knee compartment. Both modalities were able to identify the tibial osteophyte of grade 1 (OARSI and US, respectively) (arrows pointing at tibial bone side), whereas femoral osteophyte of grade 2 visualized by US was not distinguished by knee radiography (i.e. OARSI grade 0).

### 7.5 Associations with pain and function (II)

From the US-defined structural features global femoral cartilage grade reached the strongest associations with pain and WOMAC index with the IRRs increasing towards the higher US grade (adjusted IRRs [95% CI] ranging from 5.2 [2.0–13.3]–22.0 [5.4–89.7] and 3.8 [1.5–9.8]–10.5 [2.7–41.3], respectively). The strongest site-specific associations were observed between medial cartilage degeneration and pain as well as WOMAC index with the highest adjusted IRRs in grade 3, i.e. local full thickness loss, (4.7 [1.6–14.0] and 5.6 [1.7–17.9], respectively). Subjects with medial cartilage degeneration of grade 1 had significantly increased IRR for pain...
and for WOMAC index. However, in sulcus and lateral cartilage grade 1 degeneration, no significant associations were observed.

Presence of femoral osteophytes, especially in the lateral compartment, was significantly associated with both clinical outcomes, ranging from 2.2 (1.3–3.9) to 2.9 (1.4–6.0) for pain and from 2.2 (1.3–3.7) to 2.5 (1.2–5.2) for WOMAC index. Apart from osteophyte grade 2 in respect to pain, all osteophyte grades in the medial femoral site were associated with worsened present pain and symptoms in the past week (adjusted IRRs [95% CI] for pain grade-wise: 2.1 [1.2–3.8], 1.6 [0.8–3.4], 3.4 [1.4–8.4]; for WOMAC index: 2.4 [1.4–4.1], 2.2 [1.1–4.4]; 3.7 [1.6–8.8]). Subjects with any osteophytes in the lateral compartment reported worsened pain and WOMAC index, whereas only presence of medium (grade 2) and large (grade 3) osteophytes was associated with increased WOMAC index in the medial compartment. Globally, having any osteophyte of any size and/or meniscal extrusion in any site did not increase either of the outcomes after all adjustments.

All adjusted estimates of association of US-defined structural features with pain and WOMAC index are presented in Tables 3–5 in Study II. Concerning the individual WOMAC subscales, all results can be found in the supplementary tables S1–S3 of Study II. Briefly, the highest IRRs were observed for global femoral cartilage grade in relation to each WOMAC subscale (i.e., pain, stiffness and function) with stiffness being dominant especially in subjects with cartilage degeneration grade 3 in the medial site (IRR [95% CI] = 7.9 [2.8, 22.6]). Moreover, the existence of any femoral cartilage degeneration as well as femoral osteophytes was associated with all subscales. There were no associations with meniscal extrusion in any site.

7.6 Quantitative image analysis of subchondral bone (III)

Qualitatively, an increasing trend in normalized grayscale US intensity values of the subchondral bone and decreasing trend of the intensity slopes were observed as OA progressed (Fig 6). The most significant increase in US reflection intensity in comparison to KL and Noyes’ grading was observed in subchondral bone depth level 2, particularly in sulcus ($r$ [95% CI] = 0.54 [0.22–0.75], $p = 0.002$ and 0.37 (0.06–0.62), $p = 0.022$, respectively), and in medial condyle a significant increase was only seen with respect to KL assessment (0.42 [0.08–0.67], $p = 0.019$). Overall, the intensity increase in multiple bone levels as OA progressed was observed especially in sulcus area and medial condyle (Table 3 in Study III). In the sulcus and medial site, the decrease in the intensity slope was moderate and weak,
respectively, in relation to both references (Table 1 in Study III). No significant changes in US intensity nor slope were observed in the lateral condyle.

