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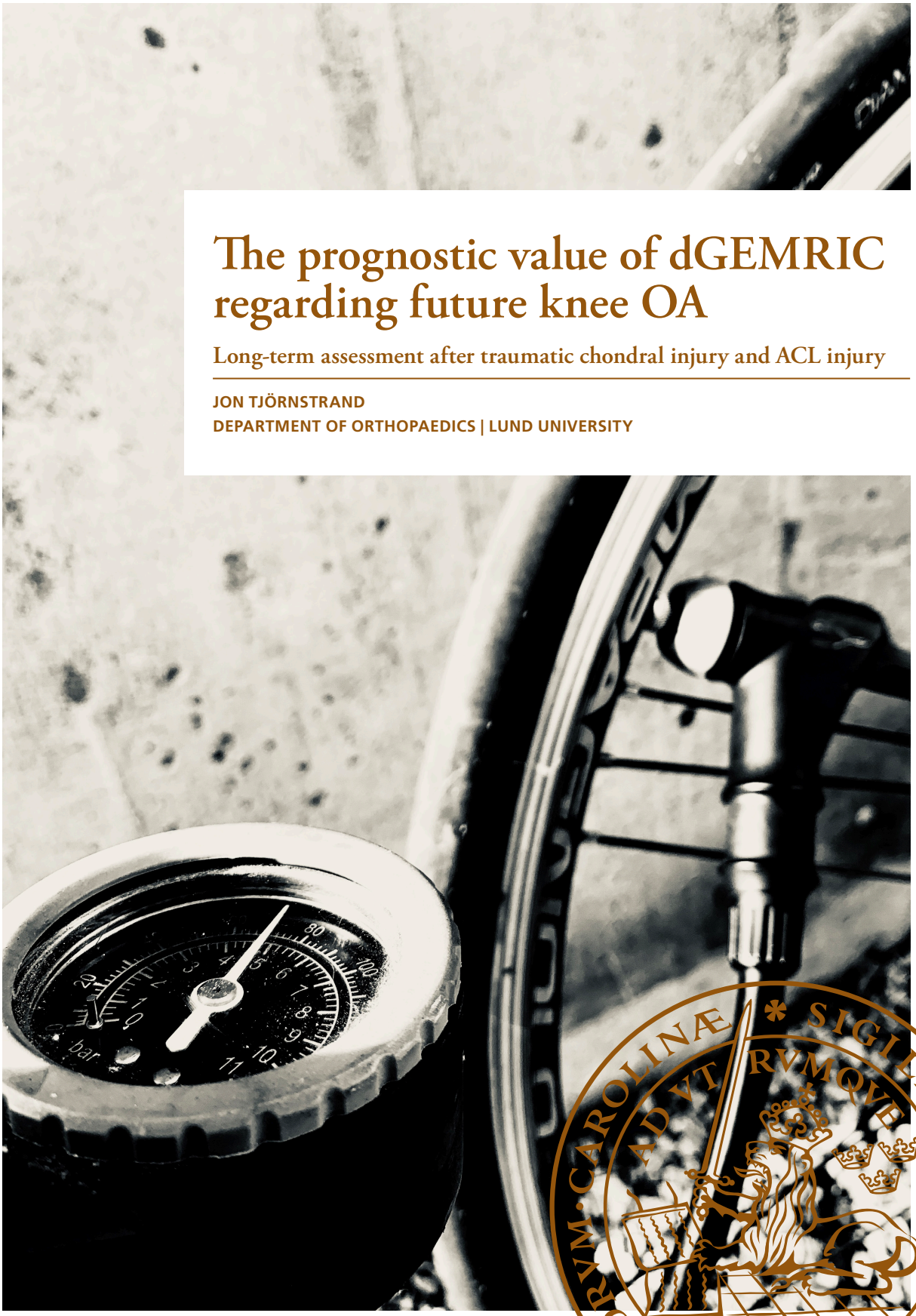

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Long-term assessment after traumatic chondral injury and ACL injury

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The prognostic value of dGEMRIC regarding future knee OA:
long-term assessment after traumatic chondral injury and ACL injury

Abstract

Background: Osteoarthritis (OA) is the most common joint disorder globally and a major cause of disabiltiy with the knee joint responsible for 80% of the disease burden. Progressive degenerative changes in cartilage tissue result in deterioration and loss of articular cartilage. Cartilage has poor capacity of healing and the detection of degeneration usually occurs at a stage of progression where changes are irreversible.

To improve the understanding of the degenerative process and to monitor early stage cartilage disease, new methods are needed. Delayed gadolinium enhanced magnetic resonance imaging of cartilage (dGEMRIC) is a technique to evaluate cartilage quality by estimation of glycosaminoglycan (GAG) content, a low dGEMRIC index indicates low cartilage quality. The characteristic resistance to compressive forces of healthy cartilage relies on high GAG content. Decreasing GAG content is regarded as one of the first alterations in progression to cartilage degeneration.

Aims and cohorts: 1) To validate the dGEMRIC measurement technique. 2) To monitor changes in cartilage quality in two cohorts of knee-injured patients: a) traumatic cartilage injury and b) ACL injury. To investigate the capacity of dGEMRIC to predict clinical and radiographic OA in both these cohorts.

Results: For 6 investigators, the inter- and intra-observer reproducibility of dGEMRIC measurements with manual definition of different regions of interest was good with CV% of <3% at repeated measures. No difference was found related to investigator experience.

A traumatic cartilage injury was associated with a high prevalence of OA after 17 years. The dGEMRIC index in the repair tissue was low already 2 years postoperatively, indicating fibrocartilage of low quality. A negative correlation between the dGEMRIC index in the adjacent cartilage and future OA suggests that the quality of the surrounding cartilage influences outcome after cartilage repair surgery.

29 patients with ACL rupture were investigated with dGEMRIC 3 weeks and 2 years after injury. dGEMRIC index was lower compared to non-injured controls. Patient that had sustained meniscectomy, or had BMI ≥25, had lower cartilage quality at 2 years.

Long-term follow-up was performed in 16 patients with cartilage repair surgery and 31 patients with ACL rupture. In the cartilage repair group, the 12 knees that had developed radiographic OA had lower dGEMRIC index ($p=0.07$), and in the ACL group low medial dGEMRIC index was associated with both medial ROA and OA symptoms ($p<0.05$) after 14 years.

Conclusion: Non-invasive assessment of cartilage quality with dGEMRIC is feasible and measures relevant differences in a clinical context. Cartilage quality assessed with dGEMRIC has a prognostic capacity relative knee OA development.

Keywords: dGEMRIC, knee, cartilage, glycosaminoglycan, GAG, anterior cruciate ligament injury, ACL, osteoarthritis, OA,

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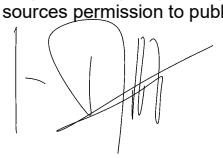
Signature  Date 2019-10-29

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Abbreviations

AC	Articular Cartilage
ACI	Autologous Cartilage Implantation
ACL	Anterior Cruciate Ligament
ACLR	Anterior Cruciate Ligament Reconstruction
AP	Ante-Posterior
APM	Arthroscopic Partial Meniscectomy
BMI	Body Mass Index
BML	Bone Marrow Lesion
cMRI	Compositional MRI
dGEMRIC	Delayed Gadolinium-Enhanced MRI of Cartilage
DMOAD	Disease Modifying OA Drugs
ECM	Extra Cellular Matrix
FCD	Fixed Charge Density
GAG	Glycosaminoglycan
Gd-DOTA^{-}	Gadoteric acid, Gadoterate meglumine (Dotarem®)
Gd-DTPA^{2-}	Gadolinium diethylene triamine pentacetate, Gadopentetate dimeglumine (Magnevist®)
GdCA	Gadolinium Contrast Agents
HTO	High Tibial (varus) Osteotomy
JSN	Joint Space Narrowing
KL	Kellgren-Lawrence OA grading score
KOOS	Knee injury and Osteoarthritis Outcome Score
MFX	Micro Fracture Technique
MRI	Magnetic Resonance Imaging
NSF	Nephrogenic Systemic Fibrosis
OA	Osteoarthritis
OARSI	OsteoArthritis Research Society International
PROMS	Patient Reported Outcome Measures
PTOA	Post Traumatic OA
RCT	Randomized Controlled Trial
ROA	Radiographic OA
ROI	Region of Interest
SF	Synovial Fluid
SOA	Symptomatic OA
T1	T1 transverse relaxation time
T1Gd	T1 transverse relaxation time after saturation with Gd-DTPA2-
T2	T2 transverse relaxation time
TCL	Traumatic Cartilage Lesion
TKA	Total Knee Arthroplasty
UKA	Uni-compartmental Knee Arthroplasty

Introduction

Osteoarthritis (OA) is a painful debilitating disease of the joints that become more prevalent in old age. An interplay of biomechanical stresses and biochemical processes break down the cartilage. Damage to the knee joint such as ligament rupture can result in the development of knee OA after 10-20 years, to affect patients already in their thirties and forties. Despite decades of research and the characterization of a multitude of complex molecular processes involved in cartilage pathology a treatment to alter the degeneration of cartilage and the development of OA is lacking.

The tools to assess and monitor cartilage developments in vivo can only detect cartilage changes on the level of morphological changes such as thinning, cracks, defects and fibrillations. These changes all imply that structural changes have occurred, and cartilage have been lost. In order for a hypothetical treatment to be effective in arresting degenerative development it needs to act before the cartilage is lost, and in order to develop such treatments monitoring of early changes in intact cartilage is needed.

Compositional MRI (cMRI) aim to detect changes in cartilage quality at a disease stage prior to cartilage loss by detecting alterations in the molecular and structural composition. A validated cMRI technique would permit the study of cartilage quality and changes in a short perspective in contrast to the 10-year period required to begin to detect radiographic OA outcome.

This thesis concern one such technology, delayed gadolinium enhanced MRI of cartilage (dGEMRIC), that estimate the density of fixed negative charge. In the cartilage matrix concentration of proteoglycans carrying negatively charged glycosaminoglycan (GAG) determines cartilage capacity to resist compression, low concentration means lower resistance. Alterations in GAG concentration can represent dynamic changes in cartilage homeostasis and a pronounced reduced content is regarded as the first step of cartilage degeneration.

This thesis found, in addition to studying methodological aspects and validation of the technique, that dGEMRIC was able to meaningfully measure cartilage quality in cohorts with high risk of OA development and that the measured cartilage quality was associated to long term outcome of OA development.

Background

The knee joint

Joint function; ligament, muscle, meniscus, cartilage

The human knee is a marvellous instrument perfected by evolution; it can endure continuous daily use under high loads for a lifetime.

The distal end of the femur and the proximal end of the tibia form the femorotibial joint carrying the gravitational force in extension. In movement flexion and extension are balanced by reciprocal muscular units, mainly the hamstrings and quadriceps respectively. The patella bone creates leverage and strengthens the extension apparatus by articulating to the frontal femoral curvature of the in-flexion range of positions. An intricate system of ligaments connects the femorotibial bones and asserts sagittal and side-to-side stability for the full range of motion; the centrally located cruciate ligaments control ante-posterior movement and the collateral ligaments restrict varus-valgus stability. To smoothly mate the convex femoral condyle to the flat tibial plateau the wedge-shaped menisci crescents the edge of the joint. Joint stability during movement is an interplay of the shape of bone and meniscal shape coupled to the tension from muscle and ligament. The contact surfaces of the bones – the articular surfaces – are clad with articular cartilage protecting the bone from erosion and distributing loads. The lubrication provided by synovial fluid and cartilage surface properties prevent the tissues from abrasion and frictional heating by practically eliminating friction. The joint is encapsulated by a thick elastic capsule protecting the joint, internally lined by the synovial membrane with the important function of producing synovial fluid to lubricate joint movement and provide nutrients to cartilage.

The knee joint function is a fine-tuned concert of all its integral parts, conducted by proprioception and neuromuscular interplay to produce a complex rolling-sliding-rotating movement with elastic give.

Articular Cartilage

Cartilage

History

Hippocrates (460–370 BC) and Galen (AD 129–199) documented the importance of cartilage and synovial fluid to prevent erosion of bone and facilitate easy lubricated motion. In 1743, William Hunter stated that '*an ulcerated Cartilage is universally allowed to be a very troublesome disease; that it admits of a cure with more difficulty than a carious bone; and that, when destroyed, it is never recovered*' (Hunter 1743). Detailed investigation of cartilage was pioneered in the 1920s by Benninghoff using polarized light microscopy to describe the radial and arcing superstructure of collagen (Benninghoff 1925).

Cartilage overview

A 2–4 mm thick layer of hyaline Articular Cartilage (AC) covers the articulating bony ends in diarthrodial joints. It provides essential biomechanical properties such as wear resistance, load bearing, superlubrication and shock absorption. AC is a highly specialized tissue of organized extracellular matrix (ECM) and low cell density. The viscoelastic capacities of ECM are dependent on the combined properties of a gel-like bulk and a rigid three-dimensional network. The main component of the gel-like matrix substance are proteoglycans that attract and hold water. Type II collagen fibres form a strong tensile structured network that counteracts expansion from the hydrated proteoglycans to create a swelling pressure resulting in ACs characteristic load-absorbing viscoelastic ability to resist compression (Mow 1992; Buckwalter 2005).

AC is avascular, aneural, with no lymphatic vessels and the only cell type found in cartilage, the chondrocyte, obtains nutrition mainly via passive diffusion over the synovial fluid from synovial capillaries. The functional properties are preserved by the chondrocytes which maintain cartilage homeostasis by inducing proteolysis and production of ECM components as a reaction to biomechanical and biochemical stimuli. In normal articular knee cartilage wet weight consist of 1–3% chondrocytes, 5% proteoglycans, 20% collagen and 70% water (Mow 1992; Buckwalter 2005).

Composition and mechanical properties

Collagen

The major structural element of the cartilage ECM is type II collagen, accounting for 75% of dry weight. There are at least 15 distinct collagen types, all of which

contain a region consisting of 3 polypeptide chains (α-chains) wound into a triple helix with stability enforced by covalent bonds along the length of the molecule. Type II collagen chains are organized regularly together in parallel to form fibrils that in turn add parallel to form bundles, by larger diameter stiffness and resistance to degradation increase. Type II collagen is extraordinarily stable and has been found preserved in 75 million year old fossils (Bertazzo 2015), and it is practically stable in human living tissue with a half-life of over 100 years (Heinemeier 2016; Maroudas 1992). The inherent molecular stability of the bundled collagen triple helix is further protected from enzymatic activity by a dense coating of aggrecans and regulatory proteins (Heathfield 2004). Irreversible degradation via enzymatic cleavage is by specific metalloproteinases, of which metalloproteinase 13 (MMP13) is the most important (Heinegard 2011).

GAGs, Proteoglycans, Hyaluronan

The gel-like bulk of cartilage matrix consists of proteoglycans composed of a core linear protein to which are attached carbohydrate polymer chains of glycosaminoglycans (GAG). Proteoglycans are organized in very large molecular aggregates that are immobile in the collagen framework. Central to the aggregate is a very long hyaluronic acid (HA or hyaluronan) chain of up to 25,000 repeats of the disaccharide unit providing a backbone to branches of proteoglycans linked to it by linking proteins (Hascall 1974). The size of the HA-aggrecan complex is added to by connections to other matrix macromolecules, so that it becomes an enormous aggregate entrapped within the collagen network. HA is present in all connective tissues. Apart from the backbone function in matrix it acts as the principal hydrant and lubricant to the movement of joint surfaces and tissues (Mow 1992; Heinegård 2009).

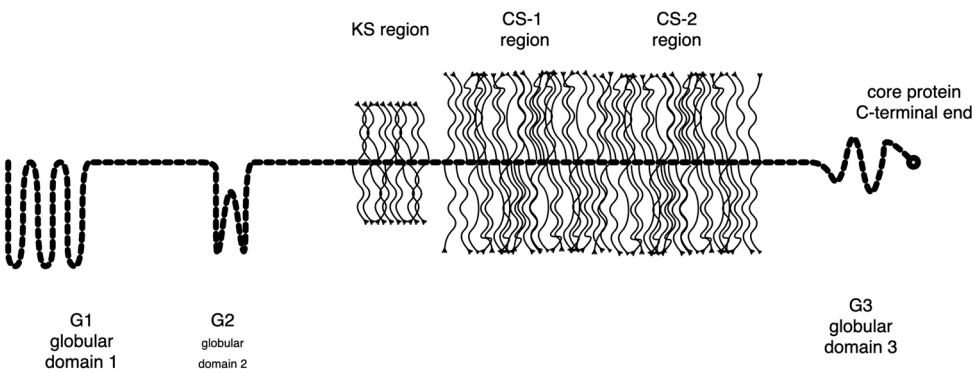


Figure 1. Aggrecan macromolecule.
The core protein has three globular domains involved in binding and interactions. Linear domains have linked glycosaminoglycans chains of which chondriotin sulfate is the most ambundant. The CS chains carry the fixed charge density, pivotal for cartilage function.

Aggrecan is the most abundant of the matrix proteoglycans, a large macromolecule of 2500 kDa exhibiting a bottlebrush structure consisting of an extended linear core protein to which GAG sidechains are attached. Near the N terminal base two globular regions G1 and G2 followed by a long linear region to be capped by a globular region (G3) at the C terminal end. To the linear region GAG sidechains are attached, each with a length of ~100 repeats of the disaccharide units, ordered in the C direction by a shorter region of keratan sulfate sidechains (KS) followed by two longer regions of chondroitin sulfate sidechains (CS-1 and CS-2). Each aggrecan contains in the order of 10,000 negative charges consisting of carboxyl and sulfate groups on the GAG sidechains. The aggrecan is immobile in the dense cartilage matrix and the strong electronegativity of the GAG sidechains imparts a high fixed charge density (FCD). Cations, mainly Na+, are attracted to the FCD and vast amounts of water molecules are attracted by osmosis (Gibbs-Donnan effect). Associated water can amount of 50 times the weight of the aggrecan molecules (Dudhia 2005; Heinegård 2009).

The chondrocyte and the chondron unit

The chondrocyte is the only cell type in the AC and is responsible for synthesizing components and enzymes essential for the metabolism and homeostasis of the ECM. Chondrocytes are unable to migrate and are encapsulated in a basket-like collagen VI structure oriented perpendicular to the surface, forming a functional unit – the chondron. It responds to stimuli from mechanical loading or damage (stresses) transduced via the chondron, ECM connections, receptors and by interstitial water flow (Mow 1999).

Cartilage ECM spatial organization. Zone and region.

The articular cartilage is 2–4 mm thick and is organized into distinct depth dependent zones; the superficial Zone (SZ), the Middle or transitional Zone (MZ) and the Deep Zone (DZ) to which can be added the calcified zone (CZ) in the deep boundary to bone. Beneath is the subchondral bone plate supporting the functional cartilage unit. Furthermore, ECM is described in relation to the chondrocyte with the pericellular region, the territorial region (chondron) and the inter-territorial region (Buckwalter 2005).

The thin SZ is exposed to joint space and is in contact with the meniscus or the opposing cartilage surface. It represents 10–20% of cartilage thickness and protects deeper layers from shear stresses. The collagen fibres of SZ (primarily type II and IX collagen) are packed tightly and aligned parallel to the articular surface. The superficial layer contains a relatively high number of flattened chondrocytes that produce lubricin and other lubricating components. The integrity of the SZ is imperative in the protection and maintenance of deeper layers. It exhibits strong

tensile properties which enable it to resist the sheering, tensile, and compressive forces imposed by articulation. The GAG content is lower in the SZ.

The MZ is the middle 40–60% of AC thickness. Chondrocytes are rounder and the cell density is low. Type II collagen is obliquely oriented, and fibrils become thicker with increased depth. The concentration of GAGs is at its highest; functionally the middle zone is the first line of resistance to compressive forces (Venn 1977). Transitions to SZ and DZ are gradual.

The DZ consists of 30–40% of thickness and has low cell density. Characteristically cells and collagen are arranged in a perpendicular direction relative to the articular surface. The DZ provides the greatest resistance to compressive forces with the highest compressive modulus due to high GAG concentration and the thickest type II collagen fibril bundles. Chondrocytes are oriented in columnar chondrons along type II collagen bundles of large diameter. At the bottom of DZ is the tidemark, a distinct delineation to the deeper calcified zone, anchoring large diameter collagen fibrils to the underlying subchondral bone.

In the MZ and DZ the ECM is spatially organized relative to the chondrocytes. The pericellular region is closest to the cell and consist of a glycan-rich coat. The territorial region has a network of type VI collagen fibrils forming a cage surrounding chondrocytes or groups of columnar oriented chondrocytes to form the chondron unit (Poole 1987). The interterritorial regions is the greatest proportion of the intercellular matrix compartment and is characterized by bulk proteoglycan and of type II collagen fibril diameter increasing with distance from chondron (Buckwalter 2005; Heinegård 2009)

Type II collagen organization, The Collagen Cathedral

The three-dimensional spatial organization of type II collagen creates the properties of the cartilage zones. Due to the regularity of the fibril orientation it was possible to use polarized light microscope already in the 1920s (Benninghoff 1925) to describe an arc-like structure of collagen reminiscent of the arcs of gothic cathedrals. The conceptual Benninghoff-arcade has remained schematically, but modern understanding is more complex and less ordered. In the adult articular cartilage thick bundles of type II collagen fibrils are rooted to the subchondral bone and ascend as perpendicular columns through DZ. When entering MZ they gradually thin and increasingly deviate to in SZ become parallel to articular surface. In addition to the orderly thick columns and arcs, thin collagen filaments crisscross the ECM in an anisotropic orientation (Eyre 2006; Hunziker 1997).

Water

Water is the most abundant component of articular cartilage, contributing up to 80% of its wet weight. Of this water 70% is associated to GAG of the ECM (interfibrillar

or pore space) and approximately 30% is associated to collagen within the collagen structure (intrafibrillar). A small percentage is contained in the intracellular space. The relative water concentration decreases from about 80% in SZ to 65% in DZ. Interfibrillar water creates a moveable interstitial water phase. The flow of interstitial water through the cartilage and across the articular surface helps to transport and distribute nutrients to chondrocytes. The negative charge and tight porosity of the gel-like matrix result in high frictional resistance to water flow through the matrix, resulting in low permeability (Maroudas 1991).

Load absorption

When load is applied, elastic deformation of the matrix is followed by cartilage 'creep' as proteoglycan-bound water moves into uncompressed regions of the matrix. When load is reduced, osmotic swelling pressures exceed applied load and the proteoglycans retract water to achieve a new equilibrium between the load applied and the swelling potential of matrix proteoglycans (Ratcliffe 1996). It is the combination of the frictional resistance to water flow and the pressurization of water within the matrix that forms the basic mechanism by which articular cartilage derives its ability to withstand significant loads, multiple times one's body weight (Mow 1992; Buckwalter 2005).

Friction and superlubricity

An amazing feature of healthy cartilage surface is its minimal friction in joint movement. Subjecting the surfaces to several hours of continuous grinding produce virtually no frictional warming – permitting running marathons and even worse abuse.

For two contacting surfaces to move relative to each other friction must be overcome. The energy of friction transfers to heat and erosion on the surfaces. During bending of the knee the joint surfaces slide against each other, the lubricated coefficient of friction of healthy joints is in the range of 0.003–0.01 (Charnley 1960; Krishnan 2004; Mow 1997) which is lower than for any other materials (Guilak 2005) and achieves the property of superlubricity where friction virtually vanish. The most important lubricants in SF are the components lubricin and HA.

Life cycle; foetal development, homeostasis, age-related change

AC is formed during foetal development and exhibits growth and remodelling during adolescence with distinct properties from the AC of adults. From age ~20 onwards the cell density of AC is maintained and exhibits no mitotic activity. The structure, density, and synthetic activity of an adult chondrocyte vary according to its zonal position. In reaching skeletal maturity AC exhibit increased diameter type II collagen bundles and the characteristic highly ordered columnar organization of

the DZ. Remodelling of type II collagen occurs in growth and adolescence but not in adults (Bank 1998). Collagen types associated with adolescent cartilage (type IX, XI) decrease in content and 'adult' collagens (type III) increase and bind more irreversibly to type II collagen structure. Collagen type VI creates local pericellular network and less well-defined microfilaments.

Healthy AC respond to physiological stresses to control homeostasis. Signalling molecules and feedback mechanisms including cellular and chondron mechanoreceptors connections to ECM filaments, fluid gradients, electrostatic change (Mow 1999) trigger chondrocyte response by increased synthesis of matrix components, enzymes and regulatory molecules. There is evidence from animal experiments that loaded joint motion is necessary for normal cartilage PG content (Palmoski 1979) and that increased exercise in the form of running, cast immobilization and remobilization induce dynamic adaptive changes in PG contents (Kiviranta 1994; Kiviranta 1988; Sivan 2014; Kiviranta 1992; Säämänen 1990; Jurvelin 1988) whereas type II collagen is static in adult (Bank 1998).

Homeostasis turnover rates of collagen are very slow, with interterritorial type II collagen (striated bundles) having 100–400 years' turnover (Maroudas 1992; Heinemeier 2016; Libby 1964; Verzijl 2000). In contrast GAG has a relatively fast turnover and is continuously synthetized with a 3-year turnover rate in distal aggrecan regions and with 24 years for the N-terminal region (Maroudas 1992; Shapiro 1991; Sivan 2014).

Ageing cartilage

With ageing, cartilage accumulates damage from oxidative stress, increase glycosylation and altered GAG configurations of shorter and fewer chains on aggrecans of decreased CS content and rate of truncated aggrecan fragments increase (Bayliss 1999). The accumulating glycosylation of collagen fibres reduce tensile strength and promote agglutinations to make cartilage brittle, it is also the reason for the curious change in colour to yellowish from slightly blue (Bank 1998; Verzijl 2000). Crosslinking of collagen bundles increase with age by increased content of type III collagen fibrils (Wu 2010).

Ageing chondrocytes show elevated oxidative stress and altered mitochondrial function that promotes cell ageing, senescence and OA. Several cell signalling pathways are altered, e.g. altered expression of the TGF-β receptors act in downregulating anabolism (matrix synthesis) and upregulating catabolism (metalloproteinases). Some chondrocytes show signs of senescence with characteristic changes of DNA methylation and low metabolic activity resulting in a decreased ability to replenish GAG and maintain cartilage structure (Loeser 2011).

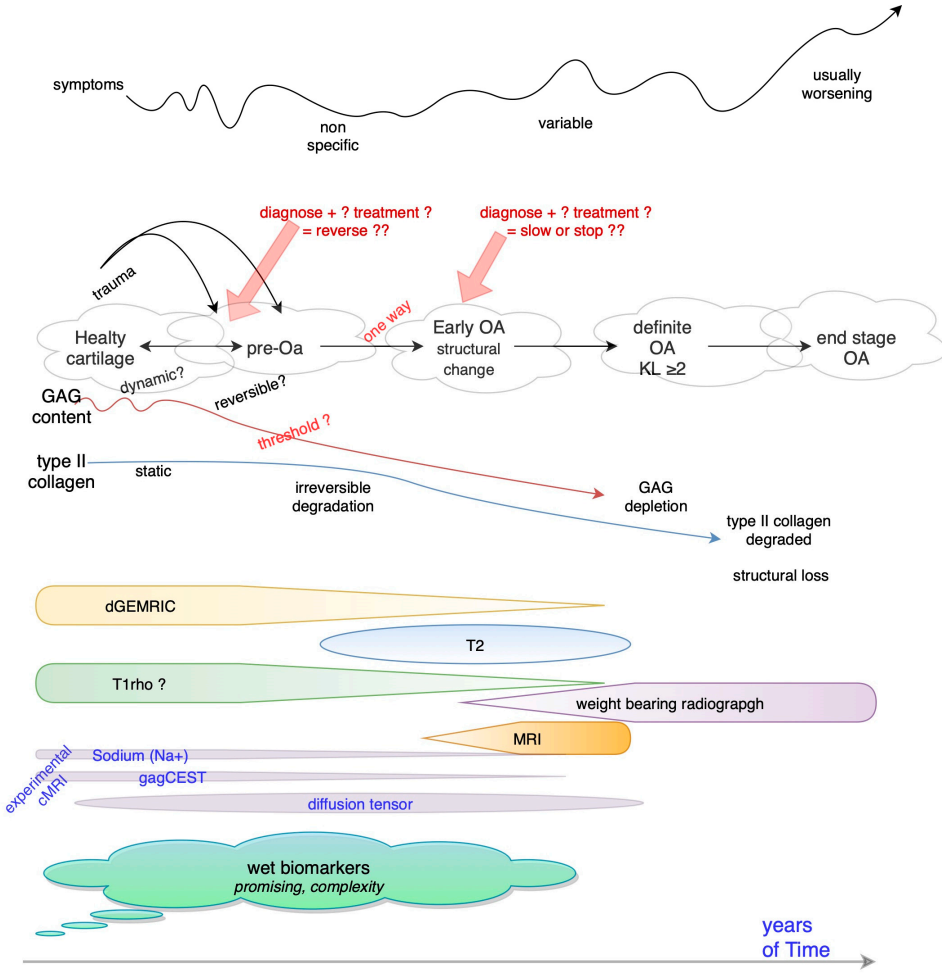


Figure II. Conceptual illustration to schematically describe the degenertive process over time and to spatially arrange classical and new investigation methods. Intended for reflection.
Time moves left to right. The top curve is of OA symptoms which typically varies. GAG content decrease early in the degenerative process. Type II collagen decrease in order and content with further progression to structural irreversible change. dGEMRIC has window of detection open to estimate GAG in healthy and pre-OA cartilage, with diminishing reliability of use in cartilage with advanced structural change and cartilage loss.

Pre-OA

The finetuned homeostasis of healthy cartilage can be altered to lose its balance between breakdown and regenerative processes and to enter a vicious circle of catabolic breakdown, resulting in cartilage degeneration. Identification of the perturbator of balance and understanding of the very early stage of alterations is incomplete. In clinical and epidemiological settings early-OA cartilage pathology is defined by macroscopically detectable early changes on arthroscopy or MRI in the

absence of definite radiological OA features (Luyten 2011). However, this definition requires structural matrix changes to have already occurred. Prior to the structural changes of early-OA a state of altered cartilage metabolism and compositional change of ECM would be the first step, for which the designation pre-OA is suggested (Ryd 2015). Severity of pre-OA change can be hypothesised to influence resilience to trauma and overload, and to be determinantal to the cartilage's response and outcome to such stresses.

Importantly this definition of pre-OA and early-OA pathology are focused on the cartilage degenerative aspect of OA, the same terms have different definitions in discussion of the symptomatic aspects of OA.

pre-OA alterations

It is not known precisely what events start cartilage change and different pathways are conceivable. In some cases, a traumatic or overuse event results in damage and reparative/regenerative processes are initiated but become overwhelmed by inflammation and breakdown for a net catabolic process. In others cases a subtle shift in homeostasis to a catabolic balance is supposed to occur. Inflammatory signal is a key factor and fragments from broken down matrix molecules activate complement and other inflammatory pathways. Altered turnover of aggrecans is an important early event of the development (Pratta 2003). Activation of aggrecanases result in GAG loss in the form of shortened GAG chains in the CS-1 and CS-2 regions and protease cleaving of the base of the aggrecan core protein at G1-G2 (Sandy 2001; Lohmander 1993). Chondrocytes responds to interterritorial GAG degradation/release with increased synthesis of aggrecans, that accumulate in the pericellular and territorial regions reimbursing total cartilage GAG content while distribution becomes altered (Arokoski 1996; Rizkalla 1992; Maroudas 1980; Mankin 1970; Hollander 1995; McDevitt 1973).

In the local environment of interterritorial organized matrix, the collagen fibrils are surrounded by linked aggrecans that form a protective coat of abundant GAG, creating a very high FCD effectively blocking diffusion of larger proteins. Furthermore, fibromodulin and other associated proteins regulate access to collagen cleavage sites (Heathfield 2004). The pruning of GAG chains and cleaving-off of the aggrecan peptide by aggrecanases (Heinegård 2009; Fosang 2008) is believed to open up access to the type II collagen bundles for enzymatic activity (Pratta 2003), enabling beneficial responses such as collagen III crosslinking and GAG replenishment to access the target locations while on the contrary also causing exposure to collagenases (MMPs) and degradation of collagen-associated glycoproteins (Heinegård 2009; Heathfield 2004). A multitude of matrix components and enzymes are active in such processes, and produced metabolites and fragments of breakdown are involved in signalling cascades, i.e. fragments from GAG breakdown (aggrecan G3 domains) bind to complement factors and interact

in inflammatory response (Heinegard 2011). Aggrecanase synthesis are upregulated by cytokines. Proteolytic cleavage degrades and remove important matrix proteins involved in protecting and maintaining type II collagen; e.g. type IX collagen, fibromodulin, decorin etc. Decreased interterritorial concentration of the crosslinking and regulatory oligomeric matrix protein (COMP) is an early feature, with deposits in pericellular space illustrating overshooting synthesis (Heinegard 2011). Decreased interterritorial FCD due to GAG loss results in reduced compressive stiffness which increases water flow and mechanical stress on type II collagen (Mow 1999; Jurvelin 1988). The matrix metabolism enters a vicious circle, in which the quality of the matrix is downgraded by proteolysis and removal of matrix components. At the same time the tissue develops an increased sensitivity to load, and the fragments of molecules released might induce and/or worsen inflammation, thereby enhancing the catabolic events in the cartilage (Heinegard 2011).