Regarding the US femoral bone mean intensity, the most statistically significant correlations with KL grade and FAS1 were observed in depth level 2 (Table 13). Furthermore, femoral bone levels 1, 4, and all, showed weak to moderate correlations with KL grade, whereas no other relationship between US femoral variables and FAS1 was observed (Table 13). The femoral bone intensity slope absolute value was significantly decreasing with both higher radiographic as well as arthroscopic grade (Table 13).

Table 13. Spearman’s rank correlations (95% confidence interval) between ultrasound femoral subchondral bone profile intensities and intensity slopes and radiographic Kellgren-Lawrence (KL) grading, and modified femoral arthroscopic score 1 (FAS1) (n = 31 for KL, n = 39 for FAS1).

<table>
<thead>
<tr>
<th>Ultrasound bone variable</th>
<th>KL grading</th>
<th>FAS1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral bone – level 1</td>
<td>0.49 (0.17–0.72)</td>
<td>0.14 (-0.19–0.44)</td>
</tr>
<tr>
<td>Femoral bone – level 2</td>
<td>0.60 (0.31–0.79)</td>
<td>0.33 (0.02–0.59)</td>
</tr>
<tr>
<td>Femoral bone – level 3</td>
<td>0.31 (-0.05–0.60)</td>
<td>0.20 (-0.13–0.48)</td>
</tr>
<tr>
<td>Femoral bone – level 4</td>
<td>0.38 (0.03–0.65)</td>
<td>-0.02 (-0.33–0.30)</td>
</tr>
<tr>
<td>Femoral bone – level 5</td>
<td>0.24 (-0.13–0.55)</td>
<td>0.05 (-0.27–0.36)</td>
</tr>
<tr>
<td>Femoral bone – level all</td>
<td>0.46 (0.13–0.70)</td>
<td>0.16 (-0.16–0.45)</td>
</tr>
<tr>
<td>Femoral bone – slope</td>
<td>0.49 (0.16–0.72)</td>
<td>0.34 (0.02–0.59)</td>
</tr>
</tbody>
</table>

\( ^1 p < 0.05, ^2 p < 0.01, ^3 p < 0.001, ^4 \text{Note, that the correlation value was calculated for the original slope values, not the absolute, therefore it is positive.} \)

Normalized US mean intensity was significantly increased in multiple femoral bone depth levels of subjects with KL grade 1 in comparison to subjects with grade 0 (Fig. 9). When comparing FAS2 grades 1 and 2, difference was found only in femoral bone depth level 2 \((p = 0.008)\). The mean intensity slope of FAS2 groups 1 and 2 statistically significantly differed \((p = 0.015)\), however no difference was observed between KL grade 0 and 1 \((p = 0.088)\).
Fig. 9. Normalized ultrasound mean grayscale intensity values in distinct femoral subchondral bone depth levels. Asterisk indicates the statistically significant differences between radiographic Kellgren-Lawrence (KL) grade 0 and 1.

KL grade 0, n = 13
KL grade 1, n = 14

* p < 0.05
8 Discussion

The current need to image and diagnose OA in daily clinical routine more sensitively and specifically than the present gold standard method, i.e. CR, has been expressed. In this regard, knee ultrasonography has been endorsed and proposed to be used either as an initial screening and/or monitoring tool (Finucci et al. 2015, Grassi et al. 1999, Hong et al. 2000, Iagnocco et al. 1992, Martino et al. 1993a, Martino et al. 1993b, Tarhan & Unlu 2003, Vojtassak & Vojtassak 2014) as well as complementary to CR (Chan et al. 2014, Iagnocco 2010, Kazam et al. 2011, Roemer et al. 2011).

In this section the main findings of the doctoral study are discussed against the current scientific literature.

8.1 Diagnostic performance and reliability of semi-quantitative knee ultrasonography (I)

8.1.1 Detection of osteophytes

The results of Study I confirmed the earlier reported ability of US to readily discern periarticular osteophyte formation in knee OA, which can be present also in radiographically normal knees (Riecke et al. 2014). In the present study, US-determined osteophytes reached higher or comparable accuracy than traditional CR when MRI was used as the reference tool. These findings are also in line with the recent study by Koski et al. (2015) who showed the superiority of semi-quantitative ultrasonography over CR in identification of tibio-femoral osteophytes. Furthermore, it is noteworthy that US, thanks to its valuable capability of multi-planar imaging, was able to identify osteophytes not visible in CR but confirmed by MRI. This observation strongly supports and verifies results of recent studies (Koski et al. 2015, Riecke et al. 2014).

8.1.2 Detection of articular cartilage degeneration

It has been reported that the semi-quantitative ultrasonography is able to predict the articular cartilage degeneration seen in arthroscopy, however negative findings in US do not exclude arthroscopic degeneration (Saarakkala et al. 2012). With the exception of the lateral femoral condyle, similar results were observed in the
present study in comparison to MRI. The somewhat lower performance of US to detect any cartilage loss, compared to full-thickness loss, might be caused by the difference between the US and MRI grading systems. In US grading the progression of cartilage thinning is assessed depth-wise, whereas in MRI grading (MOAKS cartilage I) any cartilage loss is assessed surface-wise, including partial as well as full-thickness cartilage loss. It is notable that the spatial resolution for evaluation of cartilage thickness in US is higher than in MRI. Moreover, the low NPV (61% for \( n = 159 \), and 46% for \( n = 70 \) [see Study I, Table 1 and 2]) and relatively high PPV (82% for \( n = 159 \), and 91% for \( n = 70 \) [see Study I, Table 1 and 2]) of US for detection of any cartilage loss in medial femoral condyle suggest that US may be more sensitive for detection of cartilage structural changes, but it cannot depict entire femoral articular surfaces.

The femoral and tibial cartilage volumes and their longitudinal changes are known to be strongly related (Cicuttini et al. 2001, Cicuttini et al. 2004, Saarakkala et al. 2012). Although indirectly, US was still able to detect the combined femoral and tibial cartilage changes equally well as the radiographic JSN.

### 8.1.3 Detection of meniscal extrusion

Standardized method for measurement of meniscal extrusion and definition of its pathological size in US has not been established yet. A recent study by Nogueira-Barbosa et al. (2015) demonstrated an excellent diagnostic performance of US in quantitative and semi-quantitative assessment of medial meniscal extrusion using the 2 mm threshold. In the present doctoral study, when using the 3 mm threshold, US was able to identify the extrusion with similar sensitivity, however, with lower specificity and accuracy than demonstrated previously (Nogueira-Barbosa et al. 2015). The differences might be caused by the distinct measurement setup.

### 8.1.4 Reliability

The intra-rater reliability values for evaluation of osteophytes in all four bone margins and medial meniscal extrusion are relatively consistent with values presented in earlier studies (Acebes et al. 2013, Koski et al. 2015). Moreover, the reliability has improved in detection of osteophytes in the lateral femur and tibia since the earlier study, in which the same evaluator was involved as a primary rater (\( \kappa = 0.75 \) vs. \( \kappa = 0.82 \) and \( \kappa = 0.64 \) vs. \( \kappa = 0.72 \)) (Koski et al. 2015). The reliability values for cartilage and osteophyte assessments were also similar to values reported
by Bruyn et al. (2015), even though the definition of cartilage damage differed. However, it seems that the quantitative measure of meniscal extrusion applied in the present study was more reliable than the semi-quantitative assessment used earlier (Bruyn et al. 2015). The variation in assessment of cartilage degeneration, especially in the trochlear region, might be caused by difficulty to distinguish between cartilage and soft tissue in a few overweight subjects, in which the US with the frequency of 15 MHz was more attenuated by the adipose tissue, and therefore the cartilage appearance was more blurred.