These intricate and interlocking processes are challenging to monitor in detail. Extensive scientific work for decades has identified a large number of clockworks, but a holistic-dynamic understanding remains elusive. Multiple specific fragments resulting from enzymatic degradation of matrix components have been characterized. In addition to being monitored for study of matrix in molecular and in-vitro cartilage research, the specific molecules can be studied in human fluid samples as potential biomarkers of metabolism.

Pre-OA turns into early OA, progress to OA.

At some point the minute pre-OA changes escalate further and accumulating changes over several years, matrix gradually becomes irreversibly damaged as type II collagen breaks down. Macroscopically detectable changes of thinning, softness and fibrillation ensue and transition the situation into early-OA stage. Cartilage becomes brittle and mechanical stresses aggravate delamination and can cause partial or full thickness defects that extend to denuded bony defects. Further gradual transition is defined as OA when weight-bearing radiographs show 50% cartilage loss, a state that corresponds to severe degenerative changes and is regarded as irreversible. Reduced viscoelastic dampening, increased contact stresses and mechanical micro-incongruency together with elicited inflammatory signals, induce bony change with osteophyte growth, subchondral sclerosis, bone-cyst and metaphyseal bone shape change with widening and flattening of the joint.

Osteoarthritis

Osteoarthritis (OA) is the most common chronic condition of the joints (Jordan 2014). It is also one of the most common reasons for disability globally and its overall prevalence is increasing (Cross 2014; Nguyen 2011) doubling in the last deacdes (Wallace 2017). OA can affect any joint but the knee is the most affected accounting for more than 80% of OA total disease burden (Vos et al. 2012). OA affects at least 19% of adults aged 45 y and older (Lawrence et al. 2008).

Knee OA is the clinical manifestation of a cartilage dysfunction causing its gradual loss from the joint surfaces, resulting in pain and functional loss.

What is OA to the patient? Symptoms

Individuals afflicted with OA typically have variable symptoms including knee pain, morning stiffness, impaired function with reduced mobility and restricted range of motion, crepitus and swelling. It is common to have symptoms without detectable cartilage change but it is also common in individuals with no symptoms to have structural changes that can be detected by MRI or arthroscopy. Even definite radiographic features of OA can be free of symptoms. To diagnose a patient of having OA disease the combination of symptoms and pathology needs to be present.

Knee OA can cause partial or complete inability to work, difficulties with activities of daily living, and impaired quality of life, leading to very significant social-economic costs. One third of older population seek consultation to primary care in a 7-year period for OA symptoms (Jordan 2014).

OA pathology is not limited to the cartilage. It affects all the tissues of the joint, including synovium, meniscus, subchondral bone, capsule, ligaments, tendon, musculature and nerves.

Incidence and prevalence

Definition of OA is an elusive subject. While end stage disease is no problem to diagnose, there is no single point or test to decide when a line is crossed from minor complaints to manifest disease (Felson 2004). Symptoms typically vary over time and affect individuals differently, and thus a clear definition of what constitutes OA symptoms is not fixed. Radiographic changes follow a continuous progression but the detailed definition of division between unaffected joint and very early ROA change is problematic, as is the transition from early to definitive ROA. Different criteria for both SOA and ROA are used in publications but in broad terms criteria are agreed upon (Pereira 2011; Lane 2011).

OA is a slowly progressive disease with discordant symptoms and mild radiological presentation in the early stage. This results in a low prevalence in the younger age group, with a linear increase into being common in old age.

The incidence of developing OA is age dependent with overall incidence of 0.02%, in women of 30-39 years of age, 0.1% in 40-49 years old, 0.27% in 50-59, 0.65% in 60-69 and peaks in 70-89 at 1.1%. For men the incidence in age brackets are 0.04% in 30-39, 0.12% in 40-49, 0.25% in 50-59, 0.49% in 60-69 and peaks in 70-80 at 0.84% with 0.62% in 80-90 (Oliveria 1995). Another expression of incidence is the lifetime risk, for men the estimated lifetime risk of developing symptomatic OA is 40%, for women 47% and for obese (>30 BMI) the risk increase to 60% (Murphy 2008).

Many prevalence studies select patients by questioning for OA symptoms and performing radiographs of positives, which severely underestimates cross-sectional ROA numbers by not diagnosing symptom-free ROA. In younger patients SOA+ROA prevalence is reported at 1.5% in Swedish males 35-55 years (Petersson 1997). Similar rates were found in a study of rural Greeks with a prevalence in the 19–44 age bracket of 0.5% in males and 0.3% in females, increasing in the ages 45–49 to 1.2% and 2.4% respectively (Andrianakos 2006). No strict cross-sectional prevalence population study of ROA exists for ages under 45 years, but prevalence is inferred to be very low in the 30–40 age bracket. Cross sectional prevalence of ROA is reported as 10% at 45-50 years of age and 15 to 30% in the 50-65 age range (van Saase 1989; Turkiewicz 2015; Jordan 2007). In advanced age ROA prevalence increases more in women, reaching 30-60% in ages 60-80 years and 30-40% for men (Dillon 2006; Felson 1987). A large global meta-study found prevalence of OA (ROA and SOA present) in Europe and North America (best data quality) to be very uncommon under age 30, with the curve steeply rising from 35 years of age to reach 9–11% in 55 year old males and 15–17% in females, levelling out at after age 60 to peak at age 75 at 11–12% in males and 17–19% in females (Cross 2014).

Discordance

There is a considerable discordance between clinical and radiographic findings regarding knee OA. Firstly, up to 50% of subjects in the general population with radiographic knee osteoarthritis do not have pain. Secondly, 30–50% of subjects who complain of knee pain, and who are at or above the age when osteoarthritis starts to become common (about 55 years), have no definite radiographic evidence of osteoarthritis (Hannan 2000; Bedson 2008). However in individuals with symptoms a consistent relationship between increasing severity and persistence of pain or disability and the prevalence of ROA is found, with a threshold effect in the prevalence of ROA at the point when pain became persistent and of high intensity (Duncan 2007). MRI of asymptomatic knees is reported to have OA features in over 40% in subjects over 40 years of age (Culvenor 2018).

Cause of OA, risk factors

Philosophically all individual OA cases may have some causation in their history, but OA is multifactorial and it is more useful to describe risk factors. When a patient presents with early OA some prognostic guidance can be estimated from assessing present risk factors and correctable risk factors can be addressed to decrease the risk of progression. The nature of risk factors aids our understanding of the disease process.

Age

Age is often stated as the strongest risk factor for developing OA as incidence and prevalence increase with age (Felson 2000), but age in itself is not the risk, rather age increase the cumulative exposure to various risk factors, accumulated mechanical stress and biologic changes that occur with aging.

Ageing cartilage show changes in the ECM, accumulated glycation end-product, reduced aggrecan size, reduced hydration and increased collagen cleavage that alter the mechanical properties of cartilage and make it more susceptible to degeneration and wear (Loeser 2016; Lotz 2012). Age-dependent changes exterior to the cartilage tissue affect OA development. Age-related sarcopenia and decrease of stereognosis control of muscle due to less responsive neuromuscular action and micro-incoordination combine to reduced proprioception (Papalia 2014). BMI increases with age by about 0.2 kg/m2/year in Swedish and Norwegian cohorts (Caman 2013; Jacobsen 2015).

Genetics

Genetic factors have been found to explain 40–65% of the variance of OA development in twins (Spector 1996). The majority of hereditary attribution is interpreted to be in the total phenotype of interplaying cartilage matrix components and the biomechanics influenced by heritable morphology of knee shape and body constitution. Specific rare genetic mutations and polymorphisms are described for collagens, aggrecans and regulatory components of ECM (Kannu 2009). Errors in post-translational and extracellular assembly are typical mechanisms. Various forms of dwarfisms, malformations and dysfunction are among manifestations, many mutations are incompatible to life. Syndromes such as Ehlers-Danlos and Marfans are associated with increased OA risk due to alterations of collagen structure or pathways, as are rare genetic defects such as in the metabolic deposit disorder alkaptonuria/ochronosis (Gaines Jr 1989; Virchow 1866). Ethnicity-dependent variation in prevalence is hard to isolate, and most population studies are conducted in European or North American populations. Prevalence in 2010 was highest in the Asia Pacific high-income region, followed by Oceania and North Africa/Middle East and lowest in South and Southeast Asia (Cross 2014)

Alignment

In the normal anatomical configuration, the load line over the knee joint in standing extension is through the centre of the knee in perpendicular alignment with the centre of the hip joint and the centre of the ankle joint. Moderate deviation in the medial or lateral position of the load line, varus and valgus deformity, increases stress on the respective compartment. Whereas malalignment does not cause degenerative change to occur, malalignment increases the risk of and rate of progression to OA when degenerative changes has started. The progressive loss of cartilage thickness means that the deformity becomes more pronounced and stresses increase, aggravating further degenerative change (Williams 2005; Sharma 2001).

Sex

Primary knee OA is more common in women overall and increasingly more common over 50 years of age, indicating a post-menopausal connection. In younger age groups knee OA is more common in men due to post-traumatic OA and work related risk historically being more common in men (Dillon 2006; Felson 1987).

Fitness

There is an intricate interplay of the muscular functions of the lower limb and OA. Quadriceps weakness, reduced neuromuscular control, altered gait and resulting micro instability are suggested to increase cartilage stress. Sarcopenia and quadriceps strength are predictors of later development of OA (Slemenda 1997; Thorstensson 2004; Oiestad 2015). Muscular strength correlates to cartilage quality estimated by dGEMRIC (Ericsson 2009). In patients with OA neuromuscular training have beneficial effect on pain and function, however it has not been proven that neuromuscular training decrease ROA progression (Oiestad 2015) as it seems to not alter loading patterns related to structural change.

Obesity

Obesity and overweight act as dose-dependent risk factors for developing OA, on the order of 35% risk increase for 5 extra units of BMI (Jiang 2012). Excessive adipose tissue is associated with increased cartilage loss and increase in inflammation dependent on adipokine cytokines is described (Neumann 2016). The additional load acts to accelerate cartilage breakdown once OA process has started(Anandacoomarasamy 2008). Increased weight of only a few kilograms significantly increases the risk of developing OA in studied twins (Cicuttini 1996). Weight loss has been shown to ease pain, to improve function and to decrease low-grade inflammation (Richette 2011); 5 kg weight reduction decreased the risk of developing knee OA by 50% (Christensen 2007). Unfortunately, population trends over recent decades are of average BMI increase in the population, doubling the prevalence of obesity over the last decades (Murray 2017).

Exposure, occupation, trauma

Knee OA is more common in patients with a history of heavy work (Lindberg 1987; Andersson 1988), and occupational squatting, kneeling and climbing stairs have been linked to higher OA frequency (Cooper 1994). In archaeological material femuro-tibial OA is uncommon in medieval times, suggesting causation to be related to modern lifestyle (Rogers 1994; Wallace 2017). Effects of sports are conflicting, with both salutary factors such as lower BMI and better muscular control set against exposure to risk factors of overuse and trauma (Hunter 2009). Runners have been shown to have lower risk (Conaghan 2002) of OA while soccer and other high-impact sports have increased risk (Roos 1994). Former elite athletes have increased risk of knee and hip OA regardless of high or low impact sports (Tveit 2012).

Salutary factors.

The existence of risk factors that can be corrected means that their opposite condition is a potential benefactor. Salutary factors for knee OA are normal BMI, with well-trained and conditioned muscles to counteracts risk (Slemenda 1997). Exercise and sport, if one is spared of knee trauma, seem to have a counteracting effect on OA (Hunter 2009), with possible exception of the top elite athletes (Tveit 2012).

Prediction of OA risk

While risk factors can be demonstrated on the group level, reliable individual predictors of OA are lacking for predicting both onset of OA structural change and progression of pathology severity as for the onset and progress of symptoms. Research are focusing on developing biomarkers to this effect.

Treatment of OA

Currently no treatment can block the progress of OA structural change and no surgery can reliably produce restoration of hyaline cartilage. Mitigation of symptoms by weight optimization (Messier 2011) and training of knee function and strength is recommended, and are important interventions for all patients with OA symptoms, but lack evidence of affecting OA progression (Hochberg 2012; Oiestad 2015). Pain- and NSAID-medication can help reduce symptoms and is sometimes needed to make training possible. No disease-modifying OA drugs (DMOAD) exist yet (Karsdal 2016). Arthroscopic debridement and meniscus resection have short if any effect on symptoms (Moseley 2002; Sihvonen 2013) and risk to possibly increasing the rate at which OA progresess (Englund 2012).

For knees with isolated mild OA of the medial compartment, select patients can benefit from osteotomy of the proximal tibia to shift the mechanical load to the unaffected lateral compartment (Tjörnstrand 1981). While OA may still continue to develop, a majority of younger patients can gain on the order of 10 or more years delay to TKA (Odenbring 1989). 163 HTO operations were performed in Sweden with a mean age of 51 years (SKAR 2019).

In end stage OA with debilitating symptoms and severe radiographic change, the substitution of articulating surfaces by joint replacement is an option. In select cases of OA isolated to one compartment, uni-compartemental (typically medial) knee arthroplasty (UKA) can be considered; 1,373 UKA operations were performed in Sweden in 2018. In general, total knee arthroplasty (TKA) has good clinical outcome and is considered a low-risk operation. For the few individuals with complications the outcome can be quite severe, with reoperations and functional loss. A total of 13,885 TKA operations were performed in Sweden in 2017 for the highest incidence in the 65–84-year brackets of 0.5% in men and 0.6%in women. The prevalence rises with age to peak at 80–90 years of age with 8% in men and 9.5% in women (SKAR 2019). The average age for TKA operation is 69 years and the implant endure for the remaining life of patient in >90% of cases for that age. Younger patients (<55 years) run a high lifetime risk of experiencing a failing implant and needing a reoperation. Due to higher activity level the implant is exposed to stronger impacts and stresses. Cumulative longer remaining lifetime furthermore compounds the risk of implant failure. Additionally, restrictions regarding sports and high demand use are also advised, why TKA is not an optimal but sometimes necessary treatment in younger patients. A 2016 OARSI review noted that; joint replacement does not equate with remission or reversal of disability, but rather a lessening of disease severity in the replaced joint, it does not solve the problem completely. Most people continue to suffer some physical impairment following joint replacement and while there are improvements in pain and physical function, they do not reach the comparable level of their population peers. As many as 20–30% continue to experience pain and disability after total joint replacements and one in five require joint replacement in another joint within two years (March 2016; Gwilym 2008).

Knee injury – Post-traumatic OA (PTOA)

Joint injury is a well-established risk factor for development of OA (Gelber 2000); approximately 12% of all OA cases may be due to prior joint trauma (Brown 2006). Average age for knee replacement is 50 years for ACL injured patients compared to 67 years in non-injured (Brophy 2014). Meniscus, ACL and cartilage injuries have 50% or more risk of OA in 10–15 years (Lohmander 2007).

Increased risk of OA development after knee injuries has been scientifically described for over half a century and has been the target of extensive research efforts, yet no treatments that prevent PTOA has been put into practice (Øiestad 2018). Development is hampered by the heterogenous panorama of patients and trauma, and the long timespan from injury to outcome. Long-term RCTs of specific treatment vs no treatment are needed to allow time for definitive outcomes to develop but are exceedingly difficult to maintain. Reliable biomarkers for OA progression would allow shorter term study and thus facilitate PTOA research.

In the long term, cartilage in injured joints suffers increased stresses from suboptimal rehabilitation and structural disruptions/instability. The increased stresses in combination with inflammation signalling and catabolic cartilage processes can turn into a vicious circle of degeneration (Lotz 2010).

Injured and impacted cartilage

Knee trauma such as ACL injury or acute meniscus tear typically involves a chondral compression injury. Structural cartilage damage such as cracks, delamination and cartilage defects can result. The pre-injury quality of cartilage likely influence susceptibility to, and severity of, damage.

PTOA has historically been regarded as due to destroyed tissues with structural disruptions and mechanical instability, injuries that the orthopaedic surgeon has seen as opportunity to practise the art of mending at. Increasingly it has been appreciated that active inflammatory, catabolic and homeostatic processes are activated in cartilage matrix by the trauma force and contribute to OA development by altering cartilage metabolism (Lotz 2010; Lieberthal 2015; Larsson 2017). The eventual development of PTOA may be seen as caused by a combination of joint metabolic/katabolic processes initiated by the acute trauma, and later microinjuries caused by changes in loading patterns of the injured joint, leading to prolonged chondrocyte stress responses and ultimately mechanical attrition. If PTOA is partially a result of altered metabolism, new medical drugs could potentially be developed to resist and reverse (Anderson 2011).

ACL injury

Rupture of the anterior cruciate ligament (ACL) is a common and serious knee injury in the young active population. The ACL is a strong ligament crucial to knee stability in pivoting by opposing anterior tibial translation and hyperextension of the knee. It further guides joint position during flexion to extension movement and as a secondary actor it is important for varus/valgus and rotational stability.

The ACL is a strong structure and for it to rupture strong forces are transmitted over the joint surfaces as is indicated by high frequency of subchondral fractures and

bone marrow lesions (BML) evident in acute MRI (Frobell 2008). In animal models and in ex vivo experiments chondrocyte apoptosis and damage to the cartilage collagen structure have been reported and correlated to loading stress (Lewis 2003).

Incidence, prevalence, panorama

The incidence of ACL injury in the general population at risk in Sweden (10–64 years of age) is 0.81/1000 inhabitants annually (Frobell 2007). Exposure to sports increases risk with ACL injury in 18/1000 in Swedish soccer players annually (Roos 1995). Among athletes females are two to eight times more likely to sustain an ACL injury, females in gymnastics and soccer having the highest incidence of ACL injury at 24–33/1000 athletes annually, and a prevalence of 5–10% in elite athletes (Walden 2011; Hootman 2007; Moses 2012; Arendt 1995; Roos 1995).

The prevalence of ruptured ACL in the general population is not well known; 5% prevalence in cross-sectional MRI in a cohort aged 50–90 years has been reported (Englund 2006). Possible degenerative ruptures or injuries caused by insignificant trauma contribute to this high prevalence.

Although ACL injury is associated with contact sports, the majority of trauma (50–70%) is of non-contact (Boden 2000; Griffin 2000; Noyes 1983). Injury resulting in an ACL tear typically occurs with a coupled rotational, deceleration and valgus force (Boden 2000) during sports with pivoting movements such as soccer, basketball, handball and alpine skiing (Järvinen 1994; Myklebust 1998; Arendt 1995). The relative attribution to specific sports varies by national culture; in Sweden 3 of 4 ACL injuries occur during sport, of which 60% are from soccer (Frobell 2007).

The 2–4 times higher rate of ACL injury in female athletes compared to male athletes in the same sports (Prodromos 2007; Walden 2011) is a conundrum extensively studied and associated with (Renstrom 2008): 1) anatomical differences; a thinner ACL, general laxity, narrow femoral notch and more slope of the tibial plateau have all been associated with increased risk; 2) observed hormonal associated differences; 3) dynamic differences; less stiffness in quadriceps, a movement pattern with more valgus-adduction loading and less neuromuscular control. The dynamic differences are a modifiable risk with neuromuscular training. Exercises designed to increase strength, proprioception, movement patterns and neuromuscular control have been successful in decreasing ACL injury, especially in female athletes of up to 80% risk reduction (Walden 2012; Ettlinger 1995; Myklebust 2013; Olsen 2005; Mandelbaum 2005; Sugimoto 2014).

Treatment of ACL injury

After the initial phase of pain and joint swelling after the ACL injury has abated, patients experience instability for rotational valgus adduction movement, described

as a give-way phenomenon. Instability affect to a variable degree in daily life and in the ability to participate in sports or strenuous activity.

Optimal treatment of the ACL injury is dependent on the patients' needs and on the presence of concomitant meniscal injury. Competitive athletes typically need ACL reconstruction (ACLR) to return to sport, although this sets them up for new injuries and increased risk of developing OA. ACLR and rehabilitation typically needs 12 months before the return to competitive sports (van Melick 2016). Fifty-five percent of athletes return to the previous level of sport (Ardern 2011). A treatment algorithm of initial neuromuscular rehabilitation before evaluation of stability and function results in 50% not needing ACLR operation, with no difference in patient-related outcomes or ROA after five years (Frobell 2013). High-quality early rehabilitation and maintaining good form reduce long-term OA risk after ACL injury (Neuman 2008; Oiestad 2018; Neuman 2014; Shelbourne 2017).

Results, OA after ACL injury and ACLR

When ACLR became a feasible arthroscopic treatment, it was believed that reconstruction would prevent OA development. During the time it has taken for modern treatment to evolve it has now become clear that OA is not diminished by ACLR, (Lohmander 2007; Myklebust 2003; Cohen 2007; Salmon 2006; van der Hart 2008) Recent result suggest it may increase OA risk, an RCT found thinner patellar cartilage 5 years after ACLR vs no ACLR (Culvenor 2019).

Due to the heterogeneity of patient groups, treatments and outcome measures, the prevalence varies greatly but an overall prevalence of 50% radiographic OA after 10–20 years is agreed on (Harris 2017; Lohmander 2007). Extreme risk is represented by top athletes that had untreated ACL ruptures with 86–100% ROA after 10 years (Louboutin 2009).

Recent long-term results report 57% ROA 14 year after ACL reconstruction vs 16% in the contralateral non-injured knee (Barenius 2014). Another study reported 68%–80% ROA after 20 years with no difference in non-reconstructed vs ACLR (van Yperen 2018).

A systematic review selected studies of high quality to analyse seven prospective studies of long-term outcome and found the prevalence of ROA 10 years after ACL injury to be 0–13% and 20–43% when in combination with meniscus injury (Oiestad 2009). However, an update by the same authors using the literature period of 2008–2018 failed to reproduce the low prevalence and found ROA prevalence of 1–80% in the best ranked studies (Lie 2019).

The cartilage condition at the time of injury could be reasoned to affect OA risk, as could be inferred from one study of ROA 10 years after ACLR that reported 53.5%

ROA overall, with increased risk for patients who had arthroscopic partial meniscectomy (APM) or chondral lesions before the ACL injury (Janssen 2013).

The knee is a system. Simultaneous injuries of several knee structures imply that higher trauma energies and more damaged structures interplay to increase OA risk. Ligament instability affects loading stresses and increase susceptibility to new injuries, thus contributing to OA development. Restoring ante-posterior knee stability with ACL reconstruction might be insufficient to prevent postoperative cartilage degeneration owing to the lack of restoration of in vivo cartilage contact biomechanics at the tibiofemoral joint (Hosseini 2012).

Recent results suggest rehabilitation of knee function to be important for protecting from PTOA. ACLR patients who returned to pivoting sports had better long-term results with less ROA than non-returners, 18.5% vs 42%, and better clinical outcome, explained by being better rehabilitated and possibly having less severe injury to joint structures (Oiestad 2018). Not achieving full range of motion after ACLR and rehabilitation was a strong predictor of OA outcome. A prospective cohort study of ACLR found the factors APM, extension defect after rehabilitation and age to be predictors of OA development in a 20–33-year follow-up (Shelbourne 2017). Patients of recreational activity level with isolated ACL injury who fulfilled rehabilitation with excellent persistent compliance and who abstained from pivoting sport had 0% ROA and 68% were non-symptomatic after 15 years. If a concomitant meniscus injury was diagnosed the outcome was 16% ROA (Neuman 2008). Further investigation in this group by dGEMRIC showed no difference in cartilage quality compared to younger (25 vs 45 years mean age) activity level matched controls with no ACL injury (Neuman 2014). Keays et al. listed 10 factors as important for OA development (Keays 2010) APM, chondral damage, patellar tendon grafting, age at surgery, time delay between injury and surgery, type and intensity of post-surgery sport, quadriceps strength, hamstring strength, quadriceps-to-hamstring strength ratio, and residual joint laxity. Meniscus injury or APM is a strong predictor of long-term OA outcome (Shelbourne 2017).

Traumatic chondral lesions

Incidence, prevalence, panorama

As a result of focal point stresses and shearing motion, an area of full thickness cartilage can be ripped loose if forces are greater than the tissues can resist, resulting in a traumatic chondral injury (TCL) of bare subchondral bone. The quality of the local cartilage is probably important for susceptibility to injury, as with progression of degenerative processes softening and brittleness lowers the threshold of energy needed to cause injury.

Cartilage lesions seems to be common and is reported in 30–60% of arthroscopies (Shelbourne 2003; Widuchowski 2007; Solheim 2016; Aroen 2004; Curl 1997; Hjelle 2002). Cross-sectional MRI in a 45–60-year-old population with no ROA and no knee pain reported 40–60% rates of cartilage lesions and found knees with a lesion to have slightly lower cartilage volume (Cicuttini 2005). A recent meta study of MRI of asymptomatic knees found cartilage lesions in 11% of patients aged <40 years and in 43% of patients aged >40 years (Culvenor 2018).

Lesions in cartilage with evidence of degeneration (thinning, softening, fibrillation) are more aptly considered as events of the degenerative process that may or may not accelerate OA progression. TCL in cartilage of a healthy appearance with a history of trauma represents a risk of initiating a degenerative process for which a potential treatment would be beneficial. The exact delineation of TCL versus lesions representative of a degeneration is elusive as cartilage quality, energy of injury and efficacy of homeostatic processes are in a continuum.

Treatment

The natural history of TCL is not well known, as controlled studies of no treatment are limited and hard to perform. While the healing potential of cartilage after injury is poor, the rate of progression to OA varies (O'Driscoll 1998; Widuchowski 2011). Risk of progression relates positively to age, BMI, male gender and size of lesion (Wang 2006) overlapping with typical OA risk factors. Hypothetically risk of progression seems related to the extent of change in the surrounding cartilage on the 'degenerative continuum'. The consensus is of poor prospects for cartilage repair in degenerative knees (Brittberg 2016).

The goal for treatment of TCL is twofold: to alleviate symptoms and to avoid a degenerative process taking hold, thus preventing OA as a long-term consequence. If the latter is not achieved the justification for surgical treatment is not absolute as many patients have few symptoms from TCL (Shelbourne 2003) and rehabilitation have good clinical results (Widuchowski 2011). However, as TCL is widely reported to progress to OA (Dillon 2006; Arden 2006; Wang 2006; Løken 2010), surgeons are compelled to attempt repair despite the lack of reproduceable long-term success, even though surgical treatment strategies have over 60 years of documented history.

Surgical treatment of cartilage defects initially consisted of arthrotomy and a 'house-cleaning' of osteophytes and loose cartilage. Hardened subchondral bone was treated by abrasion. In the 1950s the English surgeon K.S. Pridie observed that cartilage defects previously treated by abrasion were covered in soft cartilage tissue at reoperations. He found this process to be facilitated by the drilling of holes in the subchondral bone, letting a blood/marrow clot form in the defect and mature to fibrocartilaginous tissue (Pridie 1959). Insall later reported on Pridie's results,

including histological evidence from samples retrieved at later arthrodesis (Insall 1974) and with the era of arthroscopic knee surgery taking off, the technique of debridement and drilling as a feasible option made the Pridie drilling procedure widespread. In the 1980s J.R. Steadman evolved the technique by introducing a sharp curved awl to make holes to standardized depth with the benefits of avoiding potential thermal injury and due to the curve of the instrument achieving better access in the arthroscopic procedure (Steadman 2001). Further iterations of the technique have included a return to drilling with closer spacing with smaller drill diameter.

Pridie's drilling and Steadman's microfracture are Mesenchymal Stimulation Techniques (MST); through the penetrations of the subchondral bone plate, blood and bone matrix components leak into the volume of the debrided cartilage injury. A clot forms containing pluripotent mesenchymal stem cells and a cell signalling milieu promoting differentiation of fibroblasts into fibrocartilage producing phenotype. The clot matures into fibrocartilage characterized by lacking in zonal organization and low type II collagen content (Erggelet 2016). Functionally it partially substitutes for hyaline cartilage but is less resistant to wear. Many variations on MST exist with little difference to the achieved biological process and clinical outcome. As microfracture is the most frequently used technique currently the literature typically discusses "MFX" even when MST is actually performed by drilling.

A more extensive procedure is the implantation of complete osteochondral grafts (Osteochondral autologous transplant – OAT). When successful this restores the TCL with hyaline cartilage, with the caveat of uncertainty of the fate of the adjacent cartilage interfacing integration to transplanted cartilage. Allogenic grafts imply donor site morbidity. One widespread approach is to use multiple small osteochondral plug grafts to cover the area in a mosaic fashion (Mosaicoplasty – MP), allowing in-between spaces to be covered by cartilage growth presumed to be a mixed form of hyaline and fibrous cartilage.

In 1994 Mats Brittberg et al. described a new approach to achieve better-quality cartilage repair tissue by utilizing differentiated chondrocytes (Brittberg 1994). The technique described, termed Autologous Chondrocyte Implantation (ACI), harvests hyaline cartilage from a less important locale and via in-vitro cell culture clonally expands autologous chondrocytes in order to implant a large number of chondrocytes into the defect at a second-stage procedure. The procedure is demanding in needing external resources and being a technically complex two-stage procedure. Reports show varied results and the costly technique has been subject to extensive debate. The original concept (1st gen) used a periosteal flap sutured to cover the defect and to confine the injected chondrocytes' further development, as a 2nd gen collagen flaps and characterized phenotype chondrocytes were developed (Saris 2009). Further innovations currently explored (3rd gen) are matrix-assisted

chondrocyte transplants (MACT) where various three-dimensional matrix scaffolding material is seeded by chondrocytes and implanted to fill the defect along with experimental use of cartilage growth factors.

Result, OA after cartilage injury and repair

MFX and ACI techniques have many positive reports and appear to provide good clinical outcomes for the medium and long term in reported case studies (Steadman 2003a; Peterson 2010; Vasiliadis 2010). Long term RCTs of surgical treatment vs no treatment are lacking. Good-quality RCTs of MFX vs ACI show no clear difference in medium and long term (Kraeutler 2017).

In a short and mid-term perspective, several studies of MFX reports good results, with a large proportion of patients returning to high levels of activity (Mithoefer 2009; Erggelet 2016; Steadman 2003b). However, 10–15 years after the operation, results deteriorate with 40-48% of patients having radiographic OA (Gudas 2012; Gobbi 2014; Ulstein 2014; Knutsen 2016). Theoretically, the failure might be explained by the fact that repair cartilage after MFX lacks type II collagen and does not show the zonal organization of hyaline cartilage (Mithoefer 2009; Erggelet 2016; Knutsen 2004).

By contrast, the ACI technique was developed to yield repair tissue with a hyaline-like structure that potentially also had mechanical properties that resemble healthy cartilage. A recent review of nine ACI studies (Pareek 2016) with 9–13 years' follow-up reported on average 81% successful results, defined by no diagnosed graft failures and good or excellent clinical results. However, the only study that presented radiographic follow-up (Martinčič 2014) 10 years postoperatively found OA in 45% of the cases, i.e. similar to results reported for MFX.

MFX and 2nd gen ACI showed no difference after 5 years with a high rate of failure in an RCT (Vanlauwe 2011). An RCT of ACI vs. MFX with a 15 year-follow up had 37.5% failures and in the remaining knees 50% had radiographic OA with no difference between the treatment groups (Knutsen 2016). For both ACI and MFX, a high failure rate has been associated with large or multiple lesions, as well as with older age (Pareek 2016; Erggelet 2016). General factors such as age, obesity and activity level that increase OA risk are also risk factors for a negative outcome of cartilage repair. The quality of the cartilage at the time of injury could be reasoned to be a factor of detrimental importance for outcome. Yet this is often not clearly reported, partly because compositional quality assessment is difficult without biopsy. Studies relies on general description of appearance and on arthroscopic scoring (e.g. Outerbridge), and by stratification of age and injury mechanism. If the hypothetical importance of pre-injury cartilage quality is accepted, it follows that studies and clinical decisions have general problems by not stratifying for pre-OA and potential misclassifying of degenerative lesions as TCL. The importance of the

surrounding cartilage quality has also been demonstrated by Solheim in a clinical MFX study (Solheim 2016). Follow-up after 10–14 years demonstrated that knees with visual mild degeneration of surrounding cartilage at the primary arthroscopy had a worse outcome than knees with normal-appearing cartilage. Animal studies have shown superior results for immediate versus delayed surgery related to degeneration of adjacent cartilage (Saris 2003).