8.2 Prevalence and co-occurrence of ultrasound-defined osteoarthritic features and their association with symptoms (II)

8.2.1 Prevalence

To the author’s knowledge, there has been only one epidemiological study on prevalence of US-defined features (Abraham et al. 2014). In the elderly population-based cohort, 30% of subjects had osteophyte in either knee. In the current study, osteophytes were present in two thirds of all subjects. This difference is, however, justifiable by different study design. Hong et al. (2000) examined a group of 20 OA patients, in which cartilage changes and osteophytes were more frequently present in the medial site (80–85% and 65%, respectively) than in the lateral site (50–70% and 60%, respectively). The site-specific prevalence in the OKOA population of Study II was similar. Since there are several OA structural phenotypes (McGonagle et al. 2010), it is no wonder that the absolute prevalence values are in disagreement between particular populations.

Although the prevalence of the US-defined OA features was predominating in the symptomatic subject group, pathological changes were frequently seen also in the asymptomatic subjects. This observation suggests the presence of the non-painful knee OA phenotype within the OKOA asymptomatic population.

Interestingly, in both groups the proportion of subjects with laterally extruded meniscus was the same (87%). Furthermore, the median extrusion values did not differ. The mean normal lateral extrusion reported by Verdonk et al. (2004) was 3.77 (SD = 1.76) mm, which is more than the pathological threshold used in the current study. Therefore, further research on determination of the threshold(s) for pathological meniscal extrusion is recommended in the future.
8.2.2 Co-occurrence

In the OKOA population of Study II, presence and severity of the femoral cartilage degeneration and osteophyte formation were mostly concomitant. In a few subjects, however, the atrophic OA phenotype (Roemer et al. 2012, Roemer et al. 2015) was observed with US. The high co-occurrence of medial cartilage changes and meniscal extrusion is in line with results of a study by Ragab et al. (2012). This finding also suggests the contribution of meniscus displacement to the cartilage loss as proposed by the recent longitudinal studies (Blöcker et al. 2015, Pelletier et al. 2007). However, this hypothesis could not be confirmed in the current study due to its cross-sectional design.

8.2.3 Associations

It was observed that already early femoral cartilage degeneration was associated with clinical symptoms. It is known that nociceptive tissues in the knee joint are the likely biological source of pain in OA (Hunter et al. 2013), however, normal cartilage is lacking this characteristic. Pain sensitization in early OA might be, therefore, a consequence of joint inflammation developed as a response to the released pro-inflammatory mediators from slowly degrading cartilage (Houard et al. 2013). This hypothesis has been recently reinforced by study of Kaukinen et al. (2016) who showed that in the exactly same OKOA population the MRI-detected synovitis and effusion remain significantly associated with knee pain even after correction for other structural OA features. Furthermore, the significant positive relation of structural cartilage degeneration to pain was diminished in the medial femoral condyle and considerably decreased in the lateral femoral site after adjustments (Kaukinen et al. 2016). As described in Chapter 3.2, vascular ingrowth accompanied by nerves occurs at the osteochondral junction and eventually invades the degenerated non-calcified cartilage and osteophytes (Ashraf & Walsh 2008, Mapp & Walsh 2012). Subsequently, especially in severe cartilage loss, pain may be caused by mechanical irritation of the newly grown sensory nerves, or exposed nociceptors of denuded subchondral bone (Hunter et al. 2013).

Chen and colleagues have reported significant associations of cartilage degeneration with WOMAC and VAS pain. In addition, VAS pain associated with cartilage changes degree-dependently (Chen et al. 2015). Cartilage thinning, determined as present or absent, has also positively associated with the WOMAC index (Razek & El-Basyouni 2015). Albeit using different US grading system, we
were able to observe similar outcomes. In contrast, despite the use of the same assessment criteria as in a study by Chen et al. (2015), Malas et al. (2014) did not observe any relationship between WOMAC subscales and the severity of cartilage damage (Malas et al. 2014). However, it is important to mention that none of these studies applied adjustment of the statistical analysis for possible pain confounders, especially inflammatory features. Moreover, synovial effusion was also individually found to be associated with clinical symptoms in a study by Razek & El-Basyouni (2015).

More severe symptoms were observed in patients with osteophytes in the lateral joint compartment. Likewise in earlier studies, presence of osteophytes was related to increased WOMAC index (Razek & El-Basyouni 2015) and the predominant lateral knee morphological changes, including osteophytes, positively correlated with the KOOS symptom subscale (Riecke et al. 2014). However, in the latter study, no relationship with a pain subscale was found. Again, as mentioned above, vascular and nerve growth could be the rational source of pain. Furthermore, bony osteophytes can irritate the adjacent synovium (Hunter et al. 2008b). It is also possible that the marginal osteophytes may contribute to the knee functional impairment through restriction of range of motion of the affected joint (van der Kraan & van den Berg 2007).

US studies assessing the link between meniscal extrusion and clinical outcomes reported either no (Razek & El-Basyouni 2015, Wu et al. 2012) or only weak (Chan et al. 2014, Malas et al. 2014) associations. Also in the present study, medial meniscal extrusion showed associations with pain and the WOMAC index only when adjusted for demographic confounders, whereas with further adjustments for global femoral cartilage and global osteophyte grades, the associations became nonsignificant. Again, these results are in line with the observations of Kaukinen et al. (2015). Therefore, the meniscal extrusion might have been only a coexistent abnormality to another painful and disabling OA processes within the knee joint.

As discussed in Chapter 8.2.1, the relatively wide confidence intervals, especially in the global femoral cartilage grade associations, may be explained by the existence of the non-painful OA phenotype among the asymptomatic subjects.

8.3 Quantitative image analysis of subchondral bone (III)

In Study III, the ability of quantitative knee US image analysis to detect OA changes in the subchondral bone was investigated. Present in vivo results verified
the earlier *in vitro* findings (Saarakkala *et al.* 2006, Saïed *et al.* 1997) that the US reflection and backscattering from the cartilage-bone interface and underlying subchondral bone increases with OA progression. This finding indicates the presence of subchondral bone sclerosis, in which bone density increases. Consequently, the difference in acoustic impedances between the sclerotic subchondral bone and overlying articular cartilage (or soft tissue if the cartilage is totally lost) is larger than at the normal bone-cartilage interface. Therefore, more US is reflected back, which is demonstrated by increased US image intensity. This finding is further supported by Leicht & Raum (2008) who observed a significant increase in the acoustic impedance of the subchondral bone just beneath the surface in comparison to deeper bone regions.

With the current quantitative approach, the likely OA-related changes in US reflection were detected sensitively, particularly in the subchondral bone depth level 2, corresponding to the depth range of 0.35–0.7 mm within the subchondral bone. In the deeper bone regions, strong US attenuation greatly decreases the signal-to-noise ratio (Kossoff 2000), thus explaining the lower intensity. Furthermore, the decrease in the intensity slope was observed in more severe OA which may reflect the enhanced subchondral plate thickness (Goldring & Goldring 2010a, Goldring & Goldring 2010b). Another reason could be the higher US beam scattering at the cartilage-bone interface due to the surface disorientation and/or disruption, resulting in the wide high reflection band appearance in US images.

In bone, most of the OA changes appear in weight-bearing regions (Burr 2004a, Loeser *et al.* 2012). However, in the present study increased US intensity and decreased slope were detected especially in the sulcus region. To select the medial and lateral condyle bone region, which would be at the same time perpendicular to the upcoming US beam and weight-bearing, was rather challenging due to their naturally curved shape. Therefore, the early OA changes in these locations might have been missed.

Generally, the quantitative US was better and more frequently correlating with CR than arthroscopy. In the author’s opinion, CR should also be considered as the true reference in this study since it directly depicts the subchondral bone, and its evaluation is part of the KL grade (Kellgren & Lawrence 1957). In contrast, during the traditional arthroscopy the subchondral bone can be assessed only after the cartilage tissue is completely lost (Noyes & Stabler 1989). On the other hand, some correlations with arthroscopy were still found, likely indicating the concomitant degenerative processes in the cartilage and subchondral bone in some subjects.
8.4 Limitations