Despite some reports of well-documented good long-term outcome (Vasiliadis 2010), the overall view is that MFX and 1st gen ACI are not successful in preventing long-term development of OA. Moreover, it is not proven that treatment has lower OA outcome than no treatment. It remains to be studied whether selected subgroups of patients would have a higher long-term success rate. A multitude of 3rd generation strategies are in continuous technical advance, with some notable reports of well-documented medium-term success (Siebold 2018). Long-term RCTs comparing treatment vs no treatment are needed but are hard to conduct as patients tend to search alternative treatment. Reliable biomarkers would be most useful in this context to evaluate adjacent cartilage and to monitor treatment.

Furthermore, decreased loading of the repair tissue can be achieved with HTO, and alternatives to biological repair treatment are custom metal filler implants designed to fill out the defect in order to reduce mechanical stress in adjacent cartilage.

Meniscus

Panorama, incidence, prevalence

The menisci are wedge-shaped fibrocartilages interposed in a crescent-like configuration between the tibia and femur in both the medial and lateral compartments of the knee joint. Menisci provide joint congruity between the curved articulating surfaces of the femoral condyles and the flat tibial plateau, contributing to load distribution during weight-bearing, and knee joint stability during torsional movements (Messner 1998). The most peripheral 1/3 is vascularized and is relatively rich in fibro-chondrocytes, whereas the inner 2/3 have limited vascularization, few cells and little healing capacity. The fibrocartilage main fibrillar constituent is Type I collagen with type II, III, VI and elastic microfibrillar glycoproteins in small amounts. The matrix of the inner 2/3 is rich in proteoglycans attracting hydration for a meniscus water content of 70%. The type I collagen has a complex organization of strong circumferential fibres to which radially spoking and branching fibres of meniscus body interior are anchored, and an interconnected laminar surface-oriented network of superficial and middle layers.

Like cartilage undergoing degenerative change, menisci are subject to develop degenerative alteration. The elastic properties of fibrocartilage wane, meniscus

matrix reduces in viscoelasticity and collagen breakage increases, affecting structural integrity. Laminar tears and complex tears are regarded as typical degenerative tears. There is some variability with occasionally finding healthy menisci in end stage OA knees, but generally cartilage and menisci degeneration escalate in tandem, with degenerative meniscus change being more pronounced than macroscopic cartilage degeneration. An emerging concept suggests a meniscal pathway to knee OA (Englund 2012), where the degenerative joint alterations begin in the meniscus and via altered biomechanical stress and biochemical signalling possibly initiate and definitively augment cartilage degeneration (Melrose 2017).

Degenerative meniscus injury is very common in middle-aged patients, with cross-sectional MRI studies suggesting a 1/3 prevalence of degenerative meniscal tears in middle-aged and 1/2 in aged population (Englund 2006). Results from a meta study of MRI in asymptomatic knees found meniscus tears in 4% of patients aged <40 year and 16% aged >40 years (Culvenor 2018). Ruptures, degeneration and partial meniscectomies affect the meniscus integrity to cause extrusion of the meniscus. Extruded meniscus has decreased biomechanical function and results in increased cartilage contact point stresses causing risk of accelerated degeneration. In younger populations the panorama of meniscus injury is different with healthy menisci sustaining tears as a result of significant knee trauma and frequently associated with ACL injury. In 40-60% of ACL injuries concomitant meniscus injury is present (Fithian 2002; Frobell 2010).

Treatment

Historically meniscus injuries were treated by complete surgical removal of the meniscus, resulting in OA. The high incidence of OA after total meniscectomy was first recognized in 1948 by Fairbank, who found a 50%–66% incidence of OA on the ipsilateral and a 5% incidence of OA on the contralateral leg (Fairbank 1948). As a consequence, and with development of arthroscopic technique, a more considerate treatment of the meniscus emerged with suture of tears of favourable location in good-quality menisci and resection and debridement to stable tissue of meniscal tears that are not expected to have healing potential. Complete surgical removal or even partial resection of torn or diseased menisci significantly increases the risk of premature onset of osteoarthritis (OA) (Englund 2001; Jorgensen 1987; Monk 2017; Englund 2012). For degenerative meniscus tears, APM and placebo operation had the same clinical outcome in an RCT (Sihvonen 2013). In younger patients with no macroscopical degeneration a traumatic meniscus injury is treated by suture if it is localized in the vascularized peripheral zone and of vertical or unstable radial tear type. APM is an option for tears of the inner zones. The combination of ACLR with APM is a 2–4 times multiplier of the risk of OA development (Shelbourne 2017; Jomha 1999; Oiestad 2009; Lohmander 2007).

Diagnostic tools in OA

Biomarkers

Any substance, structure, or process that can be measured in the body or its products that monitors or predicts the incidence or outcome of a disease is a biomarker. Biomarkers can be measured in samples of fluids and tissues, 'wet biomarkers', or consist of measures obtained from patient characteristics, 'dry biomarkers', such as gait analysis, functional testing, PROMS and imaging techniques.

Reliable validated biomarkers capable of predicting OA development and of monitoring cartilage degenerative processes would profoundly advance science in several respects: basic research, development of DMOADs, the optimization of preventive medicine, aid diagnostics and identifying risk. Furthermore, predictive biomarkers would help indicate individuals in most need and benefit specific surgical treatments.

Wet biomarkers

Fluids from the compartments SF, serum and urine can be analysed to detect joint metabolites and signalling molecules. Fragments from anabolic and catabolic processes (metabolomics), e.g. collagen or proteoglycan fragments, can be measured. The expanding multitude of potential wet biomarkers include specific matrix components, signalling molecules, activated complement, by-products of synthesis etc. What levels of metabolites represent normal turnover versus degenerative processes is a challenging field of research.

Acute knee trauma has a long history of research focus because it provides a set point to temporally orient the study measurements and outcome evaluations. Acute knee trauma such as ACL injury releases GAG into SF with a longitudinal decrease (Dahlberg 1994). After an ACL injury a pattern of elevated SF and serum biomarkers is seen with inflammatory cytokines TNF and IL-6 highly elevated and products of aggrecan and type II collagen proteolysis. Aggrecan fragment (ARGS neoepitope) is found in SF and c-terminal crosslinked telopeptide of collagen II (CTXII) in urine. The pattern is both immediate and long term with elevation of inflammation persistant and has been detected 5 years after ACL injury (Sward 2012; Struglics 2015). ACLR operation in the acute post-injury phase represents a second trauma to the injured knee, resulting in a prolonged elevation of already high

SF levels of inflammatory cytokines such as TNF, IL-6, -8 and -10, and IFNγ, an effect that persisted over 5 years (Larsson 2017). However elevated inflammatory markers did not predict OA development after 5 years (Roemer 2019).

While wet biomarkers have the potential to be detected very early in the OA process, the specificity vs normal turnover is unclear. Processes are localized in joints, and to particular sites of cartilage activity, serum and urine samples are not joint-specific (systemic biomarkers). Samples of SF are specific to one joint (local biomarkers), but in large joints such as the knee parallel localized processes can have mixed results due to simultaneous anabolic and catabolic action. From a broad spectrum of potential biomarkers for prediction for OA to progress to a more severe ROA grade, the best candidate biomarker was serum C-terminal crosslinked telopeptide of type I and II collagen (CTXI and CTXII). The level in plasma predicts progression of OA in 2 years (AUC =0.67, OR =1.2) (Kraus 2017).

Although great progress has been made in wet biomarker research, with corresponding gains in understanding of mechanisms of cartilage breakdown and metabolism, clinically useful reliable biomarkers of OA progression have not emerged as specificity for clinical utility is lacking (Kraus 2015; Watt 2018).

Dry biomarkers

While clinical measures, e.g. quadriceps strength, malalignment, BMI etc. have been proving to correlate to disease progression, the most promising and feasible dry biomarkers have been shown in imaging. Radiographic features and MRI features correlate to disease progression, but those features are *a priori* structural changes representative of cartilage destruction. A common problem with the dry biomarkers is that they are measurements of phenomena occurring in the middle to late part of the OA development timeline (figure II). Dry biomarkers for very early cartilage change previous to cartilage destruction were lacking prior to the development of MRI (see section of cMRI).

PROMS, OA symptoms, knee function, activity level.

Symptoms and discomfort focused on the knee can for several reasons be something other than OA. In clinical practice a judgement call of symptoms, physical test and factoring in of patient age and risk factors is usually enough to diagnose OA, and if needed diagnosis is confirmed by standing radiographs. A method to reliably classify patients by diagnosis of OA or no OA is problematic by questioning of symptoms alone – a complication to the researcher.

Several approaches to document patient symptoms in a meaningful and repeatable method have been tried. To avoid bias the patient should answer questionnaires independent of investigators or caregiver, hence it is common to use patient reported

outcome measures (PROMS) that can feasibly be administered via mail or electronically.

Self-reported knee function by the modified (1985) Lysholm score (Tegner 1985) has well-documented validity, reproducibility, responsiveness (Briggs 2006) and has been widely used historically in the context of ACL, meniscus and cartilage injury. The Lysholm score covers 8 subscales representing aspects of knee function for a score of 0–100, with 100 corresponding to full knee health. Results of ≤84 are regarded as an indication of having knee problems during daily life and representing a usable cut-off point for dichotomization (Rockborn 1995).

Symptomatic OA (SOA) is challenging to define. The level of symptoms and of discomfort that should qualify as OA varies in scoring systems used.

Knee injury and Osteoarthritis Outcome Score (KOOS) (Roos 2003; Roos 1998a) is a knee-specific instrument, developed to assess the patients' opinion of their knee and associated problems. KOOS evaluates both short-term and long-term consequences of knee injury. It contains 42 items (5-point Likert scale) in 5 separately scored subscales: Pain, other Symptoms, Function in daily living (ADL), Function in Sport and Recreation (Sport/Rec), and knee-related Quality of Life (QOL). KOOS does not produce a single score to interpret; instead each subscale can be viewed separately and for presentation the subscale scores are commonly normalized to a 0–100 scale. The KOOS scale does not provide a dichotomization cut-off point to define OA, but an algorithm to calculate an operational cut-off for OA-like symptoms has been devised by Englund and the main developer of the KOOS score E. Roos (Englund 2003).

Radiography

Principles

Weight-bearing plain radiograph is the gold standard for diagnosing radiographic OA (ROA) in the knee. Significant loss of cartilage volume is apparent by reduced distance between the femoral and tibial bones, the narrowing of the joint space (JSN), indicating OA.

A classification for grading radiographic images of the knee-joint was developed by Fairbank in 1948 (Fairbank 1948) and although the principal OA features described are still used the Fairbank grading system is of limited use today. Kellgren and Lawrence introduced a radiographic classification system in 1957 (Kellgren 1957) and a grading system for knee OA in epidemiological context was presented in 1963 (Kellgren 1963) and later further additions and adjustments were added. The Kellgren-Lawrence grade of OA (KL) has dominated literature, and a score of ≥2 is

generally accepted as definition of radiographic OA. The Swedish radiologist Ahlbäck of Lund University introduced a grading system including osteophytes and with emphasis on the JSN, establishing the patient to stand during exposure in order to load the knee joint by body weight to increase the reproducibility and sensitivity of JSN (Ahlbäck 1968), in contrast to the original KL publications that had the patients lying down for exposures. Over the years several versions and updates of definitions of the KL criteria (e.g. irregularities in the wordings of osteophyte definitions) have been used and weight-bearing radiographs has only gradually been incorporated as the norm (Schiphof 2011; Schiphof 2008). Such irregularities are of concern when comparing reports as even large studies such as NHANES III (Dillon 2006) have reported KL score based on non-weight-bearing radiographs.

Standing weight-bearing radiographs in ante-posterior (AP) projection with the knee in semiflexion of 20–30 degrees and the beam parallel to the tibial slope is a feasible method with high reproducibility (Peterfy 2003). Errors can be introduced by not controlling for knee flexion. Osteophytes are more prominent in extension radiographs (Wolfe 2002) and JSN is more sensitive in semiflexion (Boegård 1997).

A slight change of JSN is less specific as it can be influenced to a large extent by extrusion of a degenerative meniscus or meniscectomy and only in higher grade JSN becomes sensitive and specific to cartilage loss (Hunter 2006; Amin 2005). Furthermore, patients might inadvertently reduce the load on a painful knee compartment, masking a reduced joint space by not compressing (Mazzuca 2002). In practice, meniscus extrusion and cartilage reduction occur in lockstep on the pathway of OA development (Crema 2012; Gale 1999). In the longitudinal evolution of radiographic OA change the appearance of osteophytes is suggestive of OA change, and a harbinger of more change to come (Felson 2005; Wolfe 2002).

The categorial classification scales (e.g. KL) are of value for OA definition in a clinical setting but are crude for monitoring longitudinal change and describing early degenerative change. Semi-quantitative scoring of individual features is more sensitive for detecting change/progression (Felson 1997; LaValley 2001; Culvenor 2015; Gossec 2008; Sheehy 2015). To increase detail of OA grading and to better allow for characterization of longitudinal change in repeated investigations, OARSI published an atlas and a scoring system in 1995, updated in 2007 (Altman 2007), for OA change to various joints. Scores are applied to each individual radiographic feature according to the atlas. For the knee joint the frontal semi-flexed weight-bearing image is used, femoral and tibial marginal osteophytes on both the medial and lateral compartment edges are individually assigned scores of 0 (no change) to 3, as is the JSN for medial and lateral compartment of 0 (no JSN change) to 3 (bone erosion). Thus, for the femorotibial joint 3 medial and 3 lateral scores are recorded and can be used to sensitively grade the severity of OA change and enable longitudinal comparison. Osteophyte score can be considered as a monotonic

variable for grading of OA severity – a bigger osteophyte is worse than a small one which is worse than none – that is not influenced by the confounders that relate to grading by JSN. Inter-reader variability has previously been examined and reported with a good inter-rater kappa value of 0.52–0.78 for OARSI scoring (Roos 1998b; Neuman 2008).

In order to use the OARSI scoring to dichotomize radiographic OA (no-ROA or ROA), criteria approximate to KL ≥2 were formulated by Englund (Englund 2003) and used in several studies (Lohmander 2004; Neuman 2008; Englund 2005; Culvenor 2015; Neuman 2014; Owman 2014a; Owman 2008). These criteria are slightly more sensitive than KL, classifying a higher rate as ROA (Culvenor 2015) and have shown good reproducibility. The medial and lateral compartments are analysed separately. For OA to be present in the respective compartment the sum of scores in that compartment should be 2 or more for either JSN, osteophyte or the JSN and osteophyte combined (Englund 2003).

Limitation of radiography

The most important limitation of radiography is its insensitivity in terms of detecting early articular cartilage loss in knee OA, which means that changes do not become visible until perhaps decades after the onset of the disease, when the cartilage changes in the joint are beyond repair. Radiography only detects when cartilage is significantly absent (>50% gone), which is why it is insensitive to early OA changes and cartilage injury. Joint space narrowing is sensitive to patient compliance to bear full weight, which can be problematic if there is severe pain. The radiation dose of 1–2 μS for one ante-posterior exposure is small relative to the natural background radiation of 0.5 mS/year and the occupational limit of 20 mS/year. The current best practice of grading of features by an atlas of weight-bearing semiflexion radiographs and classification by algorithmic dichotomization is still not perfect, especially for early stage OA. Efforts to increase sensitivity, selectivity and reproducibility are ongoing. Automated computer algorithms for classification of radiographic features using machine learning (Tiulpin 2018) trained on cross-reference CT and MRI 3D features holds promise. Currently whole joint 3D investigation has the drawback of not being weightbearing. Semi-flexed weightbearing radiographs remain the gold standard and have the benefit of being readily available and widely used as the preferred method of investigating OA in clinical practice.

Arthroscopy

Advances in fibre-optic light and video technology made arthroscopy available for general use since the 1970s. Arthroscopy has the ability to visualize all the surface area of articular cartilage in the knee joint and to use instruments to further investigate the properties of cartilage, meniscus and ligaments by probing and

pulling, and to enable surgical procedures (e.g. debridement, MFX, meniscus suture). However, the quality of the investigation is dependent on the surgeon's skill. Although arthroscopy is able to visualize all surfaces of the knee articular cartilage all surgeons are not able to do so in all cases. In stiff knees the medial posterior compartment can be difficult to assess, and horizontal meniscal tears and tibial cartilage injuries can remain undetected. Methodologically distinct and meaningful classification of cartilage injuries is difficult, and several classification systems have been used (Brismar 2002). Another shortcoming of arthroscopy is that it is primarily the appearance of the cartilage surface that can be judged. Thickness is difficult to assess, and instrumentally palpated softness is hard to quantify. Probes to measure indentation stiffness and thickness exist but have seen limited use. Arthroscopy is an invasive investigation with slight morbidity in postoperative knee pain/discomfort, and it exposes the patient to risk from anaesthesia and complication risks on the order of 1–2% for more serious complication such as pyogenic joint infection or venous thromboembolism (Friberger Pajalic 2018).

Magnetic resonance imaging

Principles

Nuclear Magnetic Resonance Imaging (MRI) provides non-invasive imaging of all the structures of the knee, including the cartilage, subchondral bone, menisci, ligaments, and muscle. In addition to morphological information pathological properties such as oedema and bleeding can be detected. Images are produced from signal acquired from hydrogen atom nuclei interacting with tissue in a strong magnetic field. The interaction of hydrogen to tissue is influenced by the tissues molecular content, density, regularity and diffusion parameters. Hydrogen in fat and water are the main component of MRI signal.

MR Physics and technique

Spin is the intrinsic property of elementary particles as described by the theory of quantum mechanics. Protons and neutrons have spin as a function of their constituent quark's and gluon's spin. In multinucleon atoms proton-proton and neutron-neutron pairs form, and paired nucleons cancel out their spin value. Atoms with even numbers of protons and neutrons thus have zero nuclear spin, e.g. ^{12}C and ^{16}O. Spin of the atom can be conceptually expressed as the nucleus rotating around its axis. In an external magnetic field, the nucleus rotation axis will orient to precess around the direction of the field (the axis rotates around the field direction vector).

The frequency of the precession (Larmor frequency) is specific to the combination of nucleons and proportional to the strength of the magnetic field.

These phenomena are exploited in the magnetic resonance imaging technique (MRI). The superconductor magnet of an MRI scanner produces an extremely strong magnetic field that align sensitive atoms of a body put inside the field to form tiny net magnetization in every single position. On a measurable scale net magnetization will be the collective magnetization of very large numbers of atoms.

Hydrogen (^{1}H), consisting of one single proton, possess spin with strong gyromagnetic ratio and is thus one of the most sensitive elements to field interaction. The properties and the abundance in tissue of hydrogen in water and fat make ^{1}H the principal nucleus of MRI, dominating the net magnetization. For ^{1}H in a 1.5 T field the Larmor frequency is in the radiofrequency (RF) range at 63.9 kHz.

An RF-pulse (electromagnetic wave) with the Larmor frequency of ^{1}H that is applied in perpendicular to the direction of the strong magnetic field rotate the vector of net magnetization away from the field. With enough time and strength, the RF pulse will flip the net magnetization transverse (90°) to the field direction with a coherent rotation at the Larmor frequency. The rotating magnetic movement create induction current in a coil placed nearby which can be measured as the MR signal.

When the brief RF pulse is turned off net magnetization is at maximum in the 90° plane. The gradual turning back of net magnetization to the strong field is T1-relaxation and occurs by ^{1}H shedding gained energy via interactions to the molecular surroundings, the efficiency of energy loss varies dependent on the tissue composition and state. The loss of signal in the 90° plane is T2-relaxation and depends on loss of coherence of individual magnetization due to fast interactions to local field inhomogeneities and spin-spin interactions, and slower interaction to molecular surroundings.

To create an image from the MR signal additional gradient magnetic fields are applied with use of orthogonal gradient coils during signal generation to induce small spatial linear variations to the magnetic field. Because of the Larmor frequency being proportional to field strength these gradients will allow three-dimensional spatial specificity by targeting the frequency of the RF pulse to excite a certain slice, and to create spatial resolution within that slice. Pulse sequences are advanced protocols used to repeatedly apply RF pulses, gradients, timing events and MR signal sampling to collect enough signal data for an image. Complex Fourier transformation calculation are performed to generate the image from the signal data.

Visualisation of morphology and fluid distributions are needed for clinical use. By careful adjustment of different parameters of the pulse sequence, the generated image can be made to highlight different aspects of the tissue. E.g. differences in T1-relaxation times can be enhanced (T1-weighting) or differences in T2-relaxation

times can be enhanced (T2-weighting), but several other image contrasts are also possible. By repeated measurement of the relaxation signal for different timepoints and fitting values to a known recovery curve a quantitative value of relaxation-time can be calculated for a specified volume. MRI can reveal many characteristics of tissues but understanding of exactly what is measured, and the correct interpretation is not fully elucidated.

Image contrast can be further enhanced in other ways. Certain paramagnetic substances have small local magnetic fields that can act on nearby hydrogen atoms to enhance relaxation (i.e. shorten tissue relaxation times). This property is utilized in MRI contrast agents to enhance signal in T1-weighted images in proportion to its distribution. Other nuclei than hydrogen e.g. Sodium (^{23}Na), can also be measured by targeting their specific RF resonance, but this requires special hardware that is usually not installed in standard MR scanners.

Use in the knee

MRI has been widely used clinically for more than 30 years and significant progress has been made thanks to higher field strength, better coils, much improved computer capacity and development of advanced imaging acquisition sequences. Applied on the cartilage of the knee, clinical MRI allows precise detection of cartilage defects and lesions that can be reliably (Hunter 2011) monitored for progress using scoring protocols (e.g. WORMS), although detectable changes represent cartilage at a stage where damage is already irreversible and tissue already lost. Furthermore, as with other imaging techniques, no information on tensile properties (softness, stiffness, brittleness) are obtained. Clinical MRI can detect focal defects and diffuse cartilage loss but has limited ability to detect earlier changes in cartilage composition.

Compositional MRI, cMRI

Compositional MRI (cMRI) are advanced MRI techniques designed to measure biochemical or structural properties of the examined tissue with spatial precision. In cartilage the initial pre-OA processes of diminished GAG content and collagen alterations occur without macroscopic alterations to morphology, cMRI techniques enhance our understanding of early disease thanks to their capability to detect ultrastructural tissue alterations that are not conceivable by visual assessment. Focus on early disease means focusing research efforts at a stage of potential reversibility. Once joint damage has progressed to stages of cartilage loss and destruction, cMRI will likely only play a secondary role in joint assessment.

Potential cMRI applications are: 1) to monitor homeostatic changes in healthy cartilage; 2) to identify the earliest changes of pre-OA; 3) to monitor the effect of

trauma and treatment; 4) to monitor the effect of attempts to modify pre-OA and early-OA progression such as experiential disease modifying OA drugs (DMOAD), physiotherapy and surgical interventions; 5) to perform pre-treatment stratification to characterize joints that are likely to benefit from a treatment; 6) to monitor result from treatments such as ACLR, APM and cartilage repair in order to shorten time to assessment of outcome; 7) to become a tool to indicate treatment with a potential future effective DMOAD in analogy of DEXA in osteoporosis.

A reliable and valid cMRI would be the ultimate cartilage biomarker combination of specific localization and specific compositional information.

Pre-OA and early-OA processes that cMRI attempts to assess include GAG content, water content, changes/damage to the collagen network structure, increased permeability, loss of network rigidity and unchecked swelling. Specific measurement of GAG/FCD content may be the earliest detectable event (Pratta 2003) and also is important as early GAG changes may be reversible. Collagen change might turn out to be very prognostic but represent a slightly later stage situation with less potential for restitution.

Technical progress enables continuous evolvement of cMRI techniques carrying specific strengths and weaknesses in feasibility (cost, access, safety), reliability (repeatably and reproducibility) and validation (sensitivity, selectivity). So far cMRI is a developing field with limited clinical use, but nevertheless it has provided great insight for cartilage science.

T1ρ (T1rho)

T1ρ describes longitudinal spin-lattice relaxation in the rotating frame and involves a complex acquisition protocol to measure interaction to lower frequency molecular movement. Spin-lattice exchange of bound-water to large macromolecules such as the aggrecan-bound GAGs has a low interaction frequency to which T1ρ is sensitive (Akella 2004; Akella 2001). T1ρ has the advantage of estimating GAG content (Mosher 2004) without the use of contrast because disruption of the proteoglycan content of the matrix leads to increase in water molecule motion.

In-vivo imaging and postoperative retrieval ex-vivo analysis have shown histological correlation of T1ρ and GAG concentration (Wong 2013) and OA grade (Regatte 2006; Tsushima 2012). In-vivo studies suggests a correlation of high T1ρ values to cartilage of suspect lower GAG content and/or early degenerative change (Li 2011) and in patients with mild OA compared to healthy controls (Stahl 2009). After ACL injury a higher 3T T1ρ signal was found in lateral tibia cartilage with underlying BML (Bolbos 2008), and ACL injury with higher T1ρ was associated with worse KOOS in 1-year outcome (Su 2016). Marathon running causes T1ρ to be significantly elevated after vs previous to the race and did not recover fully on repeat measure after 3 months (Stehling 2011; Luke 2010). Unloaded regions of

knee cartilage had higher T1ρ values than regions of weight-bearing in healthy controls (Bolbos 2008). Higher value in asymptomatic hips with CAM lesions than in controls with no deformity was demonstrated (Anwander 2016). In asymptomatic active athletes with chronic PCL injury, T1rho values were increased compared to non-injured controls(Okazaki 2015).

Results of prognosticating progression in the short term have been published; knees with progressing cartilage lesions on MRI over 2 years had significantly higher T1ρ than non-progressing lesions (Prasad 2013), and cartilage in hips with impingement that progressed to OA had higher T1ρ values (Gallo 2016).

A contrary finding of preoperative T1ρ not correlating to explant histology GAG analysis, while in the same samples preoperative dGEMRIC had good correlation to histologic GAG (Tiel 2016), encourages further study for validation.

T1ρ has been combined with T2 and when no increased T2 signal was detected, T1ρ was sensitive to GAG change, suggesting different sensitivity windows (Keenan 2011).

Despite promising results T1ρ has drawbacks in no proven biochemical correlate, complicated protocols, sensitivity to disturbance of inhomogeneities and because the need of high RF power becoming limited by specific adsorption(Tiel 2016; Oei 2014).

T2 relaxation

T2 relaxation times are primarily dependent on the water and collagen content of the extracellular matrix as well as its ordered orientation (anisotropy) of the collagen fibres. Normal cartilage deep zone matrix is highly anisotropic with organization governed by type II collagen bundles acting to direct movement of bound water and facilitating decay of the T2 signal. Altered cartilage loses order and increases free water content, resulting in delayed T2 spin-spin decay. T2 signal is a sum of several components: increased water content, collagen microstructure disorganization and to some extent GAG loss all elevate T2 value (Mosher 2004). T2 relaxation has been regarded as the best method to measure type II collagen content (measured by signal intensity) and orientation (expressed by anisotropy). As it is not as strong in measuring GAG content it could thus be less sensitive to the earliest signs of cartilage alterations. T2 relaxation quantification is feasible as it needs no special equipment and has good inter-site repeatability. With increased accessibility to 3T MRI, several T2 relaxation studies have been reported, several sequence variations have been used, which makes direct comparations between studies complicated. Good reproducibility has been demonstrated (Mosher 2011).

Several in-vivo studies have demonstrated association of T2 findings with cartilage macroscopic change in knees with early OA (Li 2009b; Dunn 2004; Apprich 2010) to a histologic grading scale of retrieved specimens(Regatte 2006; Reiter 2012) and

GAG concentration (Wong 2013). In a 3T system a threshold of 47 ms T2 relaxation time was found to differentiate the arthroscopy classification score of normal appearing and degenerative cartilage (Soellner 2017). Associations of T2 to change in cartilage exposed to risk factors have been demonstrated; T2 signal increased after a marathon race but returned to pre-race value after 3 months (Luke 2010), while knees with individual risk factors for OA had higher T2 values (Joseph et al. 2011), and 3 years after ACL injury T2 was higher than in the contralateral knee (Bae 2015).

Results of studies on the Osteoarthritis initiative (OAI) cohort indicate a prognostic capacity of T2. Knees with new cartilage lesions or progressive lesions over 2 years had significantly higher T2 (Prasad 2013). Long T2 values of tibiofemoral cartilage may predict worsening of OA associated MRI findings after 3 years (Joseph 2012), and the appearance of radiographic OA over a 4-year period (Liebl 2015). In more advanced OA of KL ≥2 measurement of T2 could not predict progression (Eckstein 2011).

Some concerns about the validity of T2 relaxation measurement have been raised: T2 signal varies with depth and the cartilage deep zone signal is short whereas the transitional and superficial zone signal is long (Mosher 2004; Smith 2001), complicating the precision and repeatability of ROI definition. The weight-loading status of the joint affects water to collagen ratios and a period (~30 min) of unloading is required to equilibrate for reliable conditions of the deep zone (Apprich 2010; Nishii 2008b; Fernquest 2019). The dependence on anisotropy has peculiar effects on the collagen angle versus the B0 magnetic field, with a magic angle effect of distortion at certain angles (55° to the field) of concern in the spherical hip joint, which also have to be considered in the femoral condyles. The linear relationship to cartilage OA change is not clear (Koff 2007), and the depth of observation range seems limited(Regatte 2006). In one study no difference was found in T2 of dysplastic hip joints between healthy, early OA and mild OA (Nishii 2008a).

T2* mapping is suggested to be more sensitive to early collagen change (Stahl 2009) and is shown to better measure deeper cartilage layers as well as being faster (Tao 2018). T2* is influenced by small local susceptibility-induced magnetic fields which play a significant role at the bone-cartilage interface and within the anisotropic fundamental cartilage microarchitecture. T2* mapping might be a more sensitive imaging strategy for identifying cartilage degeneration of deep and calcified layers. Advanced sequences allow for T2* mapping and a 3D image of the joint surface (Bittersohl 2009b), although there are limitations in the propensity to artefacts and magic angle effects (Hesper 2014).

Ultrashort echo-time T2* (UTE-T2*) uses very short echo times (TE) of benefit in the deep calcified layer and meniscus (Olsson 2018; Bae 2010; Du 2013). In a recent study 2 years after ACL reconstruction, UTE-T2* profiles show both injured and contralateral knee of the patient to differ from knees of uninjured controls (Williams

2018). However current clinical application is still limited due to spatial resolution and signal-to-noise ratio (SNR) and technical complexity (Oei 2014).

sodium MRI

Sodium ($^{23}Na+$) is distributed by equilibration in the cartilage matrix in concentration relative to the fixed charge density and thus correlate to GAG concentration. The properties and relaxation rates of $^{23}Na+$ is challenging with weaker gyromagnetic ratio and much lower concentrations than ^{1}H, resulting in less resolution and low SNR (Shapiro 2002). Repeatability (Newbould 2012) and distinction of OA knee versus controls have been reported in pilot studies (Wheaton 2004; Widhalm 2016). Cartilage sodium MRI is attractive as the signal relates directly to the GAG concentration but the need for higher field strengths, dedicated sodium coils and long exposure means that it remains experimental so far (Zbýň 2016).

gagCEST

Chemical exchange saturation transfer (CEST) has complex sequences that enhance bulk water signal. To use this on cartilage, a strong magnetic field is required, with results reported from 7T. In theory gagCEST measure an enhanced water signal related to exchange of protons (including NH, OH, and NH2) on the GAG chains, and has been experimentally used in vivo (Ling 2008). In one study gagCEST of cartilage in OAT correlated to MRI morphology grade (Krusche-Mandl 2012). While promising, gagCEST is currently an experimental 7T technique.

diffusion weighted MRI (DWM) and diffusion tensor MRI (DTI)

Diffusion coefficients are increased in early degenerative disease of articular cartilage (Mlynárik 2003). Diffusion weighted MRI indirectly measure a quality of matrix depending on GAG change. Diffusion tensor imaging (DTI) can also measure diffusion anisotropy of cartilage and is sensitive to the proteoglycan by diffusivity and to collagen architecture by anisotropy. DTI has been shown to be able to detect differences between OA knees and controls (Raya 2014). DTI has the drawback of requiring high field strength and long exposure. So far it is experimental.

dGEMRIC

dGEMRIC basics

Delayed Gadolinium-enhanced MRI of cartilage (dGEMRIC) estimates the GAG content of hyaline cartilage. The negatively charged Gadolinium contrast agent (Gd-$DTPA^{2-}$) is repelled from tissue with a strong negative charge. Normal cartilage has high negative fixed charge density (FCD) due to the abundance of negative GAG chains of the aggrecans, and the equilibrated concentration of Gd-$DTPA^{2-}$ thus becomes low. GAG depletion reduces the FCD and the equilibrated concentration of Gd-$DTPA^{2-}$ becomes higher. Gd-$DTPA^{2-}$ shortens the T1 relaxation time of the tissue and results in low (short) T1 value for a low FCD and high (long) T1 time value for a high FCD. The T1 relaxation time in a select cartilage volume thus becomes a value (T1Gd index) of estimated FCD/GAG, functioning as a biomarker for cartilage quality.