In general, the most limiting factor of knee ultrasonography is the lack of the appropriate acoustic windows, due to which the deeper joint structures cannot be evaluated. Patellar shadowing is one example restricting the visibility of the femoral cartilage, particularly in the lateral site while the knee is in full flexion. Moreover, US imaging is highly operator-dependent, hence some bias might have been introduced by keeping an inconsistent angle between the US probe and the ROIs. On the other hand, in the case of operator uncertainty, regarding the semi-quantitative assessment, the same anatomical region can be promptly compared collaterally (Grobbelaar & Bouffard 2000). A mechanical scanning approach proposed by Ohashi and colleagues might be also a solution in the future (Ohashi et al. 2012a).

One of the specific limitations of Study I was the lack of knee radiographs of the OKOA asymptomatic subjects which were not obtained for ethical reasons. Furthermore, radiographs from eight subjects of Study III were missing. Consequently, the sample sizes for the performance cross-comparison with CR in Study I, and quantitative subchondral bone assessment in Study III were limited. With regard to Study I, the assumption of coincident degeneration of the femoral and tibial cartilage was made as they cannot be distinguished and individually assessed from CR. In addition, JSN can be further enhanced by meniscus displacement, which is even more pronounced during the weight-bearing image acquisition. Therefore, some bias, when comparing JSN only with cartilage or meniscal damage evaluated by US or MRI, might have occurred. Moreover, the US and MRI grading system definitions for evaluation of cartilage and meniscal damage differed as per se.

Perhaps the greatest limitation faced in Study II was the lack of examination of the US inflammatory features. The reason the inflammation was not included into the study protocol was the primary focus on the structural changes. Although it is known that synovitis, effusion and synovial hypertrophy are often associated with painful knee OA (Chan et al. 2014, de Miguel Mendieta et al. 2006, Hall et al. 2014, Razek & El-Basyouni 2015, Wu et al. 2012), inflammation can also often develop as a consequence of other structural damage, including cartilage, bone and meniscus, within the knee joint (Bonnet & Walsh 2005). In addition, factors such as mechanical and psycho-social, which are believed to play an important role in pain aetiology (Hunter et al. 2013, Vincent et al. 2012), were not accounted for. Thus, whether the pain and symptom deterioration was more likely structure-casual,
inflammatory, mechanical or psychological, or an interaction of all of these factors in the OKOA study population, cannot be concluded. Lastly, the use of analgesics was not restricted during the study, therefore the reported pain and disability levels might have been lower than the true ones.

In Study III, the sample size was relatively small since the data collection was planned using an earlier study protocol and reviewed in this doctoral thesis. Therefore, data with Noyes’ grade 0 and with more severe KL grades were lacking. Consequently, multiple comparisons between FAS2 groups or KL groups were not able to be performed. Yet, US was able to detect differences in subchondral bone reflection between normal and doubtful radiographic OA. These results should be, however, interpreted with caution since the reliability analysis was not performed in this study.

Neither inter-rater reliability nor operator dependency of the introduced semi-quantitative and quantitative knee US assessment was investigated. Moreover, the US was evaluated from stored videos. This might lead to higher reliability values than in repeated real-time examinations, as the reliability was not dependent on the actual examination. Considering the hand-held scanning multi-planar nature of the introduced imaging method, the lack of these analyses exhibits a significant limitation of the doctoral study.

8.5 Clinical implications

Despite its limitations, the results of the current doctoral thesis provide strong evidence and highly support the use of the semi-quantitative knee US assessment as a complementary diagnostic technique in a daily clinical routine. The added diagnostic value of ultrasonography over CR is certainly its capability to directly visualize and distinguish between structural changes of the cartilage and meniscus (Adams et al. 1999, Finucci et al. 2015, Gale et al. 1999, Hunter et al. 2006). Early identification of meniscal extrusion is highly important since it strongly contributes to the subsequent articular cartilage loss (Adams et al. 1999, Bloeker et al. 2015, Pelletier et al. 2007). Moreover, the dynamicity of US in the sense of a probe moving continuously over the ROI, the freedom of imaging plane selection and immediate repeatability may help to identify and localize the tissue damage more accurately, and thus serve as an explanatory tool of knee pain and other clinical symptoms (Laslett et al. 2016, Nazarian 2008).