The value of T1Gd can be measured in different ways. A widely used method, which is also considered gold standard, is the 2D inversion recovery technique. The method is applied in a single, few mm thick slice. An inversion RF pulse inverts the direction of the net magnetization 180°. The nuclei are then allowed to recover (T1 relaxation) for a specific inversion time (TI), after which the amplitude of the magnetization is measured by a 90° spin echo readout. This procedure is repeated for 5 or 6 different TI, chosen to correspond to a recovery varying from a few percent to more than 70% (e.g. TI = 50, 100, 200, 400, 800, 1600 ms). The amplitudes in the resulting images are then fitted to a known recovery curve, giving the value of T1. The fitting can be performed either on a voxel-by-voxel basis or in a selected region of interest in the tissue.

Gadolinium, gadolinium contrast agents

The rare-earth element Gadolinium (^{64}Gd), is a lanthanide heavy metal. Gd is toxic and is not readily eliminated, causing bio-accumulation. Toxicity of free ionic Gd^{3+} is due to disruption of biological processes, e.g. by interaction with calcium-ion channels. Gd^{3+} has strong paramagnetic properties which in the MRI setting facilitates the spin-lattice relaxation of nearby ^{1}H excited by the RF-pulse to shorten T1 relaxation. This effect is because of the unique arrangement of atomic properties, the electron 4*f*-shell of Gd^{3+} have 7 unpaired electrons to create a strong local magnetic field that interact with ^{1}H, to function as the principal MRI contrast media. To allow safe medical use Gd^{3+} must be bound to an inert organic ligand that facilitates diffusion and elimination, creating Gd-bound contrast agents (GdCA). Various forms of commercially available GdCAs have been introduced since the 1980s, with some different properties such as size, diffusion characteristics and

electronegativity (Gd-DOTA^{-} Dotarem®, Gd-DTPA^{2-} Magnevist®) or electroneutrality (GD-HP-DO3A ProHance®, GD-DTPA-BMEA Omniscan®). GdCAs are usually administered intravenously and are eliminated through the urinary, and, to a lesser extent, biliary system. In subjects with normal renal function plasma half-life is approximately 1.5 h, and it is completely removed from the urine in 7 days (>90% in the first 12 h) (Aime 2009). Gd-DTPA^{2-} is readily diffused and has a molecular weight of 548 Da.

Principle and development, validation

The pioneer developers of the dGEMRIC method were Adil Bashir, Deborah Burstein and Martha Gray in collaboration with Alice Maroudas. In vitro a strong negative correlation was observed between T1Gd and GAG concentration (Bashir 1996) and it was proven to be dependent on the electronegativity, as the electroneutral GdCA Gadoteridol shows no correlation to GAG concentration (Bashir 1997). The technique was further validated in vitro and in vivo (Bashir 1999; Mlynárik 1999; Nissi 2004; Trattnig 1999; Nieminen 2002) and was able to monitor GAG replenishment in-vitro (Allen 1999). dGEMRIC also correlated to mechanical properties in-vitro (Kurkijärvi 2004; Samosky 2005; Baldassarri 2007; Lammentausta 2006; Lammentausta 2007), findings that are in line with the observed role of GAG in cartilage compressive stiffness (Jurvelin 1988; Kempson 1976).

Parameters and protocols for repeatable research in human subjects for knee (Multanen 2009; Burstein 2001; Tiderius 2003; Tiderius 2001) and hip (Bittersohl 2009c) were subsequently developed.

Protocol specifics

To achieve repeatable results for human in vivo study, a protocol of dose, timing and procedure had to be established. Joint movement facilitates GdCA transport to synovial fluid (Winalski 1993), and flexion-extension movement brings larger cartilage area in contact with more fluid. Furthermore, cyclic compression accelerates fluid exchange in cartilage matrix, therefore subjects need to perform mild loaded exercise to facilitate Gd-DTPA^{2-} distribution after intravenous administration. Ten minutes of weight-bearing knee exercise has been shown to be required after the injection of contrast to facilitate homogenous uptake in femoral cartilage (Burstein 2001). In healthy knees a broad peaked cartilage signal was archived at 2–3 h post-contrast, and the triple dose (0.3 mmol/kg) had the strongest signal (Tiderius 2001). In osteoarthritic cartilage a slightly shorter time to peak was found and 90 minutes post-contrast was defined as the optimal time point for the start of image acquisition (Tiderius 2003). Whereas the dose of Gd-DTPA^{2-} is calculated dependent on weight the distribution is mainly to plasma volume, resulting in higher plasma concentrations in obese versus lean subjects. Bias from BMI has a nearly linear function enabling a

correction procedure (Tiderius 2006). Sex have no bias and age appears to have only limited correlation (r2=0.04) in mixed sample of several cohorts (Dahlberg 2012). Bilateral sequential dGEMRIC of ACL injury with the non-injured knee investigated as a control detected no systematic error dependent on the order of knees examined (Fleming 2010). Time-consuming pre-contrast T1 imaging for ΔT1Gd of cartilage was not found to be necessary (Bittersohl 2009c; Li 2009a) and to induce more variability (Tiderius 2004b), ΔT1Gd is more sensitive when the contrast-free T1 component is highly variable, as has been demonstrated in evaluation of fibrocartilage after cartilage repair (Watanabe 2006), while others have found T1Gd to be sufficient (Trattnig 2009). Intraarticular administration is more invasive and have been used less with less validation but appears feasible in reports of both hip (Boesen 2006; Zilkens 2014; Bittersohl 2010) and knee (Hangaard 2017; Hangaard 2018).

Analysis

Spatial variations of dGEMRIC value are found in regions of the knee cartilage. The optimal image orientation to study femoral cartilage representative of the greatest load and most prevalent of lesion (Boegård 1997) is in the sagittal plane of the centre of the respective medial and lateral femoral condyle. The tibial cartilage is partly covered from femoral contact by the meniscus. The cartilage of interest is the femoral cartilage from the point of contact to tibial cartilage in extension and extending posterior to the point of contact at 30° of flexion. Manual definition of this region as a region of interest (ROI) on the T1 image and its repeatability and feasibility is the subject of **Study I** of this dissertation (Tiderius 2004b). Variability in the definition of cartilage ROI has been assessed also in repeated dGEMRIC investigation within 5 days in volunteers with healthy knees of full thickness cartilage ROI was 5–7 CVRMS% (root-mean-square value of CV) (Multanen 2009) and 5–8 CVRMS% in a study of dGEMRIC investigations repeated at 2-week intervals in long-term ACL copers (Siversson 2010). In healthy hip joints a variability of 4–7% has been reported (Bittersohl 2009a).

dGEMRIC results

dGEMRIC have produced results demonstrating it a clinical research tool regarding early cartilage change and early degeneration/OA to function as an imaging biomarker.

Correlation of in-vivo dGEMRIC to ex-vivo cartilage measurements:

A strong correlation of T1Gd to GAG content was found between dGEMRIC prior to arthroplasty and the GAG content of the explant by histological analysis (Tiel 2016), and a similar study in the hip found correlation of T1Gd to explant GAG content (Zilkens 2013). Explants from femur at TKA with variable stage of OA were

examined in vitro, showing that dGEMRIC value had strong correlation to biomechanical properties of compression and T2 to have weak insignificant correlation to biomechanical parameters (Juras 2009).

Correlation to OA grade / stage

A more advanced radiological OA stage had lower T1Gd than less advanced stage and affected compartments had lower T1Gd than unaffected contralateral compartments of the same knee (Williams 2005). In a longitudinal dGEMRIC investigation of 148 women >40 years old examined three times over 2 years, a decrease in regional T1Gd correlated to cartilage swelling as a marker for cartilage OA progression in knees with radiographic OA (Crema 2014). In hips at risk of degeneration due to FAI morphology, T1Gd and T2 correlated in regions at risk of cartilage lesion (Fernquest 2019). T1Gd differed between ROA and noROA and correlated to radiographic KL grade in the patello-femoral joint (Nojiri 2006).

Variation related to activity, symptoms and adaption

In healthy volunteers with no knee symptoms dGEMRIC correlates to activity level with the highest values in elite runners, medium values in normal activity profile and the lowest values in subjects of sedentary activity profile, suggesting a dose-dependent adaptation. Additionally the range of observed values was narrow in the group of moderate activity and wider in both runners and sedentary subjects (Tiderius 2004a). Higher T1Gd was also found in high activity professional dancers (Williams 2004). Correlation of dGEMRIC to varus valgus alignment and to grade of radiographic change have been reported (Williams 2005). Untrained healthy volunteers who started regular running increased longitudinal dGEMRIC value during a 10-week programme, suggesting an adaptative response (Van Ginckel 2010). In patients with mild OA, increased activity with a controlled 4-month physiotherapy programme was shown to increase dGEMRIC value in a longitudinal study, suggesting a potential for the early-OA affected cartilage to improve (Roos 2005; Hawezi 2016). In patients with an arthroscopic partial meniscus resection 1–5 years prior to dGEMRIC investigation, low T1Gd values compared to healthy volunteers were found. In addition, low dGEMRIC values correlated to high BMI and to reduced thigh-muscle strength (Ericsson 2009).

Injury

Three weeks after ACL injury dGEMRIC was 12-14% lower than in healthy volunteers and sampled synovial fluid had increased GAG levels (Tiderius 2005). A 13% lower dGEMRIC value was found 2–6 months after ACL injury compared to the contralateral non-injured knee (Fleming 2010). Klocke et al. examined 13 patients with ACL injury with T2, T1rho and dGEMRIC at 3T and concluded that cartilage had increased water content by higher T1rho value and that no significant

GAG loss was detected by T1Gd (Klocke 2013). In a study of recurrent patella dislocation, lower T1Gd correlated with longer duration of the condition, implying a more affected cartilage (Watanabe 2009). Structured non-operative treatment of ACL injury was shown to have T1Gd values on par with healthy knees, 20 years after injury (Neuman 2014). One interesting case report exist of were a researcher had for validation purposes, as part of a group of healthy knees, performed dGEMRIC of his own knee and 1 weeks later accidently sustained a PCL injury. He then proceeded to perform three longitudinal dGEMRIC investigations. Normal pre injury T1Gd dropped by 15% post injury, 19% 3 months post injury and recovered to pre injury level 6 months after injury (Young 2005).

Immobilization

In patients with ankle fracture that were prescribed 6 weeks of non-weight-bearing dGEMRIC, mean values were unchanged after 6 weeks, after which return to weight-bearing was allowed. At 4 months mean dGEMRIC value was significantly lower and stayed at the same level after one year. dGEMRIC values were of a narrow range at the baseline investigation but had a significantly wider range at all follow- ups (Owman 2014b).

Osteotomy

dGEMRIC has been used to study cartilage response to HTO, documenting a pre-operative lower dGEMRIC value of the medial compartment compared to the lateral, but follow-up assessments have not been able to convincingly detect an adaptive response of the cartilage post-operation (Besselink 2018; Rutgers 2012; d'Entremont 2015), probably due to medial OA stage being too advanced to allow for cartilage restitution.

Cartilage repair

The monitoring of cartilage repair is an attractive target for cMRI, and several studies have employed dGEMRIC to evaluate repair tissue, to monitor maturation and to assess the status of the adjacent cartilage. A fundamental challenge is that repair tissue is variable and typically does not result in the well-organized zonal hyaline cartilage, thus validation of dGEMRIC measurement does not automatically apply as is suggested by mixed results in the literature.

The first pilot study examined 10 knees and found repair cartilage T1Gd value to be lower than in adjacent cartilage but to increase with length of follow-up time, range 6–30 months (Gillis 2001). Kurkijärvi et al. evaluated ACI grafts in 12 patients after 15 months and found conflicting results of poor T2 values and T1Gd values indicative of GAG replenishment (Kurkijärvi 2007). Watanabe et al. found good correlation of dGEMRIC assessment and biopsy analysis of ACI repair tissue in 9 knees after 2 years, and the estimated GAG content was lower than in normal

hyaline cartilage (Watanabe 2006). Trattnig et al. found dGEMRIC indicative of lower GAG content after 3rd gen ACI (MACT) compared to MFX after 31 months (Trattnig 2008). dGEMRIC 13 years after ACI in 36 knees had T1Gd values on a par with adjacent cartilage but reported subchondral cysts, intralesional bone, BML and other irregularities in the repair tissue with low average KOOS scores (Vasiliadis 2010).

Conceptually the status of the cartilage adjacent to an injury would influence outcome of an attempted repair. To evaluate this hypothesis dGEMRIC and T2 were accessed in a cohort of average 33-year-old patients prior to attempted ACI repair. In this baseline assessment no average difference in the injured knee cartilage compared to contralateral knee was found despite 1–3 years' history of cartilage defect (Aroen 2016).

Correlation to future OA.

Owman et al. have presented two different cohorts of middle-aged patients at risk of developing knee OA. In patients selected by the arthroscopic finding of fibrillation as a manifestation of early-OA, a low dGEMRIC index was associated with radiographic OA development 6 years later (Owman 2008). Patients selected by medial APM of a degenerative tear and examined 3.7 years later with dGEMRIC had values correlating negatively with the grade of radiographic OA features 11 years later (Owman 2014a).

In the hip joint a low preoperative dGEMRIC index of hip cartilage was found to be the strongest predictor for a OA progression after periacetabular osteotomy in patients with hip dysplasia (Cunningham 2006; Kim 2012). Similarly, a high preoperative dGEMRIC index before hip arthroscopy was correlated to a favourable clinical outcome 2 years postoperatively (Chandrasekaran 2015). In hips with FAI, baseline dGEMRIC predicted the radiographic OA development at the 5-year follow-up (Palmer 2017).

Eckstein et al. investigated different possible predictors of cartilage degenerative progression measured by cartilage thinning as outcome. Applying ROC curve statistics, dGEMRIC as a predictor had AUC=0.68 p=0.02 whereas T2 relaxation had poor AUC at 0.59 p=0.3 (Eckstein 2011).

dGEMRIC limitations

Whereas dGEMRIC can be performed on a standard MRI system, there are practical limitations with a complex protocol of standardized joint movement after injection, 1.5–2h delay from GdCA administration to MRI time and relatively long image acquisition sequences (15–20 min), which is not only stressful to the patients, but also long time increases the risk of motion artefacts. Techniques have been

introduced that allow for shorter acquisition protocols and 3D imaging (Trattnig 2008; Siversson 2010; Trattnig 2007).

Questions remain about ensuring that the contrast agent fully penetrates the cartilage based on in-vitro diffusion tests (Salo 2012; Nieminen 2002). In-vivo mobilization/movement aids the distribution but thick cartilage is a concern (Hawezi 2011), a potential remedy would be to adjust the ROI so that it does not extend to the deepest layer of cartilage after appropriate validation (Li 2012). Other potential contrast distribution variations would be related to equilibration to synovial fluid compartment versus plasma compartment and plasma compartment volume versus variation in body constitution, a conversion factor addresses the BMI/body constitution variation (Tiderius 2006). Increased turnover alters the spatial variation of GAG content of pericellular and interstitial regions, potentially affecting contrast diffusion and T1Gd signal (Stubendorff 2012).

Fundamentally dGEMRIC is an investigation to detect early compositional change of hyaline cartilage matrix to estimate change of the GAG concentration. In more advanced stages of OA when structural change plagues the tissue the substrate of investigation (densely packed GAG) vanish and results will prove progressively less reliable. It remains to quantify what level of GAG depletion marks the edge of meaningful range of T1Gd. In advanced degeneration and other abnormal cartilage tissue, a potential increase in baseline T1 variability has a potential for measurement error.

Safety concerns.

The first 20 years of clinical use of GdCAs were quite uncomplicated, with a general perception of high safety, especially in comparison to traditional radiographic contrast agents such as iodine – a substance with notorious risk of anaphylaxis and renal complications. Widespread use of high-dose (0.3 mmol/kg) GdCA for neuro-oncology and MRI-angiograph became common. GdCAs are considered relatively safe as regards acute reactions: 1/1000 have immediate hypersensitivity reaction with pruritus or urticaria; 1/5000 have more severe hypersensitive reaction such as bronchospasm or facial oedema; 1/20,000 have anaphylaxis. More than 100 million patients have been exposed to GdCAs with a reported number of associated deaths of 40 (Jung 2012; Behzadi 2018; Ramalho 2016). GdCAs have been used for over 30 years in over 100 million patients and were used in roughly 30–45% of all clinical MR studies 2015 (Kanal 2016).

Toxic reactions: Nephrogenic systemic fibrosis, gadolinium depositions.

In 2006 a rare mysterious condition of systemic progressive fibrosis in patients with kidney disease was linked to GdCA exposure (Marckmann 2006; Perazella 2009; McDonald 2015) and was termed Nephrogenic Systemic Fibrosis (NSF). The pathologic process is not fully elucidated. Activation of CD34+ fibroblast is

demonstrated and in addition to impaired kidney function immunological processes are involved. NSF is a progressive, incurable and usually fatal disorder characterized by skin thickening, painful joint contractures, and fibrosis of multiple organs including the lungs, liver, muscles, and heart. Several hundred cases were reported worldwide. Since mid 2009 very few new cases have been reported, as screening for renal insufficiency become the norm before administration of GdCA (Ramalho 2016). In patients with normal kidney function GdCAs continued to be regarded as safe to use in most patients, with occasional case reports of nephrotoxicity, acute tubular necrosis, acute pancreatitis and possible encephalopathy in patients with exceptional cumulative dose exposure.

Since 2014 renewed concerns regarding GdCA have emerged, with reports of depositions in bone and brain in patients with normal renal function. Accumulation in dentate nuclei and globus pallidus regions of basal ganglia was seen in unenhanced T1 scans (Kanda 2014) and confirmed in autopsy specimen (Kanda 2015a). These findings resulted in extensive research with MRI, biopsy and animal studies that have established dose dependence and repeated dose-dependent accumulation, even in patients with normal renal function. Deposition is reported in tissues of skin, bone matrix and brain and with lesser certainty spleen and liver. The presence of deposits is termed Gadolinium Storage Condition (GSC) and is not considered to have demonstrated neurotoxicity or other tissue-toxic effects in significant numbers. In the affected brain locations symptoms of parkinsonism and disturbed motor learning could presumably occur from neurotoxicity, but such symptoms have not been described (Pullicino 2018; Tedeschi 2017).

Recently reports of diffuse symptoms possibly linked to GSC causes renewed concern: gadolinium deposition disease (GDD) represents symptoms in patients with normal renal function who have received a GdCA agent. It is suggested to involve inflammatory response to Gd deposition and producing symptoms of pain in bone, skin and subcutaneous tissue in distal extremities, and some reported cases of progression to skin and subcutaneous tissue thickening and discoloration in distal extremities, with some patients experiencing affected mentation (brain fog) (Semelka 2016). GDD differs from NSF in having a semi-acute onset with remaining pain and diffuse symptoms, while NSF is slowly progressive and severe. Overall symptomatology and pathogenesis are not yet well established. Susceptibility is suggested to be dependent on genetic variation, and a pathobiology with activation of inflammatory pathways involving CD34+ fibrocytes is hypothesized. A limited number of GDD cases, 200–300, are known and roughly 1000 undiagnosed cases are estimated (Ramalho 2016; Tedeschi 2017; Semelka 2016).

The culprit of Gd deposition is the linear GdCAs having a binding to Gd that under physiological conditions is less stable than previously realized. Macrocyclic

gadolinium chelates have higher molecular stability and are demonstrated to have significantly fewer or no brain depositions compared to linear chelates (Kanda 2015b; Radbruch 2015) and to be more completely eliminated (Lancelot 2016).

National regulatory bodies have responded to the emerging safety concerns and have restricted the use of linear GdCA such as Gd-DTPA^{2-} and recommend to instead use macrocyclic GdCAs with consideration (FDA_document: 2018; EMA_document: 2017).

Future of dGEMRIC

The best candidate to substitute for Gd-DTPA^{2-} in dGEMRIC is the macrocyclic electronegative Gd-DOTA^{-} (gadoteric acid, Dotarem®). Gd-DOTA^{-} has been tried as substitution for Gd-DTPA^{2-} in dGEMRIC of hip, wrist and knee cartilage with comparable result (Rehnitz 2017; Zilkens 2012).

Intra-articular administration of GdCA after ultrasound-guided aspiration of the knee synovial fluid increases safety by reducing the dose to 1/1000 of the intravenous dose (Hangaard 2017; Hangaard 2018) and has been performed also in the hip (Zilkens 2014; Boesen 2006). While early results are promising, further validation of this route of administration is warranted.

Gadolinium agents have to be further evaluated for safety concerns but at the present time macrocyclical GdCA appear safe to pursue. The collective findings from dGEMRIC studies demonstrate a sensitive technique for monitoring cartilage quality reflecting cartilage GAG change in health, post-trauma, post-surgery, pre- and early-OA and to have prognostic ability regarding OA development. The search for an optimal biomarker of cartilage change continues and the dGEMRIC experience has shown the potential of image biomarkers and added immensely to the knowledge of the in-vivo processes and importance of GAG change, to be expanded on by future research.

Aims of the thesis

General aim

To evaluate dGEMRIC as a diagnostic and prognostic tool in patients with a high risk of developing knee OA.

Specific aims

Study I

To establish the intra- and inter-observer variability in T1 with a standardized ROI drawing technique for femoral knee cartilage.

Study II

To investigate the prognostic value of dGEMRIC for long-term radiographic and symptomatic osteoarthritis after a surgically treated cartilage injury on the medial femoral condyle.

Study III

To evaluate the knee cartilage quality after an ACL injury using dGEMRIC, both cross-sectionally and longitudinally, and to examine the influence of an associated meniscus injury and ACLR.

Study IV

To investigate the prognostic value of dGEMRIC for long-term radiographic and symptomatic osteoarthritis after ACL injury.

Methods

Patients and surveys

Study I

The data sets from previously performed dGEMRIC examination of twelve volunteer males were used, mean age 24 (range 23–29) years, with no history of knee injuries or operations. The patients had been examined in a previous study (Tiderius 2004a). The six investigators were selected to represent differentiated methodological competence: two experienced skeletal radiologists and one orthopaedic surgeon with experience of the MRI workstation, one junior orthopaedic surgeon and two medical school students with no experience of the MRI workstation.

The four sets of images, i.e. medial and lateral of pre-contrast and post-contrast, of the 12 volunteers were subject to measurements twice within 5–10 days by each of the six investigators independently. The volume, height, and length were then measured from the recorded ROIs separately.

Study II:

Fifteen patients (10 men and 5 women) with 16 knees (one woman had bilateral injuries) of median age 37 (range: 30–47) years with surgically treated cartilage injuries of the medial femoral condyle were included.

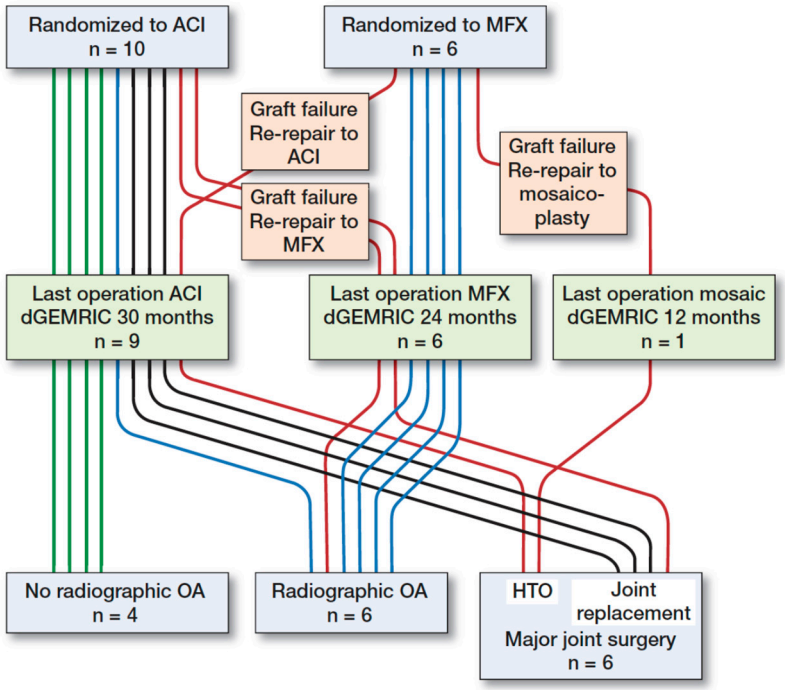


Figure III. Flow chart of the patients of study II.

The 15 patients were recruited from a randomized study designed to compare treatments for full thickness cartilage defects, who had been discontinued after 3 years as it had proven more difficult to find patients with injuries matching the inclusion criteria than anticipated and due to logistical challenges. The study's inclusion criteria were: isolated full thickness cartilage injury of the medial femoral condyle and a history of normal knee function and no symptoms before injury. Exclusion criteria were: radiographic evidence of OA or evidence of cartilage degeneration at arthroscopy, concomitant disease, injury or malalignment. Patients were randomized to either MFX or ACI treatment and surgery were performed in 1997-2000. The mean size of the treated traumatic chondral injury was 268 mm^2 (range 120–600 mm^2). Due to early clinical failure 2 patients in each group had to be re-operated within the first 2 years (*figure III*). One patient initially treated with MFX was reoperated with mosaicoplasty as a salvage procedure. In that patient, cartilage-bone plugs were harvested from unloaded joint regions and implanted to the injury site. This resulted in 9 knees treated with ACI, 6 knees treated with MFX drilling and one knee treated with mosaicoplasty at the time of dGEMRIC examination. All knees were examined with dGEMRIC a median 27 (12–57) months after the cartilage repair procedure. The 4 early graft failure reoperations

were performed 12–15 months prior to the dGEMRIC examination. dGEMRIC values were compared to values of a reference cohort of 19 asymptomatic individuals (mean age 24 years) that had previously been investigated with an identical MRI protocol (Tiderius 2001).

The relevant available medical records, including surgical reports and archived radiographs were studied in all patients until the 17-year follow-up. An average 17 years after operation, patients were investigated by radiographs and PROMS.

Study III

Of an initial cohort of 40 patients with acute ACL injury, 29 patients (19 men and 10 women) of mean age 27 (range 14–40) years could be included in the 2-year dGEMRIC follow-up.

Patients were included prospectively between February 2000 and June 2005 at irregular intervals depending on the compliance of the emergency unit physicians at Skåne University Hospital in Malmö and examined by dGEMRIC and clinical reassessment within 3 weeks (range: 3–47 days) of the trauma. The inclusion criteria were: closed physes, MRI verified acute ACL rupture, and the exclusion criteria were: radiographic OA, previous injury.

Thirty-seven of the 40 patients were re-examined with dGEMRIC on average 2 years (range 7–60 months) after the initial investigation. Seven patients had to be excluded because of motion artefacts in the MR images, defined as having >10% of the pixels within the ROI outside a T1 interval of 0–1300 ms. One patient had to be excluded because his opposite knee was examined by mistake at follow-up.

dGEMRIC results of 24 healthy volunteers (14 men), 22–29 years of age (mean 25), who had participated in a previous dGEMRIC study (Tiderius 2004a) were used as age- and activity-matched reference values to represent ‘healthy’ cartilage (mean activity level 3, BMI 22.5 ± 2.3 kg/m^2).

Meniscus injury and bone marrow oedema (BML) was cross-sectionally identified by the radiologist’s diagnosis of meniscus injury on the diagnostic series MRI performed prior to the dGEMRIC sequences at the two examinations.

The study had no influence on the treatment of the ACL injury. Treatment followed standard treatment algorithm; patients performed supervised neuromuscular knee rehabilitation and were examined regularly as out-patients for assessment of rehabilitating progress and individual evaluation of the need for ACLR based on subjective instability or high knee-demands.

Study IV

Thirty-one patients (19 men and 12 women) with a mean age of 27 (15–40) years from the initial cohort of 40 patients with acute ACL injury could be included for a 14-year follow-up.

All 40 patients could be located but 2 (of whom one had not attended 2 year follow-up either) did not respond to repeat contact attempts and 3 replied but did not show up for radiographic investigations. Of the 35 patients that attended the radiographic investigation 2 had not completed the 2-year dGEMRIC examination and 2 had technical artefacts in the dGEMRIC examinations.

Self-reported PROMS

KOOS, ***studies II–IV***

To characterize symptomatic OA (SOA) we used the operational definition described by Englund and Roos (Englund 2003), aiming to identify individuals symptomatic enough to possibly seek medical care. The definition of a symptomatic knee required that the score for the KOOS subscale Quality of Life (QOL) and 2 of the 4 additional subscales should be equal to or less than the score obtained as follows: at least 50% of the questions within the subscale were answered with at least a 1-step decrease from the best response (indicating no pain/best possible function, etc.) on a 5-point Likert scale. After conversion to a 0–100 scale (0 worst, 100 best), the cut-offs were as follows: pain 86.1, symptoms 85.7, ADL 86.8, sport/rec 85.0, and QOL 87.5. This operational definition of SOA is not specific to medial or lateral symptoms.

Lysholm, ***studies II-IV***

Modified (1985) Lysholm score (Tegner 1985) as self-administrated PROMS was used to assess knee function and symptoms. The Lysholm score covers 8 subscales representing aspects of knee function for a score of 0–100, with 100 corresponding to full knee health. A result of ≤84 is regarded as an indication of having knee problems during daily life and representing a usable cut-off point for dichotomization (Rockborn 1995).

Activity scale

In **Study III** we used an 'in-house' 4-grade Likert scale to document activity level during the year before investigation. The scale was selected in order to be comparable to recent other local studies, it is not validated but is quite similar to the Tegner activity scale in character (Tegner 1985), rescaled to four levels: 1 sedentary, 2 moderate exercise with regular activities on average 2 times weekly, 3 regular

physical activity more than 2 times weekly, 4 regular activities at elite/competition level (Tiderius 2005).

Arthroscopy and surgery

Surgical Treatment of Cartilage Defects. ***Study II***

Orthopaedic centres in southern Sweden were invited to refer potential participants to the orthopaedic centre of the university clinic. Diagnostic arthroscopy had thus been performed before patients entered the study. Three different surgeons participated in the study. At the start of the study arthroscopy was performed and the status of cartilage and injury was classified, after which patients were randomized for ACI or Pridie (MFX) treatment. ACI was performed according to the method described by Brittberg et al. (Brittberg 1994). A quantity of 500 mg hyaline cartilage containing no bony tissue was extirpated from the medial rim of the medial femoral condyle cartilage on the edge of the intercondylar notch and sent by courier for immediate processing at the cartilage laboratory facility. After 2 weeks of cell culture expansion the chondrocytes were returned to be implanted via an anteromedial mini-arthrotomy. The cartilage lesion was debrided to stable edges, a periosteal flap was sutured over the lesion and sealed by fibrin glue and the in-vitro expanded chondrocytes were injected under the flap. The knees randomized to MFX had the defect debrided to stable edges and the lesion drilled with a Ø2 mm drill with a c-c of 4–6 mm. Both groups performed the same postoperative protocol of supervised physiotherapy with 6 weeks of unloading followed by 6 weeks of progressive weight-bearing as described for postoperative management of ACI (Brittberg 1994). According to the initial study protocol, patients were scheduled to evaluation arthroscopy with the option of cartilage sample. As it turned out, 6 patients did not accept the investigation and in the 10 knees evaluated cartilage assessment and samples of fibrocartilaginous tissue were extracted only in 6 patients.

During the time from dGEMRIC to the 17-year follow-up 6 patients had major knee operations for intolerable OA knee symptoms, with no involvement from the study; 2 patients were treated with HTO, 2 with UKA and 2 with TKA. In addition, at the time of the follow-up study 2 patients were considering TKA.