Since OA is a highly heterogeneous disorder, a combination of diagnostic imaging modalities is recommended to identify all features involved (Braun &
Cost-efficient and rapid non-invasive ultrasonography could, therefore, reveal early cartilage-, meniscus- or osteophyte-specific changes, especially when the degeneration is radiographically doubtful and/or the source of pain is not obvious. Furthermore, knee US might help in recognition of the individual structural OA phenotypes.

Indeed, more time might need to be dedicated by the clinician for patient examination when applying US in the clinical practice. On the contrary, the immediate real-time imaging, which is readily accepted by the patient, might speed up the diagnosis and prevent unnecessary costs of other diagnostic interventions (Couturier et al. 2016, Nazarian 2008).

The author believes that the present study will contribute to a paradigm shift in the field of OA diagnostics and follow-up from being only reliant on out-dated CR evaluation methods to clinical application of novel and more sophisticated imaging techniques, such as non-invasive ultrasonography.

### 8.6 Suggestions for future studies

In order to confirm the results of Study I, superiority of US over CR in diagnostic performance and detection of early knee OA signs, CR should be obtained also from knees of asymptomatic subjects. Furthermore, intra- and inter-operator and inter-rater reliability of the introduced US assessment should be examined. Recruitment and longitudinal follow-up of a larger birth cohort or cohort with well-defined age range could help to further explore the diagnostic potential of knee ultrasonography. In these subjects all possible OA features, structural as well as inflammatory, which are accessible by the non-invasive US imaging should be evaluated. Thus, the entire diagnostic role of knee ultrasonography among other imaging modalities would be provided.

The quantitative analysis from Study III should be validated by applying the algorithm on US data with higher resolution and image quality acquired by a high-end US device (also applicable to OKOA study), and larger subject group with all KL grades. Furthermore, 3-D mapping of the subchondral bone US reflection variation could be studied and compared with quantitative results from, e.g. cone beam computed tomography (CT), the most sensitive method for assessment of the subchondral bone structure.

There has been a lot of discussion about whether US is able to depict the weight-bearing area of the femoral condyle. In the first study, Aisen and colleagues claimed that the central femoral condyle is well accessible while the knee is in full
knee flexion (Aisen et al. 1984). On the contrary, Østergaard et al. (1995) reported the opposite. A later study, however, confirmed the claim of Aisen and colleagues by comparing the non-invasive ultrasonography with the post-operative ultrasonography and histology in the late OA subjects, and with CT images in the control group (Martino et al. 1993b). Yet, doubt exists among researchers and clinicians. Therefore, by application of fusion imaging with MRI or CT the actual area depictable by US could be mapped for distinct knee flexion angles (Finucci et al. 2015, Gutierrez et al. 2015).

Last but not least, US has the great advantage of dynamic imaging, i.e. to depict active or passive movement of the tissue structures. This ability has been addressed in a few studies examining the meniscal extrusion (Acebes et al. 2013, Ko et al. 2007, Patel et al. 2012) and snapping knee (Marchand et al. 2012) showing its high potential for assessment of knee OA. The author suggests that further dynamic imaging studies should be conducted for assessment of other knee structures, such as patellar cartilage, as well.
9 Conclusions

This doctoral thesis showed that the non-invasive knee ultrasonography, particularly the presented wide-area US scanning method, provides important information on structural OA abnormalities and their association with clinical symptoms. Especially, the strong evidence of US diagnostic ability to assess knee OA greatly suggests the deployment of US as a complementary diagnostic tool in daily clinical practice. Based on the results of the study, it can be specifically concluded that:

1. Site-specific, semi-quantitative, wide-area US scanning is an accurate method for assessment of knee OA. US can reliably detect the tibio-femoral osteophytes, medial meniscal extrusion and morphological articular cartilage changes in the medial femoral condyle. US is superior to CR in the detection of femoral osteophytes and medial meniscal extrusion, and is able to directly discern femoral cartilage morphological changes from meniscal extrusion.

2. The most prevalent feature identified by knee US imaging was femoral cartilage degeneration, especially in medial condyle, followed by lateral meniscal extrusion, presence of any osteophytes and medial meniscal extrusion. Femoral cartilage changes were strongly and degree-dependently associated with pain and functional impairment. Presence of lateral femoral osteophytes, but not necessary their size, was also associated with increased pain and disability of studied subjects.

3. As indicated by the significant correlations of quantitative femoral US with radiographic KL grade, and femoral arthroscopic assessment, the non-invasive quantitative US analysis is a promising technique for detection of early OA changes at the femoral cartilage-bone interface and the subchondral bone plate. Specifically, it was confirmed that the US reflection and backscattering at the cartilage-bone interface and underlying subchondral bone increases in OA.
References


Peterfy CG, Guermazi A, Zaim S, Tirman PFJ, Miaux Y, White D, Kothari M, Lu Y, Fye K, 
Qvistgaard E, Torp-Pedersen S, Christensen R & Bliddal H (2006) Reproducibility and inter- 
reader agreement of a scoring system for ultrasound evaluation of hip osteoarthritis. 
Radin EL & Rose RM (1986) Role of subchondral bone in the initiation and progression of 
Ralphs JR & Benjamin M (1994) The joint capsule: structure, composition, ageing and 
agreement and correlation with Western Ontario and McMaster Universities 
An ultrasound score for knee osteoarthritis: a cross-sectional validation study. 
Roemer FW, Eckstein F, Hayashi D & Guermazi A (2014) The role of imaging in 
defined atrophic and hypertrophic phenotypes of knee osteoarthritis in a population- 
Roemer FW, Hayes CW, Miller CG, Hoover K & Guermazi A (2015) Imaging atlas for 
eligibility and on-study safety of potential knee adverse events in anti-NGF studies (Part 
1). Osteoarthritis and Cartilage 23, Supplement 1: S22–S42.
Roemer FW, Crema MD, Trattnig S & Guermazi A (2011) Advances in Imaging of 
(KOOS): from joint injury to osteoarthritis. Health Qual Life Outcomes 1: 64.
- validation and comparison to the WOMAC in total knee replacement. Health and 
detects degenerative changes in articular cartilage surface and subchondral bone. Phys 
Ultrasonic quantitation of superficial degradation of articular cartilage. Ultrasound Med 
Biol 30(6): 783–792.


Appendices

Fig. 10. The representative images for semi-quantitatively US grading of articular cartilage degeneration in medial, sulcus and lateral femoral condyle. The grades are defined as follows: Grade 0 – monotonous anechoic band with sharp hyperechoic anterior and posterior interfaces, Grade 1 – loss of the normal sharpness of cartilage interfaces and/or increased echogenicity of the cartilage, Grade 2a – in addition to the above changes, clear local thinning (less than 50%) of the cartilage, Grade 2b – Local thinning of the cartilage more than 50% but less than 100%, Grade 3 – 100% local loss of the cartilage tissue (Saarakkala et al. 2012). Note that grade 3 degeneration in the lateral condyle was not observed in the Oulu Knee Osteoarthritis (OKOA) population.
Fig. 11. The representative images for semi-quantitatively US grading of presence and size of medial and lateral femoral and tibial osteophytes. The grades are defined as follows: Grade 0 – no osteophyte, Grade 1 – small osteophyte, Grade 2 – medium osteophyte, Grade 3 – large osteophyte (Koski et al. 2015).
Original publications


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1383. Raatiniemi, Lasse (2016) Major trauma in Northern Finland
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NON-INVASIVE SEMI-QUANTITATIVE AND QUANTITATIVE ULTRASOUND IMAGING FOR DIAGNOSTICS OF KNEE OSTEOARTHRITIS

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