ACLR, arthroscopies and meniscectomies. ***Study III***

ACLR was arthroscopically performed by ipsilateral gracilis/semitendinosus autologous graft in 14 patients. Meniscus tears were treated as needed when present at the ACLR procedure or when found in knees at arthroscopy for suspected tears (n=5). No meniscus tears needed suture. APM were performed for tears that risked

progression or had potential mechanical involvement and consisted of trimming of the tears to stable tissue. 10 knees had no arthroscopies as they needed no ACLR and had no significant meniscus symptoms.

*Surgery distribution in **study IV** and interim surgery during long-term follow-up period. **Study IV**.*

A slightly changed mix of patients from the initial cohort were available for 14-year follow-up compared to 2-year follow-up as described in 'Methods/patients'. The frequency of surgery from injury to 2-year follow-up was 14 ACLR, 6 medial and 6 lateral APM. At the long-term follow-up additionally 7 ACLR, 3 medial and 5 lateral APM had been performed and one knee had performed HTO due to SOA and medial ROA.

Radiography

Studies II and **IV** use weight-bearing radiographs to assess long-term radiographic OA outcome. Standing posterior-anterior radiographs were obtained using a standardized knee position with both knees at 20° flexion and weight-bearing. Fluoroscopy was used to optimize tibial plateau alignment.

Radiographs were independently read and scored according to the OARSI atlas reference material by two investigators, one senior radiologist specialized in skeletal radiology (B.L.) and one orthopaedic surgeon specialized in arthroplasty (J.T). The separate scores were compared and in cases of discrepancy images were re-read in cooperation and a consensus was reached. Dichotomization of radiographic OA (ROA or no ROA) for the respective medial or lateral compartment was achieved by use of the method described by Englund (Englund 2003). The medial and lateral femorotibial joint compartments are analysed separately. For the diagnosis of ROA in the respective compartment (medial or lateral) one of three criteria had to be met; #1: JSN ≥2 , #2: Sum of femur and tibia osteophyte score is ≥2 or more in the compartment, or #3: Sum of JSN and osteophyte score is ≥2 in the compartment. As a proxy for grade of ROA in the respective medial and lateral compartments the OARSI osteophyte score was used and treated as a semi-quantitative monotonic variable.

Some patients had moved too far to be interested in travelling to Lund University's facilities to undergo radiography. In such cases, we arranged to have radiographic investigation at a radiologic unit at the patient's locations and had electronic radiographic images transferred to the Lund University Hospital radiology database (PACS). These and were critically examined by a radiologist for quality. Ideally all patients should have been examined by the same equipment and identical procedure.

We concluded that as we did not aim to perform repeated examinations it was sufficient to use the standard flexion weight-bearing radiographs. By the same reasoning, if a patient had recently performed a radiographic examination, we accepted that investigation to spare the patient the exposure to radiation. In **Study II** for the 2 patients that did not show up to radiographs, it was possible to analyse radiographs several years old and as radiographic criteria exceeded the endpoint of OA diagnosis, dichotomization to ROA was made but we decided to not evaluate the radiographs for grade of OA by osteophytes because of the much shorter follow-up time than the rest of the cohort. In **Study IV**, for one patient operated with high tibial valgus osteotomy 11 years after the injury, the immediate preoperative radiographs were used for radiographic scoring, and the patient was dichotomized as SOA.

MRI

The ACL injured cohort in **studies III** and **IV** had the clinically suspected injury confirmed by standard 1.5 T MRI diagnostic protocol immediately prior to T1Gd acquisition. The baseline examination was planned at approximately 3 weeks after injury and the follow-up examination at 2 years. The radiologists' report of the diagnostic MRI examinations was used to document concomitant injuries (e.g. meniscus injury, cartilage injury, bone bruise).

dGEMRIC

All patients of the four studies and respective reference cohorts were examined by the same validated dGEMRIC protocol on the same 1.5 T MRI magnet.

Patients in **study II** were investigated at median 27 (12-57) months after surgery. Patients in **Studies III** and **IV** were investigated approximately 3 weeks (range 3-47 days) and 2 years (range 7-60 months) after the injury. A standard 1.5 T MRI system with a dedicated knee coil (Magnetom Vision; Siemens Medical Solutions, Erlangen, Germany) was used until April 2004 when the MRI unit was moved due to hospital rebuilding projects. The magnet was refitted with a Siemens SONATA system and calibration and phantom validation were performed. The investigations after July 2004 were all performed on the SONATA system.

Gd-DTPA^{2-} (Magnevist®, Schering AG, Berlin, Germany) was injected in the antecubital vein at 0.3 mmol/kg body weight. To optimize the distribution of Gd-DTPA^{2-} into the cartilage the patients exercised by walking up and down stairs for approximately 10 min, starting 5 min after injection. Post-contrast imaging with

subsequent T1-relaxation time calculation of the cartilage was performed 2 h after the injection. Central parts of the weight-bearing lateral and medial femoral cartilage were identified, and quantitative relaxation time calculations were made in a 3 mm thick sagittal slice on each condyle, using sets of six turbo inversion recovery (IR) images with different inversion times (TR= 2000 ms, TE= 15 ms, FoV 120×120 mm^2, matrix= 256×256, TI= 50, 100, 200, 400, 800 and 1600 ms). Total imaging time was approximately 20 min. Additionally in **study I** pre contrast TI: 100, 200, 400, 800,1600 and 2800 ms was used.

The region of interest

The most relevant region of the femoral cartilage to detect early compositional changes is the area of weight-bearing contact. On the tibial side cartilage is protected the meniscus, whereas on the femoral condyle a larger area is subject to contact stresses as the contact point moves posterior dependent on flexion angle. This area posterior to the longitudinal apex of the condyle is also where cartilage injury and osteoarthritic lesion are typically most common (Boegård 1997).

The distal-posterior femoral cartilage is thus our region of interest (ROI) to study and its definition is important for reproducible results. We defined this area as the full femoral cartilage thickness from a starting point opposite the centre of the tibia plateau on the femoral cartilage surface, continuing posteriorly on the femoral cartilage surface to the posterior edge of the meniscus dorsal horn where a 90° turn is made to the subchondral bone, thereafter the cartilage edge to subchondral bone is followed anteriorly to the AP position of the starting point where a 90 turn to connect the line to the starting point was made. The ROI was manually drawn using the T1 1600 ms post-contrast image in the MRI workstation software (2800ms in precontrast). ROI measurement of the time-image series is used for T1-relaxation time calculation using a three-parameter fit (Kingsley 1999).

In **Study II** the ROI of the medial compartment was modified to one ROI including the cartilage repair tissue, and one ROI of the standard region excluding the cartilage repair tissue thus representing the cartilage adjacent to the injury

In **Studies III** and **IV** we used full-thickness ROI drawn in the medial and lateral sagittal slice representing femoral weight-bearing cartilage according to the method described in **Study I**.

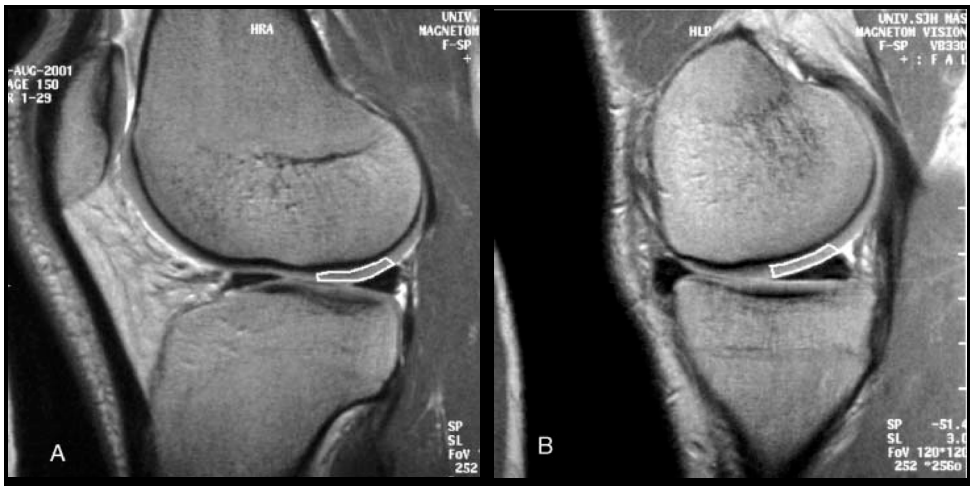


Figure IV. Examples of the regions of interest (ROI) manually defined. Lateral (A) and medial (B)
The same definition of ROI was used in **studies I, III and IV**. In **study II** the medial compartment ROI was modified to include the repair tissue in one ROI and the cartilage adjacent to the repair tissue within the normal ROI in a second ROI.

Ethics

Studies were approved by the Ethical Review Board at Lund University (Etikprövningsnämnden); **Study I**: LU#73-96 and LU#651-00, **Study III**: LU#651-00, **Studies II** and **IV**: #EPN:2014/752.

For **Studies II** and **IV** separate approval was also obtained from the Radiation Protection Committee (Strålskyddskommittén #SSFo2014-050) and the Image Research Committee (BOF053).

Signed informed consent was collected from all participants before the start of all studies. For **Study II** and **Study IV** renewed informed consent was signed when patients agreed to participate in the long-term follow-up, before the start of long-term follow-up data collection.

Statistical analysis

Data were stored and organized in Excel and Access (Microsoft), statistic calculations were performed on the Statistical Package for the Social Sciences (SPSS, IBM), graphs are SPSS, SigmaPlot 11.0 (Systat Software, San Jose, CA), Veusz 3.0.1 (GNU, Sanders, www.veusz.github.io), R Ggplot2 3.2.1 (R –

foundation for Statistical software, www.R-project.org), Illustrations and flowchart were produced using the open-source application draw.io (JGraph Ltd, Northampton, UK).

Study I

Reproducibility of one investigator's measurement can have a problem of systematic error causing uni-directional difference of the first and second measurement. This is assessed by calculating systematic error.

$$Systematic\ Error = \frac{mean\ of\ measurement\ 1 - mean\ of\ measurement\ 2}{2}$$

Furthermore, the precision of one investigator's measurement is limited and this variation is termed random error and was calculated from the difference in standard deviations of measurement 1 and 2. The Coefficient of Variation in % (C.V.%) is a measure of the intra-observer variability calculated from the random error.

$$Random\ error = SD\ of \left(\frac{measure\ 1 - measure\ 2}{\sqrt{2}}\right)$$

$$C.V.\% = \left(\frac{random\ error}{overall\ mean}\right) x100$$

The inter-observer variability is the observer's measurements deviation from the true mean of all observations for which the mean of all six investigators was used as an approximation. The variability is expressed in % and is calculated as the mean of first and second measurement performed by one investigator compared to the mean of all six investigators.

Non-parametric statistics were used for the statistical evaluation; the Wilcoxon signed rank test was used to evaluate differences between pre- and post-contrast analyses. Friedman ANOVA and the Mann Whitney U-test were used to evaluate inter-observer differences. Spearman rank sum test was used for the correlations between inter-observer variability and ROI height and length.

Study II

After testing for normal distribution (Shapiro–Wilk) and equal variance (Levene's mean test), the Student t-test was used for continuous variables. Paired t-test was used for regional measurements in the same knee; a non-paired test was used in all other instances, and 2-tailed distribution was assumed in all tests. Spearman rank correlation was used for correlation of ordinal data and continuous variables. Fisher's exact test was used to compare the distribution in cases of 2 dichotomous variables. The statistical power was low due to the few patients eligible for this study. One patient had a bilateral operation, strictly the data from the two knees are not independent. We decided to include both knees in the analysis as the hypothetical influence on the main observation, correlation of dGEMRIC to ROA, was reasoned to be irrelevant.

Study III

Student's t-test for paired and unpaired observations was used for the parametric statistics, i.e. comparisons of T1Gd values between the ACL-injured cohort and the healthy reference subjects, and in subgroup analysis within the ACL-injured cohort. The Mann Whitney test was used for non-parametric statistics (activity level). The Pearson correlation was used for the correlation between T1Gd at follow-up and the time to follow-up. All tests were two-tailed. Analysis of covariance (ANCOVA) was performed to evaluate bias factors, the result should be cautiously interpreted due to the small number of observations.

Study IV

95% confidence interval (95%CI) was used to compare difference of means (MD) and Student's t-test was used complementary.

Correlations were evaluated with Spearman rank correlation for ordinal variables (e.g., osteophyte score). Fisher exact test was used for dichotomous variables. The probability of ROA (presented in Figure 2) was determined with logistic regression using maximum likelihood estimation. For a very approximate feasibility analysis a power calculation was performed based on the results from a previous longitudinal dGEMRIC study with dichotomous ROA as outcome variable (Owman 2008). The smallest sample size for power=80% (50% incidence, α=0.05) was 16 patients and 30 patients for power=90%. The available sample size was too small to allow for multiple regression analysis and potential covariates showed no correlation when surveyed.

Results

Intra- and inter-observer variability is low when a large standardized ROI is used in dGEMRIC. Study I

In **Study I** we investigated the measurement errors of a standardized method to define the cartilage region of interest. The experience of the observer, ranging from students of medicine that were novice to MRI to expert radiologists, did not significantly affect variability. No systematic error between the observer's serial investigation (inter-observer systematic error) was found with the mean T1Gd difference for all measures < ±0.4%.

Observers have low individual variability in measurement and are in good agreement. ***Study I****.*

The variation of the measurements in post-contrast images by individual observers was very low, with mean intra-observer coefficient of variation (CV%) below 3% for all observers and mean CV% of 1.5% in medial compartment and 2.6% in lateral compartment. The observers' measurements were in good agreement with each other with the range of mean inter-observer variability between 1.3%–2.3% in the post-contrast images.

Post contrast images have less variability. ***Study I***

ROI measurement of post-contrast (T1Gd) images had lower variability ($p<0.05$) than measurements of pre-contrast (T1pre) or calculated difference of the pre- and post-contrast values ($\Delta R1$).

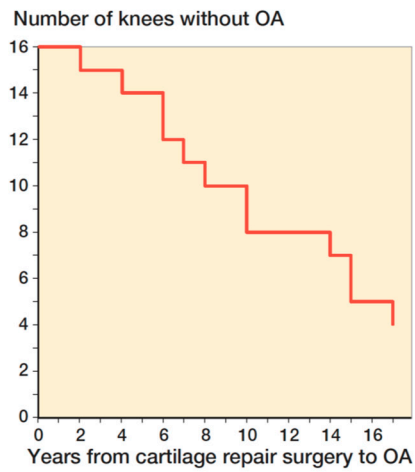
Thicker ROIs have less variability. ***Study I***

The variability was greater in the lateral compartment. The medial and lateral ROIs were of the same size, $p=0.67$, but differed slightly in shape, with the lateral ROI being more elongated, length 18.6 mm vs 16.6 mm ($p<0.01$), height 2.3 mm vs 2.5 mm ($p=0.05$). There was a larger measurement error (inter-observer variability) with thinner ROIs in the lateral compartment ($p<0.01$), but not in the medial. The mean of investigators' intra-observer CV% was higher in the lateral, 2.6%, than in the medial compartment, 1.5%, $p<0.05$.

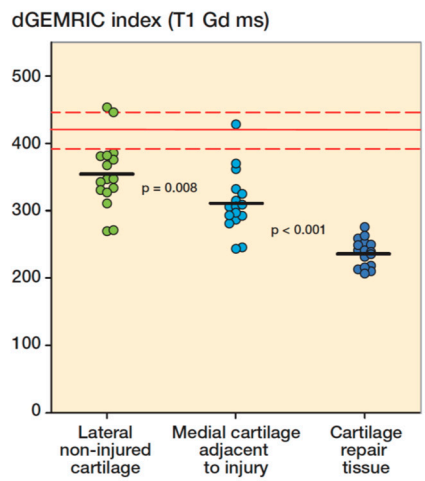
Surgically treated traumatic cartilage defects have high prevalence of OA after 17 years. Study II

In **study II** we found poor outcome at long-term follow-up of patients treated surgically for traumatic cartilage injury of the medial femoral condyle. After 17 years 75% have radiographic OA (ROA) and 88% have symptoms of OA (SOA). All of the included patients could be assessed in long-term follow-up.

Six knees had received OA surgery and was classified as having ROA and SOA; 2 had high tibial osteotomy, 2 had uni-compartmental medial knee arthroplasty and 2 had total knee arthroplasty. Six knees had radiographic OA based on OARSI scores, and 4 knees had no ROA. Thus 12 of 16 knees had failed by progressing to ROA. No radiographs had narrowing of the lateral joint space.



a.



b.

Figure V a. Study II. 12 of 16 knees progressed to radiographic OA (ROA) during the follow-up period. Knees that had performed surgery for OA (2 TKA, 2 UKA, 2 HTO) was counted as having progressed to ROA at the time of surgery, knees classified as ROA from reading of radiographs was counted from the time of the examinations.

Figure V b. Study II. Regions of femoral cartilage measured by dGEMRIC. Study II. The red line represent the average T1Gd and broken line standard deviations in a cohort of healthy volounteers examined with idential dGEMRIC protocol (Tiderius 2001). T1Gd of the non-injured lateral compartment was low compared to healthy volounteers. Medial compartment cartilage adjacent to the cartilage lesion was low comparted to the lateral compartment and high compared to the repair tissue ($p<0.01$).

The KOOS score was indicative of SOA in all but 2 knees, the two symptom-free knees had normal age-matched KOOS score and no ROA. All 4 knees that needed a second cartilage repair procedure progressed to ROA. All MFX-treated knees and 6 of 10 ACI treated knees developed ROA. BMI, sex, and size of injury were similar between knees

that developed ROA and those that did not. Patients who did not develop ROA were on average 4 years younger than patients who developed ROA (p = 0.1).

dGEMRIC detects variation in cartilage quality and character. Study II

In **study II** the three ROIs of the knee were expected to represent different cartilage quality. The non-injured lateral cartilage had higher T1Gd than the medial cartilage adjacent to injury, 354 ±51 ms vs 312 ±46 ms (p <0.008). Both values were considerably lower than values of healthy reference cartilage (Tiderius 2001). The repair tissue cartilage T1Gd mean value was very low and with little variation between knees, 237 ±20 ms, possibly representing inferior fibrocartilage. T1Gd of repair tissue showed no difference in ACI compared to MFX treated knees.

T1Gd show recovery in lateral compartment but remained low in the medial compartment 2 years after ACL injury. Study III

Three weeks after injury average T1Gd value were substantially lower (18–20% p<0.0002) in ACL injured knees than in healthy reference knees, with the medial compartment having lower T1Gd than the lateral (p=0.006). At 2-year follow-up T1Gd was still lower than in healthy references. It had increased slightly in the lateral compartment (+6% p=0.04) whereas the medial compartment showed little change (p=0.57).

Two years after injury, ACLR and no ACLR do not differ regarding T1Gd or clinical outcome. ***Study III***

T1Gd did not differ between the 14 patients treated with ACLR and the 15 patients without surgical treatment at the 2-year follow-up (p=0.92), and there was no difference in activity level or Lysholm score between groups. Age or sex did not correlate to T1Gd.

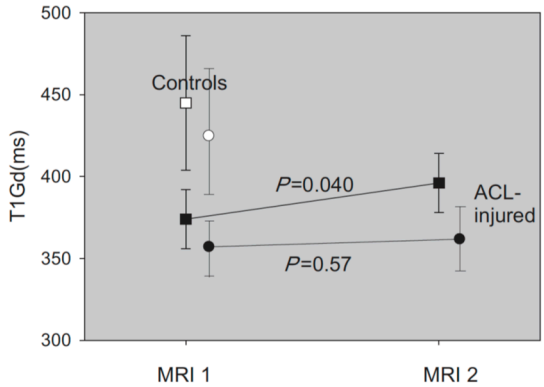


Figure VI. Study III. T1Gd in femoral cartilage after ACL injury and T1Gd of healthy reference knees. dGEMRIC perforemd at baseline 3 weeks after ACL injury (MRI 1) and 2 year follow-up (MRI 2) of medial ● and lateral ■ compartments. Healty knees as controls are marked as medial ○ and lateral □.

Meniscus injury correlates to lower T1Gd at 2-year follow-up. ***Study III***

At the 2-year follow-up APM had been performed on both menisci in 3 knees, the lateral meniscus in 5 and on the medial meniscus in 3. The meniscectomies were evenly distributed between ACLR (6 of 14) and non-ACLR (5 of 15).

At the 2-year follow-up knees with APM had lower T1Gd than knees with no APM, with difference of 84ms p=0.002 medially and 38 ms, p=0.05 laterally.

The longitudinal change from baseline to 2-year follow-up was influenced by APM. Medially knees with APM decreased T1Gd compared to knees without APM; – 30 ms and + 16 ms, p=0.09 , and laterally APM compared to knees with no APM; – 12 ms and + 35 ms, p=0.04.

There was no difference in time to follow-up between patients that had been or had not been subject to APM.

Meniscus injury identified by MRI correlates to lower T1Gd at 2-year follow-up. ***Study III***

MRI detected meniscus injury at either baseline or at 2-year follow-up was found in 10 medial menisci and in 13 lateral menisci. T1Gd in medial compartment was lower in knees with medial meniscus injury 325 ms, with a difference of 57 ms (p=0.01) to knees with no meniscus injury. Corresponding values for lateral meniscal tears were 381 ms with 27 ms difference (p=0.13).

BMI correlates to T1Gd at 2-year follow-up. ***Study III***

An increase in T1Gd value was seen between baseline and follow-up in the medial compartment of patients with normal weight (BMI <25, n=19) of + 27 ms

(p=0.013), whereas in overweight (BMI >25, n=10) mean value decreased by – 33 ms (p=0.12). In the lateral compartment the corresponding changes were + 34 ms (p=0.02) in normal weight and no change in overweight.

T1Gd increased with length of follow-up time. ***Study III****.*

A positive Pearson correlation was found between the time from baseline investigation to follow-up and the T1Gd value; medially r=0.41, p=0.026 and laterally r=0.35, p=0.061

Meniscectomy and time to follow-up persisted as significantly associated with T1Gd value at follow-up after adjustment for covariates, as did lateral meniscectomy to negative change of T1Gd. ***Study III***

BMI and length of follow-up time were recognized as potential confounders to the association of APM and T1Gd at 2-year follow-up (cross-sectional analysis). ANCOVA for the two confounders was performed with APM as fixed factor and T1Gd at 2-year follow-up as the continuous variable. The association of T1Gd and APM persisted at p=0.002 medially (unchanged) and p=0.04 laterally (from p=0.05). The correlation of follow-up time and T1Gd changed slightly, p=0.06 medially (from=0.026) and p=0.046 laterally (from p=0.06). No significant associations persisted between T1Gd and BMI medially or laterally with p values of p=0.19 medially and p=0.43 laterally.

ANCOVA with the change in T1Gd between baseline and follow-up studies (longitudinal analysis) had not substantially altered the association between APM and reduction in T1Gd p=0.14 medially (from p=0.09) and p=0.04 laterally (unchanged). No significant associations remained between change in T1Gd and either BMI or length of follow-up time.

High prevalence of OA 14 years after ACL injury. Study IV

Radiographic OA (ROA) was present in 21 of 31 (68%) of ACL-injured knees at follow-up. Of these, 7 knees had isolated medial ROA, 11 had isolated lateral ROA and 3 knees had ROA in both compartments. ROA of the index knee was present in 6 of 12 women and 15 of 19 men.

OA symptoms (SOA) were present in 13 of 31 patients (42%). Two patients had SOA without radiographic signs of OA. BMI at injury did not differ (BMI 23.3 vs 24.7 p=0.16) between patients with and without ROA development.

T1Gd value of the cartilage adjacent to the injury relate to later ROA development. Study II

In all measured cartilage ROIs in **Study II** mean T1Gd was low compared to reference healthy knees (figure V b). A slightly higher T1Gd was observed in the adjacent cartilage of the 4 knees that did not develop medial ROA, 348 ms vs. 300 ms (p= 0.07). The dGEMRIC index in the medial cartilage adjacent to the lesion correlated negatively with the radiographic osteophyte score in the medial compartment at the 17-year follow-up, r= –0.75 (p = 0.03).

Low T1Gd in the medial compartment at dGEMRIC 2 years after ACL injury is associated with worse long-time outcome regarding ROA, SOA and knee function. Study IV

Medial T1Gd 2 years after injury correlates to ipsi-compartmental ROA at 14-year follow-up. ***Study IV***

Medial compartment T1 was lower in the 10 knees that developed medial ROA, 325 ±68 ms compared to the 21 knees that did not develop ROA, 376 ±47 ms, MD 50.7 (95%CI 7.2 to 94). Corresponding value in the lateral compartment were 370 ±46 vs. 399 ±42 p=0.74 MD29.3 (95%CI –3.1 to 62).

Medial T1Gd 2 years after injury correlates to SOA at 14-year follow-up. ***Study IV***

The 14 patients who developed OA symptoms (SOA) had lower dGEMRIC index in the medial knee compartment, 327 ±61 ms, versus the 17 patients that did not develop SOA, 380 ±51, MD52.4 (95%CI 11 to 93). Also, the 14 patients who developed poor knee function had lower dGEMRIC index in the medial compartment, 337 ±54, versus the 17 who did not develop poor knee function, 381 ±52 ms, MD 48 (95%CI 7.2 to 89), compared to those that did not develop ROA or SOA. T1Gd value of the lateral compartment had no significant correlation to development of ROA or SOA (p<0.13).

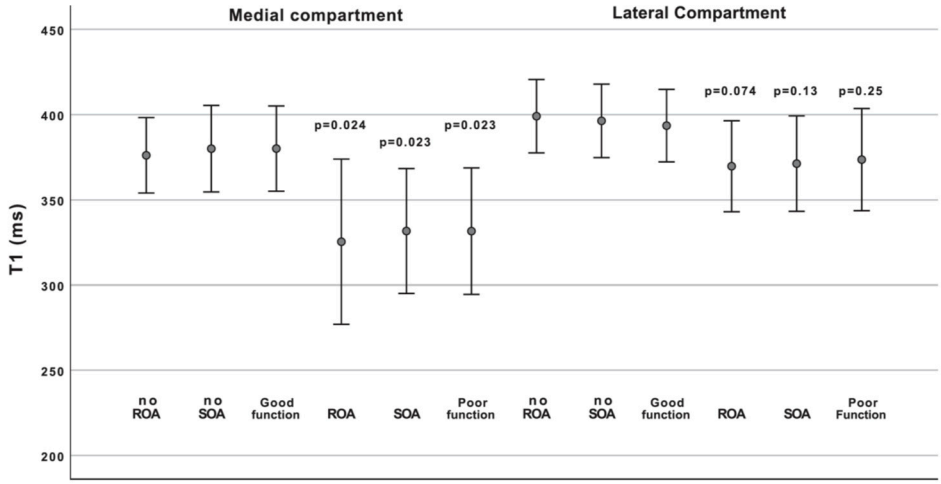


Figure VII. Dots represent average T1Gdm, bars represent 95%CI.
2 years after ACL injury, the medial compartment T1Gd was lower ($p<0.05$) in the patients that 14 years after ACL injury had developed ROA, SOA or poor knee function.

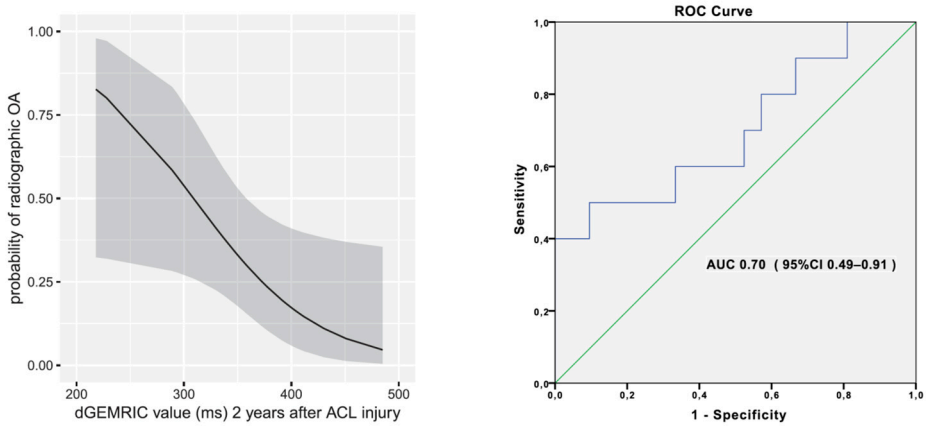
dGEMRIC 2 year after ACL injury correlates to the grade of ROA. ***Study IV***

The dGEMRIC index correlated negatively to ipsi-compartmental OARSI osteophyte score in medial ($r= – 0.44$, $p= 0.01$) and lateral ($r= – 0.38$, $p= 0.03$) compartments. Osteophyte score is a proxy for worsening grade of OA.

T1Gd in prediction of OA development. ***Study IV***

The association of medial T1Gd and ROA development was examined by receiver operated characteristics (ROC) curve statistics. AUC was 0.70 (95%CI 0.49–0.91) with a maximal sum of sensitivity =40% and specificity =91% (Youden Index) at 330 ms. Analysis of the confusion matrix with 330 ms cut-off yield a positive predictive value (PPV) of 71% and a negative predictive value (NPV) of 79%. The probability plot has wide confidence intervals. Odds ratio for a meaningful clinical difference of 50ms is 2.35 (95%CI 1.05–5.4).

Post hoc power analysis indicated that the above analysis is underpowered, a sample of 54 knees are needed to support 80% power and 0.05 significance.



a. b.

Figure VIII a. Curve of calculated probability of developing medial radiographic OA depending on dGEMRIC value of medial femoral cartilage.
14 years after ACL injury, the shaded area represents 95%CI.

Figure VIII b. Reciever operated characteristics (ROC) curve of medial T1Gd as a marker of ROA outcome.
The area under the curve was 0.70 (95%CI 0.49-0.91). Maximum Youden index was 330 ms with sensitivity=50% and specificity=91%.

Additional results, not presented in papers

ACLR and no ACLR do not differ regarding ROA or SOA in the long run. Study IV.

There was no statistical difference between the rate of ROA after 14 years for having ACLR (10 ROA in 18 ACLR knees) and not having ACLR operation performed (8 ROA in 10 no-ACLR knees) prior to the 2-year follow-up, 56% vs 80% (p=0.12). During the interim 3 more knees had ACLR, which did not alter the statistical conclusion.

Meniscus injury and ROA outcome. Study IV

Distribution of APM (within 2 years after ACL injury) and 14-year ROA in medial and lateral compartments were examined by cross-tabulation, the chi square test was p=0.067 medially and p=1.0 laterally.

Table I
Artrosopic partial meniscectomies (APM) within baseline – 2 year follow up and ROA at 14 year follow up. Medial p=0.067, Lateral p=1

p=0.067	ROA	noROA		p=1	ROA	noROA
2y Med APM	4	2		2y Lat APM	4	4
2y not Med APM	6	19		2y not Lat APM	10	13

Accumulated (2-year + interim) APM correlated to ROA in medial and lateral compartments p=0.025 and p=0.057 respectively.

Table II
Accumulated artrosopic partial meniscectomies (APM) baseline – 14 year follow up and ROA at 14 year follow up. Medial p=0.025, lateral p=0.057

P=0.025	ROA	No ROA		P=0.057	ROA	No ROA
Accum Medial APM	6	3		Accum Lat eral APM	9	2
Accum Medial not APM	9	13		Accum Lateral not APM	8	12

Is lateral ROA less symptomatic? SOA correlates to ROA in the medial but not in the lateral compartment. Study IV.

In the medial compartment ROA was present in 10 knees, of which 8 had SOA and 2 had no symptoms. In the 21 knees with no medial ROA 5 had symptoms and 16 had no symptoms. The chi square test was p=0.006 for SOA associated with ROA in the medial compartment. By contrast, in the lateral compartment of the 14 knees with lateral ROA, only 6 had SOA and 8 had no symptoms. Of the 17 knees with no lateral ROA 7 had SOA and 10 had no symptoms. The chi square test was p=1 for SOA associated with ROA in the lateral compartment.

Individual observations in patients who did not develop OA 17 years after attempted cartilage repair. Study II

The 4 knees that did not develop ROA were all treated by ACI and had a slightly higher dGEMRIC index of the adjacent cartilage 348 ms vs. 300 ms (p = 0.07). The 9 knees with ACI had slightly higher adjacent cartilage T1Gd compared to the 6 MFX knees 331 ms vs. 283 ms (p = 0.05).

Discussion

Intra- and inter-observer variability. Study I

The precision of dGEMRIC as a tool for GAG estimation depends on several factors: hardware calibration, contrast effective dose, contrast penetration, timing, image orientation, and crucially on the investigator's manual placement of the region of interest borders in the correct position within cartilage.

Study I investigates one important variable among all of these variables, the manual part of ROI drawing. The results show that a large standardized ROI yields a low measurement error, the mean T1Gd inter-observer variations being <2.3% for six different investigators and the intra-observer T1Gd variability being <2% in medial and <3% in lateral compartment. Another important finding was that expertise did not correlate to variation, which supports a more wide-spread use of the technique.

The intra-observer CV% was significantly higher for ROI in the pre-contrast image (T1pre) compared to post-contrast images (T1Gd), $p<0.05$. To use the difference of pre- and post-contrast image signal ($\Delta R1$) adds two measurements errors and consequently the intra-observer CV% of $\Delta R1$ was higher than in T1pre and T1Gd, $p<0.05$. Likewise, T1Gd inter-observer variability was lower than variability in measurement of the pre-contrast (T1pre) and the $\Delta R1$ values. One reason for the lesser error in post-contrast image ROI is that the visual contrast in the 1600 ms image used is very good at the cartilage edge, facilitating the manual outlining of the ROI. The observation of less variation in the thicker medial ROI compared to a thinner and longer lateral ROI is explained by longer borders to subchondral bone and cartilage surface, resulting in a higher proportion of voxels being potentially misplaced and cartilage region of higher variation. Generally, a small ROI has a larger proportions of border voxels that risk containing partial volume effects of subchondral bone or synovial fluid. Also, small signal irregularities are averaged out if the ROI is larger. Previously, the information from T1Gd and $\Delta R1$ were reported to be similar (Tiderius 2003) and therefore we suggested excluding pre-contrast images in dGEMRIC studies.

Generally, CV% <5% is considered as very good repeatability in imaging studies (Vignon 2003), and 2-3% variation is small compared to measured T1Gd differences in patient groups of 10-25%.

A limitation implied but not stressed enough in the publication is that the validation was performed in young healthy individuals with a thick cartilage. Since cartilage thickness decreases with the progression of OA, the measurement error will likely increase in thin degenerative cartilage.

High rate of OA at long-term follow-up. Studies II and IV

In **studies II** and **IV** the prevalence of ROA at long-term follow-up was substantial at 75% and 68% respectively.

The knees of **study II** were reported to have an isolated traumatic cartilage injury (TCL) and no sign of degeneration at inclusion/surgery, meaning that the severity of T1Gd is worse than expected. Time-delay from injury to treatment was unavoidable as patients first had to be diagnosed with the cartilage injury and then referred to the university hospital orthopedic research unit. If pre-OA changes with GAG depletion are already in progress when the surgical repair procedure takes place, if already catabolic, surgery may have a knock-on effect on the depleting processes (Larsson 2017). Also, the patients were relatively old at mean age 37 years, potentially affecting the cartilage reparative capacity. Evidently the cartilage quality was low, and the rate of long-term OA development was high. The ROA rate of 75% is comparably high relative to other studies of cartilage repair reporting ROA prevalence after 10–15 years at 40–48% in MFX treated cohorts (Gudas 2012; Gobbi 2014; Ulstein 2014; Knutsen 2016), and 45%–57% at 10-15 years for ACI (Martinčič 2014; Ulstein 2014).

Twenty-one of 31 patients had ROA in the ACL injured cohort of **Study IV**. ROA isolated to medial compartment was present in 7 knees, isolated lateral ROA in 11 and bicompartmental ROA in 3. Patients with lateral ROA had lower rate of SOA than patients with medial ROA. The prevalence of 68% having ROA 14 years after ACL injury is in range of previous studies and consistent with a recent review of the last 10 years publications (Lie 2019) and on par with Øiestad et al. who in a study with 14 years follow up of ACL injuries reported 45% SOA and 69% ROA in a matching material, with average age 27 years at injury and a similar treatment algorithm (Oiestad 2010).

In the medial compartment the ROA knees match SOA ($p=0.006$) which supports a correct calibration of the sensitivity in scoring of the radiographs. In the lateral compartment ROA did not correlate with SOA ($p=1$), which might suggest some over-classification of ROA in the lateral compartment. However there was only slightly more ROA cases 14 vs 10 compared to medial compartment, and this medial/lateral ratio is in line with what would be expected from previous observations (Swärd 2010).

Follow-up time

Some 10-20 years is needed to follow the natural course of OA. Cartilage repair studies with 10-15 year follow up have much higher OA prevalence than studies with 2-8 year follow up (Knutsen 2016; Erggelet 2016). Several long-term ACL follow-up studies have documented increased ROA prevalence from 15-20% at 10 years to 20-50% at 20 years (Lohmander 2007; Shelbourne 2017). The present studies follow up times, 17 years for cartilage injury and 14 years for ACL injury are thus adequate.

The ability of dGEMRIC to monitor cartilage quality

The T1Gd results in **studies II-IV** appear to meaningfully quantify cartilage quality and support the assumption that the T1Gd relate to GAG concentration.

The adjacent cartilage in **study II** had very low T1Gd of 312 ms 27 months after cartilage surgery (figure IX). This finding can be regarded as adequate and clinically meaningful since several possible reasons for low cartilage quality exists; GAG loss related to the injury may have occurred already before the operation, increased mechanical loading and shearing stress due to the nearby cartilage defect of fibrocartilage repair tissue, potential effect of the surgery cytokine as a second hit and nearby fibrocartilage metabolism/cytokine milieu. Furthermore, very low T1Gd of repair tissue cartilage is consistent with previous reports of repair tissue typically consisting of fibrocartilage (Knutsen 2004, Mithoefer 2009, Erggelet and Vavken 2016). Compared to previous T1Gd results the values of adjacent cartilage of the medial compartment, a high risk of developing OA is indicated.

The uninjured lateral compartment T1Gd of 354 ms in **study II** was higher than in the medial compartment but significantly lower than in normal knees (figure IX). Age difference to the control group (37 vs 24 years) is unlikely to explain the whole difference, dGERMIC data for normal knees over 35 years of age are sparse but in a cohort of ACL injured long-time-copers of mean 45 years average T1Gd was 404 ms medial and 427 ms lateral with an identical dGEMRIC protocol (Neuman 2014) and in a mixed sample of dGEMRIC investigations age only weakly correlated to T1Gd (r2= -0.04) (Dahlberg 2012). Whole joint effects of cytokines from an active catabolism medially is one suggestion for low lateral T1Gd. Relative inactivity during postoperative rehabilitation is also possible, the dynamics of transient inactivity are not well elucidated. Owman et al. found 6 weeks of unloading to induce decreased T1Gd that had not returned to baseline after 1 year (Owman 2014b) and in another study 6–8 weeks of unloading resulted in T1ρ and T2 changes that reverted after 4 months of rehabilitation (Souza 2010).

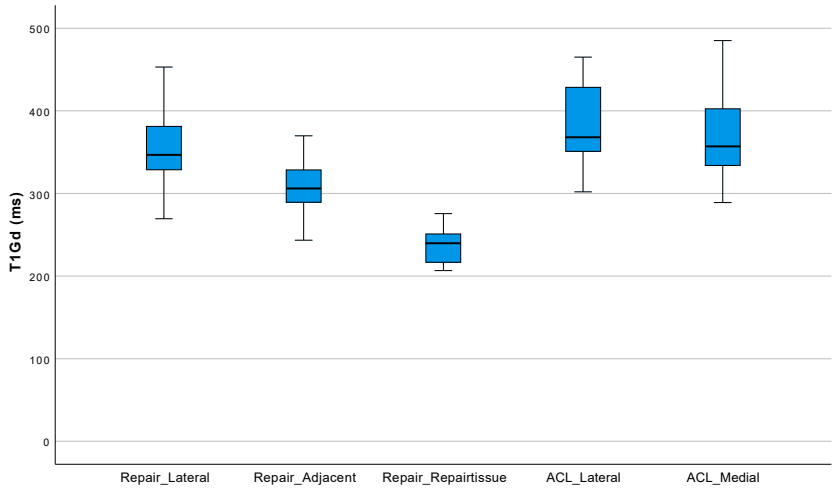


Figure IX. T1 Gd values of studies II and IV compared.

T1Gd of **study III** compared to the healthy reference cohort were low at baseline and at 2-year follow-up ($p<0.0002$). We have no indication that the ACL injured cohort had risk of low GAG content in their pre-injury state. The reference cohort had well-matched age (25 vs 26.6 years), BMI (23.3 vs 22.5) and average activity level was the same, 3= high (non-elite with ≥3 regular activity/week).

The observed low T1Gd in the acute phase after ACL injury is consistent with other studies (Fleming 2010) and was verified by increased GAG concentration in the synovial fluid of the injured knees (Tiderius 2005). Loss of GAG and other matrix component to SF fluid in the acute phase of ACL injury have been reported previously (Neuman 2017; Sward 2012; Dahlberg 1994).

Adequate and clinically meaningful measurements were demonstrated in **study III** by longitudinal dynamic T1Gd change over 2 years. Higher BMI, APM and longer time from injury to the follow-up correlated to a lower T1Gd. The strongest association was between partial APM and a low dGEMRIC value, this association persisted after covariate adjustment. However, the ANCOVA model should be cautiously interpreted as the observations of APM were few (only 6 medial and 8 lateral). The associations in medial compartment of APM and BMI to lower GAG estimated by T1Gd are consistent with these same factors being well known risk-factors for PTOA (Shelbourne 2017).

High BMI is a known risk factor for PTOA (Jones 2017; Kessler 2008) and for low T1Gd (Ericsson 2009), and although no significant association persisted in the ANCOVA model it is noteworthy (considering the model being underpowered) that a significant univariate association was found for BMI <25 with increased T1Gd

from baseline to 2-year follow-up (medially p=0.012 and laterally p=0.024 while patients of BMI>25 decreased in the medial compartment (p=0.12).

A similar ANCOVA model of the longitudinal change in T1Gd from baseline to the 2-year follow-up showed associations with APM laterally p=0.04, but not significantly medially p=0.14.

A limitation of **study III** is the unknown cartilage composition before the ACL injury. Well-conditioned athletes have significantly higher T1Gd than the person of average activity level, and conversely a person of sedentary activity profile has lower than average T1Gd (Tiderius 2004a). Thus, a significant trauma with severe GAG loss in an athlete may result in a T1Gd on par with a lesser trauma in a sedentary individual and the post traumatic value of T1Gd should not be considered a direct measure of degree of injury.

BML was present in 26 of 29 diagnostic MRI in the lateral femoral condyle. It has been speculated BML is a marker of higher trauma energy and cartilage compression injury MRI (Frobell 2008). However, the lateral compartment had higher T1Gd at baseline than the medial and had better recovery compared to medial compartment at 2-year follow-up.

T1Gd association with OA outcome and prediction, Studies II and IV.

In **studies II** and **IV**, the knees that developed medial ROA had very low T1Gd in the medial compartment. Comparable values were reported by Owman in knees that developed medial ROA in a study using an similar dGEMRIC protocol (Owman 2008). Comparing T1Gd (mean of medial and lateral compartment) of knees that developed ROA vs no ROA, Owman reported 312 ms vs 383 ms (p=0.03, n=9) compared to 300 ms vs 348 ms (p=0.07, n=16) in the present **study II**, and 325 vs 376 (p=0.02) in the present **study IV**.

A correlation of T1Gd 2 years after cartilage repair or ACL injury with marginal osteophyte score was found in **studies II** and **IV.** This finding is consistent with a comparable study by Owman (Owman 2014a) of 34 patients of mean age 41 years that had medial APM for degenerative lesion with a reported appearance of the cartilage as normal or showing mild degenerative change. dGEMRIC was performed 3.7 years after APM and radiographic long-term follow-up was performed 15 years after APM. The rate of ROA development was 80% and T1Gd correlated to medial JSN grade ($p<0.001$) and medial osteophyte score (p=0.001), results that are in line with the findings in **study IV** of correlation of medial osteophyte score to T1Gd (r=-0.38, p=0.03).

We can conclude that dGEMRIC is prognostic for OA development by providing a measure of cartilage quality by estimation of FCD/GAG. To correctly analyze the

predictive statistics a larger sample size is needed. With the available data estimations of sensitivity and specificity means that half of ROA cases will be missed but 71% will be correctly predicted by <330ms as predictor. The characteristics of the confusion-matrix (sensitivity 50%, specificity 91%, PPV 71% and NPV 91%) imply that T1Gd has merit as an individual predictor to advice risk but does not qualify as a stand-alone diagnostic test. The long follow-up time needed to evaluate ROA development introduce a directional variation due to random events (additional knee trauma) that potentially result in ROA cases not predicted by T1Gd. This effect will decrease test sensitivity. To correct for this effect, frequent clinical assessment and exclusion of cases with new trauma during the follow up time would be needed. The present studies do not have data of such granular quality.

Table III. Confusion matrix of T1Gd <330ms as predictor of ROA development.

Confusion matrix	Outcome positive ROA	Outcome negaitve No ROA	Value (95%CI)
Test positive ≤330 ms	5	2	Pos pred val 0.714 (0.19–0.81)
Test negatvive <330 ms	5	19	Neg pred val 0.792 (0.47–0.97)
Postive likelihood ratio = 5.25	Sensitivity 0.50	Specificity 0.91	Negative likelihood ratio = 0.55

One study has previously reported knee dGEMRIC predicting ROA progression over 24 months AUC=0.68 p=0.02 (Eckstein 2011). Regarding the hip, Kim et al have shown excellent predictive value of T1Gd for OA outcome after hip osteotomy after 32 months (Kim 2012). It could be speculated that OA is more difficult to predict in the knee compared to the hip due to more moving parts, including menisci of variable quality, instability, repeat trauma, malalignment and complex patterns of movement.

In summary T1Gd is a feasible biomarker for cartilage quality and can be used to measure quality, to monitor early and dynamic change, and to use as a prognostic tool. In a clinical situation individual measures of T1Gd must be interpreted wisely in context with other information and other available data – as is true for most medical measurements.

The findings in the context of cMRI

Several studies have investigated whether cMRI can be used as a biomarker to monitor cartilage change and to predict progression of degeneration and OA development. A number of studies of the osteoarthritis initiative (OAI) cohorts with have found the cMRI technique T2-relaxation to show association with progression in a shorter time scale on MRI features (Prasad 2013; Joseph 2012) and to radiographic features and ROA over 3-4 years (Liebl 2015). T2-relaxtion is related with decreased type II collagen order and breakdown which imply some degree of structural change and irreversibility and is envisioned as a later step of cartilage change than decreased GAG content. T1ρ measure is regarded as being mostly dependent on the GAG/FCD and has shown association with progression of cartilage lesions graded by MRI over 2 years (Prasad 2013) and with cartilage in symptomatic hip impingement that progressed to ROA (Gallo 2016).

An advantage of dGEMRIC is its relative specificity to changes in GAG/fixed charge density in a linear rate, whereas other cMRI has a vaguer specific mechanism. dGEMRIC has also been used clinically and experimentally for a longer history than other cMRI techniques, accumulating several studies of well-demonstrated ability to monitor cartilage change related to risk factors (Tiderius 2004a; Van Ginckel 2010; Roos 2005; Hawezi 2016; Ericsson 2009) and injuries (Tiderius 2005; Fleming 2010), and to show association with the development of radiographic features (Owman 2014a) and development of OA (Owman 2008; Palmer 2017). A study of failure after Bernese hip osteotomy for dysplasia had excellent predictive value of preoperative T1Gd of anterolateral hip joint (n=43) to failure as outcome at 32 months, with ROC curve characteristics of AUC= 0.977, and at the cut-off value of 370 ms exhibiting 100% sensitivity and 95% specificity (Kim 2012). Another study in hips following FAI and OA progression over 5 years had dGEMRIC prediction at AUC=0.75 (Palmer 2017).

The use of dGEMRIC in evaluating cartilage repair

ACI and MFX produced repair tissue of uniformly very low T1Gd that indicate low quality of the repair tissue 2 years after treatment in **study II**. While fibrocartilage of inferior quality has been the predominant finding in many prior studies, finds of cases with hyaline-like cartilage are also reported (Mithoefer 2009; Erggelet 2016; Knutsen 2004). One long-term evaluation in a cohort of mixed location of cartilage injury treated with ACI had excellent 8–18 year follow-up with T1Gd of repair tissue on a par with adjacent cartilage and reference healthy cartilage (Vasiliadis 2010). A factor to explain variable treatment result is the condition of the adjacent cartilage, which is rarely well characterized beyond arthroscopic survey and KL grade. This was recently addressed in a study with preoperative dGEMRIC and T2-

relaxation evaluation of adjacent cartilage prior to ACI and will be examined for association to future outcome (Aroen 2016).

Paradoxically, treatment of most cartilage injuries has not evolved markedly since Pridie pioneered the technique in the 1950s. MFX is the most common treatment of cartilage defects due to its feasibility. ACI is complicated and has not consistently produced better results than MFX (Knutsen 2016; Kraeutler 2017). Currently 3rd generation ACI explores numerous strategies and several studies are underway. dGEMRIC and other cMRI techniques may provide a route to evaluate the results and to stratify preoperative cartilage health to facilitate advancement of research in the field.

Study II limitation

Study II has a very long-follow up time. The 2-year follow-up parameters represent a starting point from which random events and lifestyle exposure dilute correlations. Large sample size and frequent observations can remedy this to some extent. Both strategies are lacking in this study of 16 knees over 17 years. Whereas the low number of patients hampers statistical power, we have managed to follow the fate of all knees.

The surgically treated cartilage injury is such a potent risk factor as to result in 75% ROA compared to the background age-matched population ROA prevalence of 15–30% (van Saase 1989; Turkiewicz 2015; Jordan 2007).

The 4 knees with no OA are not meaningful in a statistical sense, but their individually higher dGEMRIC and the fact that all have been treated with ACI is information best left simply described rather than analysed.

The measurements of the cartilage defects have some uncertainty; the border to adjacent cartilage is variably difficult to define on the 1600 ms image. The repair tissue is of variable thickness and structure; partial volume effects, cracks, delamination and defects are not fully discernible on the 1.5 T image and could potentially alter the measured value. For all cases of the study the measured T1Gd of repair tissue was low and with little variation, a result supporting the conclusion of fibrocartilage repair tissue low in GAG concentration.

Study III and IV, considerations and limitations

***Study IV**. Reason for investigating the 2-year follow-up rather than baseline or ΔT1Gd.*

The 2-year follow-up dGEMRIC was selected for investigating prediction of long-term OA development in favour of baseline dGEMRIC values or ΔdGEMRIC. Potentially baseline dGEMRIC has more variation due to inflammatory trauma response, variable amount of joint effusion, variable compliance to the 10-minute exercise due to pain and wide range of time from injury with investigation conducted average 22 ±8 (range 3-43) days after trauma. There was however no apparent difference in the standard variation of T1Gd at the investigations. After 2 years the majority of surgical treatment and rehabilitation has occurred and time for potential GAG restitution has passed and the joint cartilage may be at a relatively 'steady state'. A minimum of 6 months from arthroscopy was required before 2-year follow-up with dGEMRIC.

*Difficulty in characterizing all meniscus problems. **Studies III and IV**.*

Knees that did not need ACLR only performed arthroscopy if symptomatic, and only knees with mechanical instability after neuromuscular rehabilitation performed ACLR. Thus, no cross-sectional Gold Standard assessment of meniscus injury was performed and indeed more meniscus injuries were found on the MRI. APM during the baseline to 2-year follow-up was associated with ipsi-compartmental low T1Gd. Meniscus injury detected on MRI at either baseline or at 2-year follow-up was associated with lower ipsi-compartmental T1Gd (medial p=0.01, lateral p=0.13).

Meniscectomy in the interim has additional methodological concerns. ROA can induce degenerative changes in the meniscus causing APM, this is an example of autocorrelation if APM as a risk of OA development is studied. Furthermore, the interim data is potentially incomplete regarding treatment at other centres. However, adding the cases of interim meniscus injury strengthened the association to low T1Gd.

The importance of cartilage evaluation.

To adequately discuss the merits of cMRI techniques we need to consider the longitudinal sequence of changes in cartilage matrix. In healthy cartilage dynamic homeostatic changes seem to occur (Kiviranta 1994; Kiviranta 1988; Sivan 2014; Kiviranta 1992; Van Ginckel 2010; Tiderius 2004a; Kessler 2008) and warrants monitoring to understand the concepts of normal biology dynamic and to elucidate beneficial processes. The pre-OA stage (Ryd 2015) is characterized by a spectrum of cartilage compositional change but with no structural or macroscopical change. The next step, early-OA implies structural change visually detectable by

arthroscopy or MRI features in the absence of radiographic change. It is in these early phases cMRI is set to play an important role. Later stages of deteriorating and lost cartilage with radiographic progression is of less interest for compositional analysis.

Different cMRI techniques will have different window of detection. A “pre-OA window” of GAG sensitive techniques to monitor potentially reversible homeostasis and pre-OA change and a second “early-OA window” for techniques sensitive to type II collagen change and altered tissue organization to monitor the progression into irreversible OA change. A combination of image biomarkers from the two windows would provide optimal measuring, monitoring and prediction capacities. Combining cMRI for assessment of current status of the cartilage with wet biomarkers for grading intensity of metabolic activity seems a way forward

Major conclusions of the thesis

- dGEMRIC has a low intra- and inter- observer variability with regard to the critical part of manual ROI drawing.
- A traumatic cartilage injury is associated with a high prevalence of OA after 17 years.
- A concomitant meniscus injury is associated with impaired cartilage quality in ACL-injured patients.
- A low dGEMRIC index (T1Gd) is associated with long term development of symptomatic OA
- A low dGEMRIC index (T1Gd) is associated with long term development of radiographic OA
- Non-invasive assessment of cartilage quality with dGEMRIC is feasible and measure relevant variations in a clinical context.
- Cartilage quality assessed with dGEMRIC have prognostic capacity relative knee OA development.

Summary: findings and future

Findings

The studies conducted in this thesis concern the use of dGEMRIC technique to assess cartilage quality in a clinical setting. In addition to studying methodological aspects and validation of the technique, the study of two cohorts of known high OA risk found T1Gd to be low 2-3 years after injury/operation and to indicate clinically relevant variations.

The association of T1Gd to long-term development of both structural OA changes and OA symptoms, suggest dGEMRIC can be used as a much-needed proxy for outcome, enabling shorter term studies.

Future

In order for a hypothetical treatment to be effective in arresting degenerative development it needs to act before the cartilage is lost. In order to develop such treatments, techniques to non-invasively monitor early cartilage changes in-vivo is warranted. There are clinical data to support the idea that GAG can be replenished by intervention, such as physical exercise, osteotomies and -patella stabilising surgery. Dynamic GAG content is further supported by results of animal studies. Understanding of GAG metabolism and effects of possible interventions is of importance for saving cartilage from degeneration.

dGEMRIC with Gd-DTPA^{2-} as the contrast agent has safety issues and may no longer be possible to use for cartilage assessment. Macrocyclic Gd-DOTA^{1-} seem to be safe and has been shown to function as substitute for Gd-DTPA^{2-} producing similar results in similar settings. Developments in MRI technique has presented alternative contrast free cMRI techniques that are promising but so far has either a window of detection that is of more advanced cartilage alteration or less well understood correlation of MRI signal to the molecular alterations.

Studies with long term outcomes are difficult to conduct and are inherently slow to attain results. cMRI technique with a window of detection framing the earliest cartilage alterations are needed. Validation of dGEMRIC with Gd-DOTA^{1-} seems the most urgent pursuit.

Appealing future uses of dGERMIC, or an alternative cMRI technique, as a location specific biomarker of cartilage quality are many; to monitor homeostatic changes in

healthy cartilage in response to activity and risk factors, to identify the earliest changes of pre-OA and detect a point of no return, to monitor attempts to modify degenerative progression in pre-OA and early-OA with treatment such as experimental disease modifying OA drugs (DMOAD) and physiotherapy, to monitor effects of surgical interventions, to monitor the effect of trauma and treatment, to perform pre-treatment stratification to characterize patients that are likely to benefit from a treatment such as ACLR, APM and cartilage repair and to shorten the time required to assess outcome by use of T1Gd as a proxy outcome.

From available results it is clear that early alterations in hyaline joint cartilage are prognostic for degenerative development. To examine the if T1Gd can function as a predictor for individual OA risk new studies with larger cohorts are needed to perform appropriate statistics to validate prediction.

Today high field MRI 3 T is becoming common (and some ultra-high field 7 T facilities are accessible) and with advancements in computational power and MRI sequences, researchers strive to achieve contrast free cMRI sensitive to GAG and collagen. The legacy of dGEMRIC findings should motivate this development. Contrast free examination would eliminate the biases from plasma volume and synovial volume, post contrast exercise and delay for diffusion time. It would also eliminate safety issues.

Summary of papers

Paper I

Delayed Gadolinium Enhanced MRI of Cartilage (dGEMRIC): Intra- and inter-observer variability in standardized drawing of regions of interest.

Objective: To establish the intra- and inter-observer variability of our standardised ROI drawing technique in lateral and medial femoral weight bearing cartilage.

Methods: Six investigators variously skilled in MRI performed ROI drawings in images of 12 healthy male volunteers on two occasions with an interval of one week. Calculated T1 values were evaluated for intra- and inter-observer variations.

Results: The mean inter-observer variability for both compartments ranged between 1.3% and 2.3% for the six different investigators without correlation to the experience of the investigators. The post-contrast intra-observer reliability was 2.6% for lateral and 1.5% for medial femoral cartilage. The larger variability in lateral compared to medial cartilage was related to slightly longer and thinner ROIs laterally.

Conclusions: Intra- as well as inter-observer variability is low when a large, standardized ROI is used in dGEMRIC. The experience of the investigator does not affect the variability. Result support a clinical applicability of the method.

Paper II

Poor outcome after a surgically treated chondral injury on the medial femoral condyle: early evaluation with dGEMRIC and 17-year radiographic and clinical follow-up in 16 knees

Objective: To explore the results 17 years after surgical treatment of an isolated cartilage injury by radiographs and assessment of symptoms. To evaluate the influence of quality of the cartilage adjacent to the injury to long term OA development. To investigate the prognostic value of cartilage quality assessed by dGEMRIC to long term OA development.

Methods: 16 knees with an isolated traumatic cartilage injury of the medial femoral condyle had cartilage repair surgery either by microfracture or autologous cartilage implantation. dGEMRIC of the injured knee was performed 2 years after surgery, radiographic examination and assessment of symptoms were performed 17 years after the operation.

Results: Radiographic OA was present in 12 of 16 knees. Irrespective of surgical method, the dGEMRIC index was lower in repair tissue compared with adjacent cartilage in the medial compartment, which in turn had lower value than in the non-injured lateral cartilage. The dGEMRIC index in the cartilage adjacent to the repair tissue correlated negatively with radiographic osteophyte score. Knees that did not develop OA had higher dGEMRIC index of borderline significance.

Conclusion: A traumatic cartilage injury is associated with a high prevalence of OA after 17 years. The low dGEMRIC index in the repair tissue 2 years postoperatively indicates fibrocartilage of low quality. The negative correlation between the dGEMRIC index in the adjacent cartilage and future OA suggests that the quality of the surrounding cartilage influences outcome after cartilage repair surgery.

Paper III

Longitudinal assessment of femoral knee cartilage quality using contrast enhanced MRI (dGEMRIC) in patients with anterior cruciate ligament injury - comparison with asymptomatic volunteers

Objective: To longitudinally analyse femoral cartilage quality after ACL injury by repeated dGEMRIC examinations. To relate the cartilage quality changes to meniscus injury and other patient characteristics.

Method: 29 patients (10 women) were analysed with dGEMRIC 3 weeks after ACL injury and again 2 years after injury. For reference we used a control group of 24 non injured knees from a previous study(Tiderius 2004a).

Results: dGEMRIC values 3 weeks and 2 years after ACL injury were low compared to controls. Medial femoral cartilage showed similar T1Gd at the two dGEMRIC investigations, whereas the lateral femoral cartilage T1Gd increased. At follow-up T1Gd was lower in patients that had been partially meniscectomized.

Conclusions: The general decrease in dGEMRIC index of femoral cartilage in ACL-injured patients compared with references provide evidence for compositional matrix changes that seem more pronounced if a concomitant meniscal injury is present.

Paper IV

Osteoarthritis development related to cartilage quality- the prognostic value of dGEMRIC after anterior cruciate ligament injury

Objective: To investigate the prognostic value of the dGEMRIC index regarding future knee OA in a cohort at risk.

Method: 31 patients with ACL injury were examined after 2 years with dGEMRIC of femoral cartilage. 14 years post-injury patients were investigated with weight-bearing knee radiographs, Lysholm and Knee Osteoarthritis Outcome Score (KOOS).

Results: At the 14-year follow-up radiographic OA (ROA) was present in 68% and OA symptoms (SOA) in 42% of the injured knees. The dGEMRIC index of the medial compartment 2-years after injury was lower in knees that developed medial ROA, in patients that developed SOA, and knees that developed poor function. The dGEMRIC index correlated negatively with osteophytes as a marker for OA grade in medial and lateral compartments.

Conclusion: The associations between a low dGEMRIC index and future radiographic OA as well as symptoms of OA indicate that evaluation of cartilage quality with dGEMRIC has a prognostic value for future knee OA.

Populärvetenskaplig sammanfattning

Det är den fantastiska broskvävnaden som gör att våra leder kan fungera så bra. Broskvävnaden skyddar benändarna från att skava mot varandra. Dess stötdämpande förmåga och ytterst låga friktion gör det möjligt för oss att förflytta oss och att utföra tungt arbete. Även vid långvarig intensiv aktivitet, som till exempel vid maratonlöpning, fungerar lederna utan värmeutveckling och slitage. Den största och mest komplicerade av våra leder är knäleden. Den utsätts dagligen för stora belastningar och påfrestningar med kombinerade böj- och vridrörelser samt för stötkrafter vid stegisättning.

Efter att broskvävnaden färdigbildats vid skelettmognaden verkar den sedan inte kunna återbildas om den skadas strukturellt. Broskvävnaden underhålls kontinuerligt genom utbyte av dess molekylära komponenter via ett komplext system av parallellt pågående nedbrytande och uppbyggande processer. Obalanser i det systemet resulterar i ett svagare brosk. Beroende på belastningar, tidigare skador och riskfaktorer som övervikt och dålig muskelstyrka kan en långsam nedbrytningsprocess ta överhanden vilket medför att broskvävnaden degenererar. Aktiverade inflammatoriska signalsystem, nedbrytande enzymer och broskvävnadens ökande känslighet för slitage skapar en ond cirkel. Över en period om ungefär tio-tjugo år degenererar brosket alltmer för att slutligen nötas bort helt.

Ackumuleringen av påfrestningar under livet och den långsamma degenerationsprocessen medför att artros blir allt vanligare med stigande ålder. Under senare tredjedelen av livet drabbas ungefär varannan person av artros med varierande grad av symptom. Korsbandsskador och andra knäskador kan göra att nedbrytningsprocesserna inleds så att patienten utvecklar artros i förhållandevis unga år. Svullna, stela och smärtande leder medför lidande och funktionsbortfall för den drabbade. Avancerad knäledsartros som ger stora besvär kan behandlas med knäledsprotes. Även om resultatet för de allra flesta är gott finns det risk för allvarliga infektioner och andra komplikationer. Risken att behöva göra en ny protesoperation är betydande för yngre patienter eftersom protesen kan slitas ut med tiden. Avvägande av risk och nytta med operation medför att många lever med kroniska artrosbesvär i en utsträckning som begränsar deras aktiviteter och livsföring.

Idag saknas behandling mot den degenerationsprocess som leder till artros. En eventuell medicinsk behandling som bromsar eller stoppar nedbrytningen kommer

att ha bättre förutsättningar att lyckas ju tidigare den kan sättas in. Därför är det viktigt att utveckla metoder som kan upptäcka tidiga förändringar i broskvävnaden.

Broskvävnadens uppbyggnad och kvalitet

Broskvävnaden är både slitstark mot dragkrafter och tålig mot kompression eftersom den har en hård strukturell armering och tät men mjuk fyllning. Broskvävnaden utgörs av en gel som genomkorsas av ett tredimensionellt nätverk av dragfasta kollagenfibrer. Gelen består av mycket tätt packade vattenhaltiga makromolekyler (aggrekaner). Aggrekanernas struktur kan liknas vid flaskborstar. De har en skaftlik långsträckt proteinkärna, till vilken ett hundratal långa kedjor av repeterade sockermolekyler – flaskborstens borststrån– är bundna. Aggrekanerna är i sin tur i flera hundratal förankrade vid mycket långa bärande strukturer som genomkorsar broskvävnaden. Dessa på den molekylära skalan gigantiska komplex hålls fixerade i kollagennätverket genom sin storlek. Sockerarterna i aggrekanerna, borsten på flaskborsten, utgörs av glykosaminoglykaner (GAG) med stark negativ laddning. GAG attraherar positivt laddade joner som i sin tur genom osmos drar åt sig mängder av vattenmolekyler. Eftersom laddningstätheten i GAG är hög och kollagennätverket oeftergivligt byggs ett högt svällningstryck upp i broskvävnaden. Högre halter av GAG ger högre svällningstryck och bättre motstånd mot kompression.

Halten av GAG verkar kunna variera något och anpassas efter behov genom balanserande omsättning i friskt brosk. Sjunkande halter av GAG medför att brosket blir mjukare och är ett tecken på begynnande degenerativ utveckling. Mjukare brosk trycks samman och påfrestningar i det strukturella nätverket kan stimulera till ökad nedbrytning genom inflammation. Den degenerativa processen blir självförstärkande och leder till att kollagennätverket bryts ner av enzymer i överaktiverade reparationsprocesser. Det förefaller som om mindre förluster av GAG kan fyllas på igen men ett nedbrutet välorganiserat kollagennätverk verkar inte kunna återbildas.

Broskvävnadens funktion kan liknas vid det slitstarka däcket på ett cykelhjul. När däcket är välpumpat rullar cykeln bra och ojämnheter dämpas samtidigt som hjulets fälg skyddas. Lätt minskat däcktryck ökar slitaget på däcket något men ger inga större besvär. Kraftigt minskat däcktryck gör resan vansklig och riskerar haveri med både förstört däck och fälg. Att mäta och åtgärda däcktrycket i tidig fas av ”pyspunka” kan säkra god funktion i många år.

Att studera broskvävnadens kvalitet och artrosutveckling

Det finns ett stort behov av att närmare förstå och kunna mäta förlopp av uppbyggnad och nedbrytning i broskvävnad. Traditionella metoder som röntgen, artroskopi och vanlig magnetkameraundersökning kan bara avbilda ganska långt gångna förändringar där broskvävnaden är så förstörd att den delvis saknas och inte

kommer att kunna återbildas. Nya metoder utvecklas för att mäta broskvävnaden i så tidig fas av förändring som möjligt. Flera innovativa magnetkameratekniker är lovande men tenderar att upptäcka förändringar där kollagennätverket redan har börjat skadas. Kontrastförstärkt magnetkameraundersökning (eng. förk. dGEMRIC) kan studera förändringar i ett ännu tidigare skede genom att uppskatta halten av GAG. Metoden innebär att ett kontrastmedel fördelar sig i relation till broskvävnadens laddningstäthet, vilket kan användas som en indikation på broskets kvalitet.

Korsbandsskador och broskskador medför en massivt ökad artrosrisk vilket gör dem angelägna att studera. Vi har haft möjlighet att med dGEMRIC undersöka broskkvalitet i samband med sådana skador och genom långtidsuppföljning utvärdera hur uppmätt broskkvalitet relaterar till artrosutveckling.

Avhandlingen består av fyra delarbeten. I alla arbeten är kvaliteten i knäledens ledbrosk uppmätt med dGEMRIC. Det första arbetet handlar om metodens mätfel och de övriga relaterar mätresultat efter knäskador till artrosutveckling vid långtidsuppföljning.

Delarbete I

Alla undersökningar har en viss osäkerhet. För att karaktärisera mätfelet i tekniken att mäta broskkvaliteten lät vi två röntgenläkare, två ortopeder och två läkarstudenter oberoende av varandra genomföra mätningar av flera dGEMRIC-bilder vid två tillfällen. Vi jämförde resultaten och fann att mätfelet var litet och att skillnaden mellan olika undersökares mätningar var små. Undersökarens grad av erfarenhet gjorde heller inte någon skillnad på mätresultatet.

Delarbete II

Sexton knäleder undersöktes med dGEMRIC teknik två år efter att ha opererats med syfte att åstadkomma läkning av traumatisk broskskada. Vi kunde konstatera att lagningen inte skapat brosk av god kvalitet. Dessutom var omgivande broskvävnad av jämförelsevis så dålig kvalitet att en stor risk för artrosutveckling kunde förväntas. Vid röntgenundersökning 17 år efter operationerna visade det sig att 12 av 16 patienter hade utvecklat artros. De patienter som utvecklade artros hade sämre broskkvalitet vid tvåårskontrollen. Patienterna i grupperna är dock få vilket gör statistiken osäker. Artrosförändringarna blev också värre ju sämre broskkvaliteten var.

Delarbete III

29 patienter med främre korsbandsskada genomgick två undersökningar med dGEMRIC-teknik av det skadade knäet. Första undersökningen utfördes ca tre veckor efter skadan och den andra drygt två år efter skadan. Genomsnittsåldern vid skadan var 27 år. Efter en skada på det främre korsbandet var broskkvaliteten sämre

jämfört med en grupp tidigare undersökta friska personer. En förnyad undersökning visade på viss förbättring av broskkvaliteten efter två år hos de patienter som ej hade samtidig meniskskada eller var överviktiga. Korsbandsrekonstruktion ledde inte till någon skillnad i broskkvalitet vid undersökning två år efter skadan.

Delarbete IV

Artrosförekomst 14 år efter skadan undersöktes hos 31 patienter ur den korsbandsskadade gruppen. Röntgenbilderna hos mer än hälften av patienterna visade artrosförändringar och nästan hälften upplevde artrossymptom. Om korsbandsskadan opererats eller ej hade ingen inverkan på artrosförekomst. Den med dGEMRIC uppmätta kvaliteten på ledbrosket i den inre (mediala) ledkammaren två år efter skadan var klart sämre hos den grupp patienter som senare utvecklade artros, både i form av röntgenbekräftad artros i den mediala ledkammaren och hos den grupp som utvecklade artrossymptom. Graden av artrosförändringar samvarierade negativt med dGEMRIC-värdet.

Slutsatser

dGEMRIC mäter relevanta skillnader i broskkvalitet hos patienter i klinisk miljö.

Metoden att definiera mätområdet vid dGEMRIC är pålitligt med litet mätfel både för den individuella observatören och mellan olika observatörers mätningar.

Hög frekvens av artrosutveckling efter korsbandsskador och broskskador konstaterades, vilket bekräftar vad man sett i tidigare undersökningar.

Viss återhämtning av broskkvalitet sker efter den akuta försämringen i samband med främre korsbandsskada. Återhämtning sker inte om menisken samtidigt skadats.

Dålig kvalitet i ledbrosk två år efter korsbandsskada är associerat med och har prognostiskt värde för långtidsutveckling av både röntgenpåvisad artros och artrossymptom.

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Paper I

ORIGINAL ARTICLE ACTA RADIOLOGICA

Delayed Gadolinium-Enhanced MRI of Cartilage (dGEMRIC): Intra- and Interobserver Variability in Standardized Drawing of Regions of Interest

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Tiderius CJ, Tjörnstrand J, Åkeson P, Södersten K, Dahlberg L, Leander P. Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC): intra- and interobserver variability in standardized drawing of regions of interest. Acta Radiol 2004;45:628–634.

Purpose: To establish the reproducibility of a standardized region of interest (ROI) drawing procedure in delayed gadolinium-enhanced magnetic resonance imaging (MRI) of cartilage (dGEMRIC).
Material and Methods: A large ROI in lateral and medial femoral weight-bearing cartilage was drawn in images of 12 healthy male volunteers by 6 investigators with different skills in MRI. The procedure was done twice, with a 1-week interval. Calculated T1-values were evaluated for intra- and interobserver variability.
Results: The mean interobserver variability for both compartments ranged between 1.3% and 2.3% for the 6 different investigators without correlation to their experience in MRI. Post-contrast intra-observer variability was low in both the lateral and the medial femoral cartilage, 2.6% and 1.5%, respectively. The larger variability in lateral than in medial cartilage was related to slightly longer and thinner ROIs.
Conclusion: Intra-observer variability and interobserver variability are both low when a large standardized ROI is used in dGEMRIC. The experience of the investigator does not affect the variability, which further supports a clinical applicability of the method.

Key words: Cartilage; gadolinium; knee; MRI; reproducibility

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In osteoarthritis (OA), glycosaminoglycans (GAG) are lost from the cartilage matrix before macroscopic changes have occurred (8). Therefore new magnetic resonance imaging (MRI) techniques have been developed to analyze the GAG component of cartilage (5, 10, 11). One of these in particular is referred to as delayed gadolinium-enhanced MRI of cartilage, or dGEMRIC (2, 5). The negatively charged contrast agent Gd-DTPA^{2-} distributes within the cartilage matrix in an inverse relationship to the concentration of likewise negatively charged GAG. It has been shown that T1 in the presence of Gd-DTPA^{2-} ($T1_{Gd}$) is directly related to the content of GAG (2, 3).

In vivo, Gd-DTPA^{2-} is administered intravenously and distributes in the cartilage with a linear dose–response relationship, with the highest concentration approximately 2 h after injection (14). In patients with pre-radiographic degenerative cartilage changes, $T1_{Gd}$ was approximately 30% shorter compared to reference cartilage, consistent with GAG loss (15). In another study, healthy individuals with regular physical exercise had longer $T1_{Gd}$ than sedentary individuals, indicating that human cartilage can adapt to higher physical demands by increasing its GAG content (16).

Hence, dGEMRIC has shown promising preliminary results, but in order to develop the method for clinical use, a standardized protocol with reproducible results is needed. In our previous studies, quantitative T1 analysis was performed in a relatively large (150–250 voxels) region of interest (ROI) that covered the weight-bearing cartilage on the lateral and femoral condyles (14, 15). This region was chosen because it is the most prone to early degenerative cartilage changes (4, 7). In addition, such protocol allows an intra-individual reference between the lateral and

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medial compartments, the latter being much more prone to OA (6, 12).

The reproducibility in dGEMRIC depends on patient and investigator-related sources of error, of which the manual positioning of the ROI within the cartilage is one of the most important. The aim of the present study was to establish the intra- and interobserver variability of a standardized ROI drawing procedure in lateral and medial femoral weight-bearing cartilage.

Material and Methods

Images from 12 male healthy volunteers (age 23–29 years, mean 24) were included in the study. Six investigators, reflecting three different levels of competence, participated in the study. Two were students from medical school having completed their course in orthopedics but novices to the MRI workstation and the measurement function. Two were orthopedic surgeons, one used to MRI and one novice. Two were senior radiologists both experienced in MRI.

Magnetic Resonance Images

MR examinations were performed with a 1.5T MR-imaging system (Magnetom Vision; Siemens, Erlangen, Germany). Subjects were examined twice, pre-contrast and 2 h post-contrast according to the following protocol: the central part of the lateral and medial femoral condyles was identified using a routine diagnostic series. In these central parts, quantitative T1 measurements were performed in one medial and one lateral sagittal slice (slice thickness: 3 mm) using sets of six turbo inversion recovery images with six different inversion times. TR = 3000 ms, TE = 15 ms, turbo factor 7, FoV $120 \times 120\,mm^2$, matrix = 256×256, TI = 100, 200, 400, 800, 1600, and 2800 ms pre-contrast and TR = 2000 ms, TI = 50, 100, 200, 400, 800, and 1600 ms post-contrast. T1 analysis pre-contrast will be denoted $T1_{pre}$.

After the pre-contrast analysis, all subjects received Gd-$DTPA^{2-}$ (Magnevist®; Schering, Berlin, Germany) at a dose of 0.3 mmol/kg body weight, often denominated triple dose. The Gd-$DTPA^{2-}$ injection was given in an antecubital vein with the subject in the supine position in order to avoid thrombophlebitis at the injection site (14). To optimize the contrast distribution into the cartilage, starting 5 min after the injection, subjects exercised the knee for approximately 7 min by walking up and down stairs (5, 14). The post-contrast MRI was done 2 h after the injection. Post-contrast analysis included $T1_{Gd}$ and $\Delta R1$ calculations. $\Delta R1$ is the difference between post-contrast and pre-contrast T1 ($\Delta R1 = 1/T1_{Gd} - 1/T1_{pre}$) and corresponds to the concentration of Gd-$DTPA^{2-}$ in the cartilage.

Measurements

The ROI drawings were performed in the 2800 ms image (pre-contrast) and 1600 ms image (post-contrast) on the Magnetom vision system using the computer mouse. All investigators received identical oral and written instructions with regard to the positioning of the ROIs, identical to our previous studies (14, 15). The ROIs included the weight-bearing femoral cartilage that is most clinically relevant with regard to early degenerative disease. The ROI started on the surface of the femoral cartilage at a point opposite the middle of the tibial plateau regarding antero–posterior position. Thereafter the surface of the cartilage was followed to the posterior edge of the meniscus dorsal horn. From that point, the ROI continued at an angle of approximately 90° until the subchondral bone, where another approximately 90° turn was made. The subchondral bone was then followed to the antero-posterior position of the starting-point, and from there connected to the starting-point closed the ROI. A hardcopy printout documented the geometry of the recorded ROI. The ROI values were transferred to a computer for T1-relaxation time calculation using a three-parameter fit (9). The ROI volume, height, and length were recorded separately. Figure 1A, B shows typical ROIs in the lateral and medial femoral weight-bearing cartilage.

The four sets of images, i.e. medial and lateral and pre-contrast and post-contrast, of the 12 volunteers were subject to measurements twice each by the six investigators. For each investigator it was stated that the time period between measurements 1 and 2 should be 5 to 10 days.

Variability Calculations

The systematic error was used to exclude a systematic difference between the first and second measurements for each investigator. The systematic error was calculated according to formula 1.

$$Formula\ 1: Systematic\ error = (mean\ of\ measurement\ 1 - mean\ of\ measurement\ 2)/2$$

Coefficient of variation in percent (CV%) was used to describe the intra-observer variability. CV% shows the random error relative to the analyzed parameter. CV% is calculated according

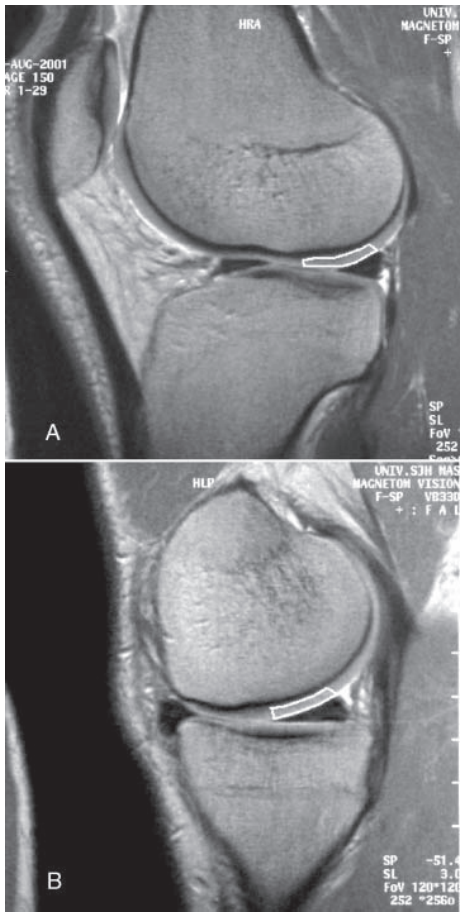

Fig. 1. A, B. Illustrations of a region of interest (ROI) in the weight-bearing lateral (A) and medial (B) femoral cartilage.

to formula 2a and b.

$$Formula\ 2a:\ Random\ error = standard\ deviation\ of\ (meas.1 - meas.2)\Big/\sqrt{2}$$

$$Formula\ 2b:\ CV\% = \frac{RandomError}{Overallmean} \times 100$$

Interobserver variability was calculated as the mean of the first and second measurements performed by one investigator compared to the mean of all six investigators as the gold standard and expressed in percent.

In order to identify subject/patient-related sources of error in dGEMRIC, the measurement error (interobserver variability) was correlated to the height and length of the measured ROIs.

Statistics

Non-parametric statistics were used for the statistical evaluation. The Wilcoxon signed-rank test was used to evaluate differences between compartments and between pre-contrast and post-contrast analyses ($T1_{pre}$, $T1_{Gd}$, and $\Delta R1$). Friedman ANOVA and the Mann-Whitney U test were used to evaluate interobserver differences. The Spearman rank-sum test was used for the correlations between interobserver variability and ROI height and length.

Results

Systematic error

There was no systematic error between the first and second analysis, as shown by a mean T1 difference of less than $\pm 0.4\%$ in both the lateral and the medial compartment, pre-contrast as well as post-contrast.

Intra-observer variability

The coefficient of variation (CV%) for the post-contrast analysis ($T1_{Gd}$) was $<3\%$ in both compartments for all investigators (Table 1). The CV% was higher pre-contrast compared to post-contrast ($P<0.05$) (mean of medial and lateral for each investigator) (Table 1). The CV% for $\Delta R1$ was significantly higher compared to both pre- and post-contrast values ($P<0.05$) (mean of medial and lateral for each investigator). With regard to the post-contrast compartmental analysis ($T1_{Gd}$), there was a larger CV% in lateral than in medial femoral cartilage ($P<0.05$) (Table 1).

Interobserver variability

As for intra-observer variability, interobserver variability (mean of both compartments) was higher for $\Delta R1$ (3.9%) than $T1_{pre}$ (2.1%) and $T1_{Gd}$ (1.8%) analyses ($P<0.05$). The mean interobserver variability for both compartments ranged between 1.3% and 2.3% for the 6 different investigators without correlation to the experience of the investigator. Individual errors are shown in Fig. 2A, B. The interobserver variability differed between the investigators ($P<0.05$) but none had consistently larger errors than others, as shown by the different outliers laterally and medially (Fig. 2A, B).

Table 1. Intra-individual variability for the different investigators in lateral and medial femoral cartilage, respectively. Coefficient of variation (C.V.%) expressed as random error divided by overall mean $\times 100$

	Coefficient of variation expressed in percent					
	pre lateral	pre medial	post lateral	post medial	ΔR1 lateral	ΔR1 medial
Student 1	3.37	3.29	2.33	1.46	4.92	4.66
Student 2	5.84	2.54	2.96	2.45	7.94	5.56
Orthopedic surgeon 1	2.32	4.08	2.08	0.99	5.27	3.54
Orthopedic surgeon 2	4.24	1.77	2.83	1.19	7.48	2.24
Radiologist 1	2.53	1.57	2.91	0.79	5.59	2.04
Radiologist 2	4.52	3.37	2.43	2.13	4.77	5.62
Mean	**3.80**	**2.77**	**2.59**	**1.50**	**6.00**	**3.94**

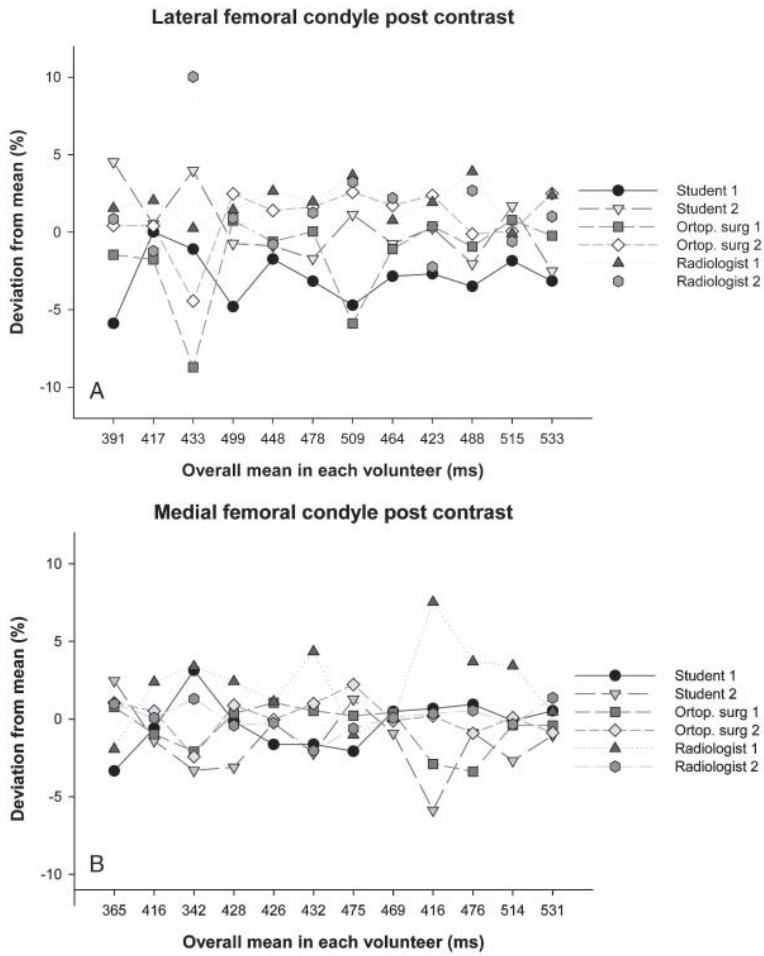

Fig. 2. A, B. Post-contrast interobserver variability illustrated as the $T1_{Gd}$ deviation in percent for each investigator in relationship to the overall mean in lateral (A) and medial (B) femoral cartilage. The mean $T1_{Gd}$ in each of the 12 subjects is indicated on the x-axis.

ROI configuration

There was no difference in ROI size between the compartments. The ROI area ($mm^2 \pm SD$) was 42.3 ± 9.6 laterally and 41.3 ± 11 medially ($P = 0.67$). However, the configuration differed slightly, the lateral ROI being longer and thinner than the medial. The length ($mm \pm SD$) of the ROI was 18.6 ± 10 in lateral and 16.6 ± 15 in medial femoral cartilage ($P < 0.01$). The height of the ROI ($mm \pm SD$) was 2.3 ± 0.5 laterally and 2.5 ± 0.7 medially ($P = 0.05$). There was a larger measurement error (interobserver variability) with decreasing ROI height in the lateral compartment ($P < 0.01$) (Fig. 3A). No correlation of this nature was seen in the medial compartment (Fig. 3B), nor was any correlation found between interobserver variability and ROI length (data not shown).

Discussion

The present study shows that a large standardized ROI yields a low measurement error, the mean $T1_{Gd}$ inter-individual variability being between 1.3% and 2.3% for the 6 different investigators. By comparison, patients with early cartilage disease had 30% higher $T1_{Gd}$ in the reference compared to the diseased compartment (15). The intra-observer $T1_{Gd}$ variability was also low, with a CV between 1% and 2% in medial and 2% and 3% in lateral femoral cartilage. These figures could be compared

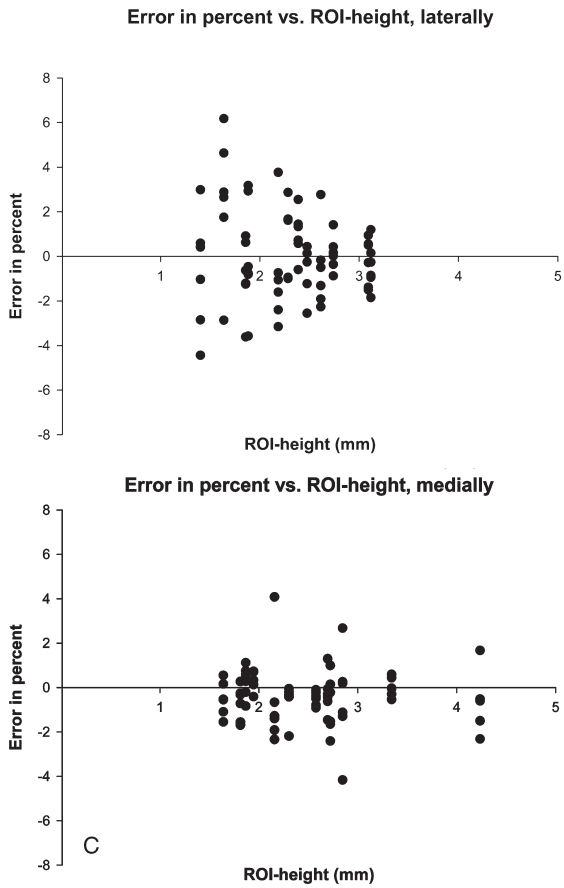

Fig. 3. A, B. Relationship between measurement error (interindividual variability) in relationship to ROI height (mm) in lateral (A) and medial (B) cartilage. On the x-axis, the height of each ROI is presented as the mean value for all six investigators.

with recent results from a study of conventional radiology of the knee. In repeated analyses of joint space narrowing on the same radiograph with a digitized image system, a CV of approximately 5% was considered very good (18).

The first data on reproducibility in dGEMRIC were presented by BURSTEIN et al., who investigated 3 healthy volunteers 2 weeks to 2 months apart (5). When the double or single dose was used, T1 of femoral cartilage was reproducible to within approximately 20%. The use of smaller ROIs may be one explanation for the lower reproducibility in that study, because, in human cartilage, areas of T1 irregularities have been reported in up to 25% of healthy volunteers (1, 3, 17). The impact of such irregularities becomes relatively smaller when a large ROI is used, because this yields a mean value for a relatively large portion of the weight-bearing cartilage. In support, it was recently shown in 4 asymptomatic volunteers who were investigated twice over a 6-month period that $T1_{Gd}$ differed less than 10% when large ROIs were used (19).

In the present study, the intra-observer variability (CV%) was lower post-contrast than pre-contrast (Table 1), possibly due to the fact that the post-contrast ROI is drawn in an image where the cartilage is saturated with Gd-DTPA^{2-}, which facilitates the outline of the cartilage.

With regard to the compartmental evaluation, post-contrast CV% was higher laterally than medially, indicating that the ROI is more difficult to draw in the lateral femoral condyle. Furthermore, there was a correlation between measurement error (inter-individual variability) and ROI height in the lateral, but not in the medial femoral cartilage (Fig. 3A, B). The larger error in the lateral compartment could be explained by a different ROI configuration, the lateral ROI being slightly longer and thinner than the medial (Fig. 1A, B).

Owing to the inclusion of two T1 calculations, the CV% for $\Delta R1$ was significantly higher than that for $T1_{pre}$ and $T1_{Gd}$ separately (Table 1). Moreover, the CV% for $\Delta R1$ more than doubled compared with $T1_{Gd}$ because the CV% was larger pre-contrast than post-contrast. Also, the inter-individual variability was larger for $\Delta R1$ than for $T1_{Gd}$ alone. It has been suggested that $T1_{pre}$ may be excluded in dGEMRIC because similar results are yielded with $T1_{Gd}$ alone (15). The considerably larger intra- and inter-variability for $\Delta R1$ that we report in the present study further supports this strategy.

When knee cartilage volume was studied, the interobserver reproducibility (coefficient of variation) varied between 7% in patellar cartilage and 11% in medial tibial cartilage (13). Reproducibility improved slightly using a semi-automatic algorithm; on average 1% (13). Although not evaluated in dGEMRIC, it cannot be ruled out that algorithms may further improve the definition and reproducibility of the ROI.

In conclusion, intra- and interobserver variability is low when a large standardized ROI is used in dGEMRIC. The experience of the investigator does not affect the variability of the ROI drawing procedure, which further supports this protocol as being applicable in a clinical setting.

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Paper II

Poor outcome after a surgically treated chondral injury on the medial femoral condyle: early evaluation with dGEMRIC and 17-year radiographic and clinical follow-up in 16 knees

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Background and purpose — The optimal treatment for traumatic cartilage injuries remains unknown. Contrast-enhanced MRI of cartilage (dGEMRIC) evaluates cartilage quality and a low dGEMRIC index may predict radiographic osteoarthritis (OA). The purpose of this study was (a) to explore the results 17 years after surgical treatment of an isolated cartilage knee injury and (b) to evaluate the predictive value of dGEMRIC.

Patients and methods — 16 knees with an isolated traumatic cartilage injury of the medial femoral condyle had cartilage repair surgery either by microfracture or autologous cartilage implantation. dGEMRIC of the injured knee was performed 2 years after surgery and radiographic examinations were performed 17 years after the operation.

Results — Radiographic OA was present in 12 of 16 knees. Irrespective of surgical method, the dGEMRIC index was lower in repair tissue compared with adjacent cartilage in the medial compartment, 237 ms vs. 312 ms ($p < 0.001$), which in turn had lower value than in the non-injured lateral cartilage, 312 ms vs. 354 ms ($p < 0.008$). The dGEMRIC index in the cartilage adjacent to the repair tissue correlated negatively with radiographic osteophyte score, $r = –0.75$ ($p = 0.03$).

Interpretation — A traumatic cartilage injury is associated with a high prevalence of OA after 17 years. The low dGEMRIC index in the repair tissue 2 years postoperatively indicates fibrocartilage of low quality. The negative correlation between the dGEMRIC index in the adjacent cartilage and future OA suggests that the quality of the surrounding cartilage influences outcome after cartilage repair surgery.

Cartilage injuries, with or without complicating ligamentous/meniscal injury, often occur after a twisting/compression trauma during sports activities. Cartilage has a limited healing potential with a complex structure with low chondrocyte density and avascularity. The best treatment for chondral defects remains controversial, despite decades of efforts (Hunziker et al. 2015). Microfracture (MFX) and autologous chondrocyte implantation (ACI) are the most commonly used techniques. In MFX, mesenchymal stem cells are recruited from the bone marrow by drilling or punching multiple holes in the subchondral bone plate of the cartilage lesion. First described in 1959 and having subsequently evolved, this is presently the most used technique (Pridie 1959, Steadman et al. 2001). ACI is a more technically demanding procedure, introduced in 1994 (Brittberg et al. 1994). This first-generation ACI includes two surgical procedures with arthroscopic harvesting of cartilage at the first operation for in vitro cultivation of chondrocytes. At the second operation, 3 weeks later, the expanded chondrocytes are injected under a periosteum flap that is sutured over the cartilage defect.

For both the MFX and the ACI technique, good 2–8 year results have been reported in younger patients with a well-defined traumatic injury. No clear difference has been observed between these treatments regarding failure rate or clinical outcome (PROMS) in randomized controlled trials (RCT) (Kraeutler et al. 2018). However, in a longer perspective, > 10 years, several studies have shown that approximately half of the patients have developed radiographic osteoarthritis (OA), after both MFX and ACI treatment (Gobbi et al. 2014, Martinčič et al. 2014, Knutsen et al. 2016).

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OA is a slowly developing degenerative disease on the timescale of 10 to 20 years. In the very early stages of the disease, molecular and cellular processes decrease cartilage quality but symptoms or radiographic changes may not yet be present.

Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) is a non-invasive method to assess and monitor such early degenerative changes in vivo. The method is based on the principle that a negatively charged contrast agent distributes into articular cartilage in an inverse relationship to the concentration of negatively charged glucosaminoglycans (GAG). In several clinical studies, the method has demonstrated a predictive potential for subsequent OA development, both in the hip (Cunningham et al. 2006, Palmer et al. 2017) and the knee (Owman et al. 2008, 2014).

The aims of the present study were to: (a) study the radiographic progression to OA 17 years after surgical treatment of a traumatic chondral injury on the medial femoral condyle, and (b) to evaluate if dGEMRIC has a predictive value in terms of future OA development.

Patient data, dGEMRIC index at 27 months, and radiographic outcome at 17 years

A	B	C	D	E	F	G	H	I
1	M	36	ACI	150	346	287	259	HTO at 3 years, presently scheduled for TKA
2	M	36	ACI	200	367	362	213	No radiographic OA, Clinical OA
3	M	37	ACI	300	334	315	253	< 2 years conversion to mosaic, HTO at 16 years, presently considering TKA
4	F	37	MFX	300	343	245	241	Radiographic OA, clinical OA
5	M	45	MFX	400	311	243	218	Radiographic OA, clinical OA
6	F	36	ACI	225	330	370	249	UKA at 6 years
7	M	36	ACI	600	381	306	210	Radiographic OA, clinical OA
8	M	31	ACI	600	269	292	241	TKA at 14 years
9	F	37	MFX	200	446	306	276	UKA at 7 years
10	F	30	ACI	200	385	309	250	No radiographic OA, clinical OA
11	M	34	MFX	250	376	281	231	Radiographic OA at 9 years
12	F	39	MFX	150	382	297	216	Radiographic OA, clinical OA
13	F	40	ACI	150	271	332	263	TKA at 13 years
14	M	38	ACI	150	347	293	239	No radiographic OA, no OA symptoms
15	M	38	MFX	180	327	325	207	Radiographic OA at 4 years
16	M	30	ACI	120	453	428	235	No radiographic OA, no OA symptoms
Mean		37.2		261	354	312	237	
(SD)		(4.7)		(151)	(51)	(46)	(21)	

A Studied knee number
B Sex
C Age at index operation
D Cartilage repair procedure
E Size of lesion, mm^2
F dGEMRIC index lateral at 27 months
G dGEMRIC index medial (adjacent to repair) at 27 months
H dGEMRIC index repair tissue at 27 months
I Outcome: radiographic OA, OA surgery, or clinical OA by KOOS score
Radiographic OA was defined using the OARSI score and clinical OA defined using the Knee Osteoarthritis Outcome Score (KOOS). 1 patient had bilateral operations (knees number 12 and 13). 2 patients (knees number 11 and 15) did not participate in the 17-year radiographic follow-up but had radiographs recorded 4 and 9 years postoperatively. Knees that had undergone OA surgery (high tibial osteotomy (HTO), unicompartmental knee arthroplasty (UKA) or total knee arthroplasty (TKA)) were dichotomized as OA diagnosis but excluded from analysis of outcome measures and radiographic change.

Patients and methods

Patients

Between 1997 and 2000, 16 knees in 15 patients were treated surgically due to a symptomatic isolated traumatic cartilage injury on the medial femoral condyle. Patients had no symptoms before the injury of the affected knee. The patients were initially included in an RCT of ACI vs. MFX that was designed for a larger number of patients. Due to logistical challenges and lack of patients, the study was not completed and the preliminary results have not been published. Exclusion criteria were: radiographic evidence of OA or evidence of cartilage degeneration at arthroscopy; patients with concomitant disease, injury, or malalignment. The group included 10 men and 5 women (1 woman had bilateral injuries) with a median age at index operation of 37 years (30–47). The mean size of the traumatic chondral injury was 261 mm^2 (120–600) (Table). Patients were randomized to either MFX or ACI treatment. However, 2 patients in each group had to be reoperated within the first 2 years (Figure 1). This resulted in 9 knees finally treated with ACI and 6 knees treated with MFX drilling. One patient initially treated with MFX was reoperated with mosaicoplasty as a salvage procedure. In that patient, cartilage-bone plugs were harvested from unloaded joint regions and implanted to the injury site.

The medical records, including surgical reports and archived radiographs, were studied in all patients until the 17-year follow-up (Figure 3 and Table). dGEMRIC values were compared (Figure 4) to a cohort of 19 asymptomatic individuals (mean age 24 years) that previously had been investigated with an identical MRI protocol (Tiderius et al. 2001).

Surgical procedures

At the first operation, the cartilage injury was verified with arthroscopy and the treatment randomized. Knees randomized to ACI had cartilage harvested from unloaded cartilage on the upper medial femoral condyle (Brittberg et al. 1994). The donor site was remote from the cartilage lesion. The second surgery, 2–3 weeks later, was a mini-arthrotomy with debridement of the cartilage lesion to stable edges in all patients. The

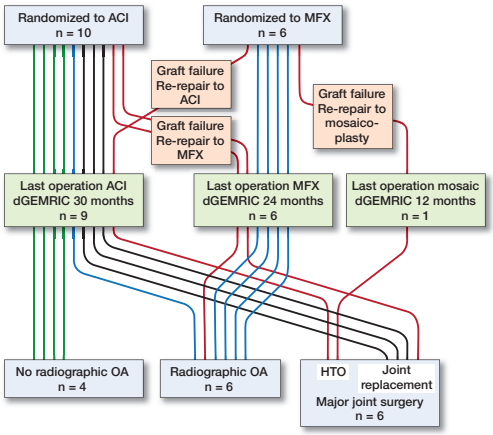

Figure 1. Flow-chart of treatment and follow-up of all 16 knees (in 15 patients).

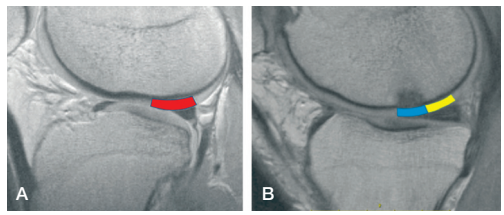

Figure 2. Illustration of how the regions of interest (ROIs) for dGEMRIC were drawn. All ROIs included full-thickness cartilage. In the lateral compartment (A), the ROI was drawn from the center of the tibial plateau to the rear insertion of the meniscus (red), according to a standardized protocol (Tiderius et al. 2004b). In the medial compartment (B), one ROI included the repair tissue (blue) and one ROI (yellow) the remaining weight-bearing cartilage to the rear insertion of the meniscus.

knees randomized to ACI had a periosteal flap sutured over the lesion and sealed by fibrin glue, under which the in-vitro expanded chondrocytes were injected (Brittberg et al. 1994). The knees randomized to MFX had the lesion drilled with a ∅2 mm drill, hole centers spaced 6 mm apart. The protocol for postoperative management of ACI described by Brittberg et al. (1994) was followed for both groups consisting of 6 weeks of unloading followed by 6 weeks of progressive weight-bearing and detailed supervised physiotherapy.

dGEMRIC

The dGEMRIC investigations were performed on average 2 years after the cartilage repair procedure using a standard 1.5 T MRI system with a dedicated knee coil (Magnetom Vision; Siemens Medical Solutions, Erlangen, Germany). Gd-DTPA^{2-} (Magnevist®, Bayer Schering Pharma AG, Berlin, Germany) at 0.3 mmol/kg body weight dosage was injected intravenously. To optimize the distribution of Gd-DTPA^{2-} into the cartilage, the patients exercised by walking up and down stairs for 10 min, starting 5 min after the injection. Post-contrast MR imaging was performed 2 hours after the injection according to a standardized protocol (Tiderius et al. 2001). Two sagittal slices covering the central parts of the weight-bearing lateral and medial femoral cartilage respectively were acquired using sets of 6 turbo inversion recovery images with different inversion times (TI = 50, 100, 200, 400, 800, and 1600 ms), from which the T1 relaxation time was subsequently calculated. Other imaging parameters were: TR = 3000 ms, TE = 15 ms, turbofactor 7, field of view (FOV) 120×120 mm^2, matrix = 256×256, slice thickness = 3 mm.

Regions of interest (ROIs) were drawn (Figure 2) in the weight-bearing central parts of the lateral and medial femoral cartilage, respectively. In the medial compartment, 1 ROI covered the area of cartilage repair tissue and 1 ROI was drawn in adjacent, non-injured weight-bearing cartilage. In the lateral compartment, the ROI was drawn between the center of the tibia plateau to the rear insertion of the meniscus according to a previously validated protocol (Tiderius et al. 2004b). Results are presented as mean T1 ms of each ROI (the dGEMRIC index).

Radiography

At median 17 years (15–19) after the cartilage repair surgery, standardized weight-bearing radiographs in 20° flexion of both knees were obtained. Blinded to clinical presentation and to the type of cartilage repair procedure, assessment of the ante-posterior radiographs was performed by 2 of the authors independently: an orthopedic surgeon specialized in joint replacement (JT) and a senior radiologist specialized in skeletal radiology (BL). In cases of discrepancy the images were reassessed by the 2 investigators together and consensus was reached. The OARSI atlas (Altman and Gold 2007) was used for the medial and lateral compartments respectively, grading radiographic change on a 4-point scale for joint space narrowing (JSN) (0–3, 0 = no evidence of JSN) and marginal osteophytes of femoral and tibial condyles (0–3 each, 0 = no bony change). Dichotomization for diagnosis of radiographic OA was defined as any of the following criteria fulfilled in either of the 2 tibiofemoral compartments: JSN ≥ 2, osteophyte score ≥ 2, or JSN grade 1 in combination with osteophyte grade 1 in the same compartment. This definition (Englund et al. 2003) approximates grade 2 knee OA based on the Kellgren–Lawrence scale. 2 patients could not partake in the radiographic examination. However, both these patients had previous visits to an orthopedic surgeon due to knee pain respectively 4 and 9 years postoperatively. Weight-bearing radiographs from these visits demonstrated OA by the above criteria.

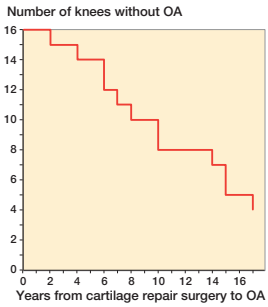

Figure 3. Temporal assessment of OA after surgical repair of a chondral injury on the medial femoral condyle. OA development was defined as either high tibial osteotomy, arthroplasty, or radiographic OA. Time points of OA diagnosis are from surgery date (HTO or joint replacement) or date of radiographic OA either due to radiographic evaluation of clinical symptoms in the intermediate time or by radiographs at the 17-year follow-up. The initial number of knees (n = 16) without OA already starts to decrease two years after surgery. At the end of the study period (17 years), only 4 knees lack radiographic OA.

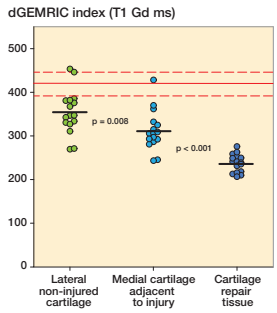

Figure 4. dGEMRIC index of the 3 investigated ROIs in each knee (n = 16): the lateral femoral condyle (354 ms SD 51), the medial cartilage adjacent to the cartilage lesion (312 ms, SD 46) and the repair tissue (237 ms, SD 20). The dGEMRIC index was lower in repair tissue vs. adjacent cartilage in the medial femoral cartilage ($p < 0.001$). The dGEMRIC index was higher in the uninjured lateral femoral cartilage than in the medial cartilage adjacent to the cartilage lesion ($p < 0.008$). Horizontal black bars are mean values. For comparison, the red solid (mean) and dashed lines (SD) represent the dGEMRIC index in healthy volunteers previously investigated with an identical protocol by our group (Tiderius et al. 2001).

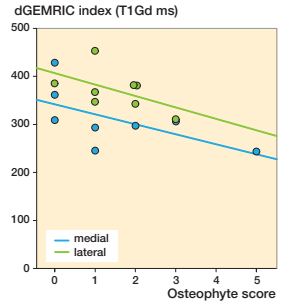

Figure 5. The dGEMRIC index of medial adjacent cartilage (blue) correlated negatively with radiographic osteophyte score at 17 years, $r = –0.75$ ($p = 0.03$). A similar trend, but not statistically significant, was found in the lateral compartment (green), $r = –0.60$ ($p = 0.1$). Note that the 6 knees that had already had surgery for OA (HTO or arthroplasty) were excluded from this correlation analysis as were the 2 knees that had only early follow-up radiographs.

PROMS

Patient-related outcome measures (PROMS) were completed at the 17-year follow-up by self-administered pen on paper forms for VAS, Lysholm, and KOOS. The algorithm based on the KOOS score described by Englund et al. (2003) was used to dichotomize for clinical OA. Patients who had been treated for OA with osteotomy or arthroplasty were excluded from PROMS analysis.

Statistics

SPSS 25 Statistics for Windows (IBM Corp, Armonk, NY, USA) and SigmaPlot 11.0 (Systat Software, San Jose, CA) was used for statistical analysis. Despite the fact that data from 1 bilateral operation are not independent, we included both knees in that patient for analysis. After testing for normal distribution (Shapiro–Wilk) and equal variance (Levene's mean test), the Student t-test was used for continuous variables. A paired test was used for regional measurements in the same knee; a non-paired test was used in all other instances, and 2-tailed distribution was assumed in all tests. Spearman's Rho was used for correlation of ordinal data and continuous variables. Fisher's exact test was used to compare the distribution in cases of 2 dichotomous variables. The statistical power was low due to the few patients eligible for this study.

Ethics, funding, and potential conflicts of interest

The study was approved by the ERB at Lund University (Etikprövningsnämnden #EPN:2014/752, LU#73-96 and LU#651-00), the Radiation Protection Committee (Strålskyddskommiten #SSFo2014-050), and the Image Research Committee (BOF053).

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Results

OA, radiographic, and symptomatic

All 16 knees could be assessed for radiographic OA (rOA) after mean 17 years follow-up (Figure 1 and Table). 6 knees had received OA surgery; 2 had high tibial osteotomy, 2 had unicompartmental medial knee arthroplasty and 2 had total knee arthroplasty. 6 knees had radiographic OA based on OARSI scores, and 4 knees had no radiographic OA. Thus 12 of 16 knees had failed by progressing to OA (Figure 3).

The KOOS score was indicative of OA (Englund et el. 2003) in all but 2 knees with a normal age-matched KOOS score and no radiographic OA (Table). All 4 knees that needed a second cartilage repair procedure progressed to OA. All MFX-treated knees and 6 of 10 ACI knees developed radiographic OA ($p = 0.2$).

dGEMRIC

All knees were examined with dGEMRIC at median 27 (12–57) months after the cartilage repair procedure. The 4 early graft failure reoperations were performed 12–15 months prior to the dGEMRIC examination.

The mean dGEMRIC index (T1Gd in ms) differed between the 3 ROIs as illustrated in Figure 4. The mean dGEMRIC index was 33% lower in the repair tissue compared with the non-affected lateral femoral cartilage, 237 vs. 354 ms ($p < 0.001$). In addition, the dGEMRIC index in the cartilage adjacent to the lesion was lower than in the non-affected lateral femoral cartilage, 311 ms (SD 58) vs. 354 ms (SD 51) ($p = 0.008$). The cartilage adjacent to the repair tissue was higher after ACI compared with MFX surgery with borderline significance: 331 ms (SD 47) vs. 283 ms (SD 33) ($p = 0.05$).

The dGEMRIC index in the medial cartilage adjacent to the lesion correlated negatively with the radiographic osteophyte score in the medial compartment at the 17-year follow-up, $r = –0.75$ ($p = 0.03$). A similar trend, although not statistically significant, was found in the lateral compartment, $r = –0.60$ ($p = 0.1$) (Figure 5). JSN did not seem to correlate in the medial compartment $r = –0.25$ ($p = 0.5$); in the lateral compartment there was no correlation as all knees had zero JSN.

There was a trend towards higher dGEMRIC index in the adjacent cartilage of the 4 knees that did not develop radiographic OA compared with OA knees, 348 (SD 61) vs. 300 (SD 35) ($p = 0.07$).

BMI, sex, and size of injury were similar between knees that developed radiographic OA and those that did not. Non-OA patients were on average 4 years younger than patients who developed radiographic OA ($p = 0.1$).

Discussion

The main finding of this study is that most (12/16) of patients that had surgical treatment for a traumatic chondral injury on the medial femoral condyle had developed radiographic OA 17 years after the surgery. In addition, 2 of the 4 patients with no radiographic OA had clinical OA according to the KOOS score. For comparison, the prevalence of radiographic knee OA in the general population of similar age (54–65 years) is between 10% and 23% (Felson et al. 1987, Turkiewicz et al. 2015).

The goal of cartilage repair is twofold: to alleviate symptoms and to avoid future OA. Despite decades of research there is no consensus regarding the optimal treatment for traumatic cartilage injuries. In a short- and mid-term perspective, several studies of MFX report good results with a large proportion of patients returning to high levels of activity (Mithoefer et al. 2009, Erggelet and Vavken 2016). However, 10–15 years after the operation, results deteriorate with half of patients having radiographic OA (Gobbi et al. 2014, Knutsen et al. 2016). Theoretically, the reason for failure might be explained by the fact that the repair cartilage after MFX operation lacks collagen II and does not show the zonal organization of hyaline cartilage (Mithoefer et al. 2009, Erggelet and Vavken 2016). By contrast, the ACI technique was designed to yield repair tissue with a hyaline-like structure that potentially also has mechanical properties that resembles healthy cartilage. A recent review of 9 ACI studies (Pareek et al. 2016) with 9–13 years' follow-up reported on average 81% successful results, defined by no diagnosed graft failures and good or excellent clinical results. However, the only study that presented radiographic follow-up (Martinčič et al. 2014) at 10 years postoperatively found OA in half of the cases, i.e., similar to that reported for MFX.

An RCT of ACI vs. MFX with a 15-year follow-up of 78 knees (Knutsen et al. 2016) had one-third failures and half of the remaining knees had radiographic OA with no difference between the treatment groups. The small numbers of patients in our study, in combination with the crossover that occurred, hampers a relevant comparison between MFX and ACI treatments. However, it should be pointed out that the 4 patients that did not end up with radiographic OA were all treated with ACI. In addition, dGEMRIC indicated better cartilage quality in the cartilage adjacent to the repair tissue in ACI compared with MFX patients. The evaluation of cartilage quality a few years after the cartilage repair procedure is a major strength of our study. Obviously, the assessment of cartilage status is equally relevant for MFX and ACI cases.

dGEMRIC is a validated in-vivo technique to estimate cartilage quality, in particular the glucosaminoglycan content. We found a low dGEMRIC index in the repair tissue both after MFX and ACI indicating fibrocartilage with low GAG content.

Several previous studies have shown that a low dGEMRIC index is associated with an increased risk of future radiographic OA, both in the hip (Cunningham et al. 2006, Palmer et al. 2017) and in the knee (Owman et al. 2008, 2014). For example, in middle-aged patients (mean age 50 years) with superficial cartilage fibrillation on the femoral cartilage, a low dGEMRIC index (circa 300 ms) was associated with radiographic OA in two-thirds of patients 6 years after the dGEMRIC investigation (Owman et al. 2008).

dGEMRIC as a prognostic tool was suggested also in our study; we found a correlation between a low dGEMRIC index in the cartilage adjacent to the repair tissue and the future prevalence of radiographic OA. This may indicate that the surrounding cartilage should be evaluated at the time of cartilage repair surgery. In support, a clinical MFX study found that visual mild degeneration of surrounding cartilage at the primary operation had a worse outcome at 10–14 years' follow-up than patients with normal-appearing cartilage (Solheim et al. 2016). Importantly, in our study, we do not know if low cartilage quality was present already at the time of surgery, or if it developed between surgery and the dGEMRIC investigation, approximately 2 years later.

Also, the non-injured lateral cartilage demonstrated lower dGEMRIC values (Figure 4) than previously observed in

healthy volunteers (Tiderius et al. 2001) investigated with an identical protocol. This finding may reflect that cartilage degeneration in the medial compartment affects the whole joint, with GAG loss also in the lateral femoral condyle. Other possible explanations for this finding are reduced loading during rehabilitation as the dGEMRIC index is known to respond to changes in activity level (Roos and Dahlberg 2005), correlate to level of activity (Tiderius et al. 2004a) and correlate to thigh muscle strength (Ericsson et al. 2009).

A strength of our study is that all included patients could be assessed regarding OA development and that the study had a strict inclusion criterion: an isolated traumatic chondral injury only on the medial femoral condyle. The main limitation is the small number of patients, resulting in low statistical power, especially regarding the comparison between the 2 surgical methods, MFX and ACI.

Furthermore, the value of PROMS was limited because several patients had major joint surgery between the cartilage repair surgery and the 17-year follow-up.

In summary, we found a high prevalence of OA at follow-up 17 years after cartilage repair. There was no evidence of hyaline-like cartilage 2 years after ACI, as demonstrated with a low dGEMRIC index. The negative correlation between the dGEMRIC index in the adjacent cartilage and future OA indicates that dGEMRIC can predict future radiographic OA and that the quality of the surrounding cartilage influences the outcome after cartilage repair surgery.

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Study design: JT, LD and CT. Analysis of radiographs: JT and BL. Analysis of MR images: JT, JS and CT. Collection of clinical data: JT. Writing of manuscript: JT, PN, JS, LD and CT. Revision of manuscript: JT and CT.

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Paper III

Osteoarthritis and Cartilage 19 (2011) 977–983

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Longitudinal assessment of femoral knee cartilage quality using contrast enhanced MRI (dGEMRIC) in patients with anterior cruciate ligament injury – comparison with asymptomatic volunteers

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SUMMARY

Objective: In this observational longitudinal study we estimate knee joint cartilage glycosaminoglycan (GAG) content, in patients with an acute anterior cruciate ligament (ACL) injury, with or without a concomitant meniscus injury.

Methods: 29 knees (19 men/10 women) were prospectively examined by repeat delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC), approximately 3 weeks and 2.3 ± 1.3 (range 4.5) years after the injury. We estimated the GAG content (T1Gd) in the central weight-bearing parts of the medial and lateral femoral cartilage and compared results with a reference cohort ($n = 24$) with normal knees and no history of injury examined by dGEMRIC at one occasion previously.

Results: The healthy reference group had longer T1Gd values compared with the ACL-injured patients at follow-up both medially: 428 ± 38 vs 363 ± 61 ms ($P < 0.0001$) and laterally: 445 ± 41 vs 396 ± 48 ms ($P = 0.0002$). At follow-up T1Gd was lower in meniscectomized patients compared to those without a meniscectomy, both medially (−84 ms, $P = 0.002$) and laterally (−38 ms, $P = 0.05$). In the injured group, the medial femoral cartilage showed similar T1Gd at the two dGEMRIC investigations: 357 ± 50 vs 363 ± 61 ms ($P = 0.57$), whereas the lateral femoral cartilage T1Gd increased: 374 ± 48 vs 396 ± 48 ms ($P = 0.04$).

Conclusions: The general decrease in cartilage T1Gd in ACL-injured patients compared with references provide evidence for structural matrix GAG changes that seem more pronounced if a concomitant meniscal injury is present. The fact that post-traumatic OA commonly develops in ACL-injured patients, in particularly those with meniscectomy, suggests that shorter T1Gd may be an early biomarker for OA.

Introduction

Rupture to the anterior cruciate ligament (ACL), with an estimated yearly incidence of 0.81 per 1000 persons aged 10–64 years[1], is associated with an increased risk for knee osteoarthritis (OA) 10–20 years after the injury[2]. The reported variability in OA incidence 0–90%[2–6], may be related to study design, different classification methods to determine radiographic OA, inhomogeneous study samples with respect to mechanisms of injury and the structures injured, variable treatment regimes and post-injury activity levels. In particular meniscectomy seems to be an important determinant of incident radiographic OA[4,6,7]. Recently in another ACL-injured cohort we showed a strong association between meniscectomy and radiographic tibiofemoral OA 15 years after the injury[4]. In these patients, a concomitant injury to the medial collateral ligament or a minor/stable meniscal tear that was left without meniscectomy, were unrelated to radiographic OA development.

It is important to acknowledge that molecular cartilage matrix changes most likely develop long before definite non-traumatic cartilage lesions, osteophytes and joint space narrowing can be detected by routine magnetic resonance imaging (MRI) or radiography. So far, studies using ACL-injured patients as a model of

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post-traumatic OA are generally hampered by the lack of methods able to identify molecular matrix changes indicative of pre-radiographic OA.

One crucial matrix molecule with regard to cartilage quality and integrity is, aggrecan. Aggrecan consists of a protein core to which a large number of highly negatively charged glycosaminoglycans (GAGs) are attached. By attracting counter ions, GAG creates a swelling pressure that is counteracted by the rigid framework of the collagen network. A valid and reliable non-invasive technique to estimate joint cartilage GAG content is delayed gadolinium-enhanced MRI of cartilage (dGEMRIC)[8,9]. In dGEMRIC, a negatively charged contrast medium (Gd-DTPA^{2-}) distributes inversely to the fixed charged density in the cartilage after an intravenous injection[10]. Accordingly, the tissue T1 value (T1Gd or the dGEMRIC index) represents an estimate of the cartilage GAG content. A long T1Gd is consistent with high cartilage GAG content[11–14].

Previously, the ability of dGEMRIC to identify molecular matrix changes in patients at risk for OA development has mainly been indicted in non-injured joints[10,15]. In 2005, we reported decreased T1Gd 3 weeks after an ACL injury compared with healthy reference subjects[8]. These baseline data are followed up in the present study where we describe the T1Gd changes that occurred within 5 years in the ACL-injured group. In addition, we explore the hypothesis that a meniscectomy will impair the estimated cartilage quality (T1Gd) suggestive of post-traumatic OA. Results are compared with the previously used healthy reference cohort.

Methods

Patients

This is the second report of a longitudinal ACL-injured cohort that has been characterized at baseline 3 weeks after the injury.

Between February 2000 and June 2005, 40 patients with a clinical positive pivot-shift and/or Lachman test and a MRI confirmed acute ACL rupture in a previously uninjured knee were included in the study. The patients were prospectively recruited depending on the compliance with recruitment of subjects into the study of the emergency unit physicians. Included patients ranged between 14 and 40 years of age and none had clinical or MRI-verified signs of knee OA at baseline (Table I).

After the knee injury, patients were treated according to the present algorithm used at the Department of Orthopedics, Malmö, Skåne University Hospital saying that patients with a suspected ACL deficiency after a knee sprain should be re-examined by an experienced orthopaedic surgeon 1–2 weeks after the injury, then referred to physical therapy before eventual decision of ACL reconstruction. The treatment algorithm has not been changed over the 5-year inclusion time. 14 of the 29 patients had been ACL-reconstructed since our previous report. It was decided that at least 6 months should pass between the reconstruction and the second MRI to avoid risk of low knee cartilage T1Gd values due to recent surgery and lowered activity level. There was no difference in the number of partial meniscectomies between ACL reconstructed (six of 14) and non-ACL reconstructed patients (five of 15). Six medial, eight lateral and three combined medial and lateral partial meniscectomies were performed during follow-up.

MRI

Patients were investigated with dGEMRIC approximately 3 weeks (range 3–47 days) and 2 years (range 7–60 months) after the injury using a standard 1.5 T MRI-system with a dedicated knee coil (Magnetom Vision; Siemens Medical Solutions, Erlangen, Germany). Initially, a diagnostic series was performed to verify the ACL rupture and concomitant cartilage and meniscus injuries. This MRI could not reveal cartilage lesions in the Region of Interest (ROI) in any of the patients. Information of MRI meniscal injuries was collected from the radiology report. Gd-DTPA^{2-} (Magnevist®, Schering AG, Berlin, Germany) was injected at 0.3 mmol/kg body weight. To optimize the distribution of Gd-DTPA^{2-} into the cartilage the patients exercised by walking up and down stairs for approximately 10 min, starting 5 min after injection. Post-contrast imaging with subsequent T1-relaxation time calculation of the cartilage was performed 2 h after the injection. Central parts of the weight-bearing lateral and medial femoral cartilage were identified and quantitative relaxation time calculations were made in a 3 mm thick sagittal slice on each condyle, using sets of six turbo inversion recovery (IR) images with different inversion times (TR = 2000 ms, TE = 15 ms, FoV 120 × 120 mm^2, matrix = 256 × 256, TI = 50, 100, 200, 400, 800 and 1600 ms). Total imaging time was approximately 20 min. The same imaging protocol was used for the healthy reference group. In the lateral and medial slices, a full-thickness ROI was drawn within the cartilage between the center of the tibial plateau and the rear insertion of the meniscus using the MRI-Mapper software developed at the Beth Israel Deaconess Medical Center (Boston, USA). All ROI drawings were blinded to per-operative findings and surgical treatment[16]. T1Gd was calculated using the mean signal intensity from each ROI as input to a three-parameter fit[17]. It has been shown that the mean T1Gd inter-individual variability in ROI drawing is 1–2% and that the intra-observer T1Gd variability coefficient of variation (C.V.%) is 1–2% medially and 2–3% laterally[16]. Repeat dGEMRIC measurement results may be influenced by biological and technical variations. In the femoral condyle cartilage, the measured 2-D T1Gd root-mean-square value of the coefficients of variation (CV_{RMS}) has been estimated to 5–8% in a cohort consisting of nine non-ACL

Table I
Characteristics of the study sample

	ACL-injured 3 weeks after injury	ACL-injured follow-up	Reference subjects	Excluded patients
	N = 29	N = 29	N = 24	N = 11
Men, n	19	19	14	8
Age, years (mean ± SD)	26.6 ± 6.8	28.8 ± 6.8	25	26.9 ± 7.0
BMI, kg/m^2 (mean ± SD)	23.8 ± 2.6	Not measured	22.5 ± 2.3	23.2 ± 1.5
Activity level (median (range))	3 (3)	3 (3)	3	3 (3)
Lysholm knee score (mean ± SD)	Not measured	88 ± 8	Not measured	Not measured
Bone-bruise at MRI 1, n	26	0	0	9
ACL reconstructed, n	0	14	0	5
Not ACL reconstructed, n	29	15	24	6
Medial meniscectomy, n	0	3	0	1
Lateral meniscectomy, n	0	5	0	0
Medial and lateral meniscectomy, n	0	3	0	0

reconstructed patients 20 years after injury, with 2 weeks between MR investigations[18]. In another dGEMRIC study from Multanen *et al.* in asymptomatic volunteers with an average interval of 5 days between the scans, the CV_{RMS} for full-thickness ROI's was 5–7%[9].

37 of the 40 patients were re-examined with dGEMRIC in average 2 years (range 7–60 months) after the initial investigation (Table I). Seven patients had to be excluded because of motion artifacts in the MR images, defined as having >10% of the pixels within the ROI outside a T1 interval of 0–1300 ms. One patient had to be excluded because his opposite knee was examined by mistake at follow-up. Three patients declined to perform the second MRI. The 11 excluded patients did not differ regarding patient characteristics (Table I). The local institutional review board approved the study and written informed consent was obtained from all participants.

Questionnaires

The activity level during the year preceding the injury and at follow-up was registered on a four level scale (1 = sedentary lifestyle; 2 = moderately exercising individuals with regular physical activities on average twice weekly; 3 = regular physical activities more often than twice a week; 4 = regular physical activities at an elite/competitive level)[8].

The Lysholm knee score was used to document knee symptoms at follow-up[19]. In the Lysholm score the highest obtainable and best score is 100, consisting of the following sub-groups of knee symptoms: locking 0–15 points, instability 0–25 points, pain 0–25 points, swelling 0–10 points, decreased ability to climb stairs 0–10 points, limp 0–5 points, walking aid 0–5 points and squat 0–5 points.

The activity level and the Lysholm score were mainly used to characterize the study population and to present scores in line with a common ACL-injured cohort treated with or without ACL reconstruction.

Reference group

dGEMRIC results (T1Gd) from the ACL-injured patients were compared with 24 healthy volunteers (14 men) who have been reported previously[8]. The reference group was investigated with dGEMRIC at one occasion and was not scheduled for repeat dGEMRIC. The reference group had a mean age of 25 years and similar BMI (22.5 ± 2.3 kg/m^2). Their activity level (mean and median activity level 3) was matched with the ACL-injured group as described previously[8]. They had no history of a knee injury, no clinical or MRI-related signs of OA at examination and were all from the same geographic region as the ACL-injured cohort.

Statistical analysis

Student's *t* test for paired and unpaired observations was used for the parametric statistics, i.e., comparisons of T1Gd values between the ACL-injured cohort and the healthy reference subjects and in subgroup analysis within the ACL-injured cohort (Table III). The Mann–Whitney test was used for non-parametric statistics (activity level). The Pearson correlation was used for the correlation between T1Gd at follow-up and the time to follow-up. Analysis of covariance (ANCOVA) was used to adjust for differences in BMI and follow-up times between patients. All tests were two-tailed. The statistical analysis was performed with SPSS for Windows 14.0 and 17.0 software package (SPSS Inc., Chicago, IL, USA).

Results

Demographics (Table I)

The mean activity level (mean ± SD) at baseline in those who remained non-ACL reconstructed (n = 15) vs the ACL reconstructed (n = 14) was 2.9 ± 0.7 and 3.1 ± 0.9 ($P = 0.40$). The activity level at follow-up in non-ACL (n = 15) vs ACL reconstructed (n = 14) patients, was 2.7 ± 0.7 and 2.8 ± 1.0 ms, $P = 0.81$ (Mann–Whitney test).

The Lysholm knee score (mean ± SD) for all patients (n = 29) at follow-up was 88 ± 8, which is considered good. The Lysholm score did not differ significantly between non-ACL reconstructed (n = 15) vs ACL reconstructed (n = 14) patients, 86 ± 9 and 90 ± 9, respectively ($P = 0.33$).

Overall T1Gd results

The healthy reference group, who was investigated with dGEMRIC at one occasion, had longer T1Gd (mean ± SD) values than the ACL-injured patients (n = 29) both at their baseline and follow-up examination. At follow-up, T1Gd was 363 ± 61 ms medially in the ACL-injured group vs 428 ± 38 in the reference group ($P < 0.0001$) and 396 ± 48 vs 445 ± 41 ms laterally ($P = 0.0002$) (Fig. 1).

In the ACL-injured patients (n = 29), the medial femoral cartilage T1Gd (mean ± SD) did not change between the two dGEMRIC investigations: 357 ± 50 vs 363 ± 61 ms ($P = 0.57$) (Fig. 1, Table III). In contrast, in the lateral femoral cartilage T1Gd increased: 374 ± 48 vs 396 ± 48 ms ($P = 0.04$) (Fig. 1, Table III).

T1Gd and meniscectomy

Cross-sectional T1Gd analysis at the second MRI showed that ACL-injured patients who underwent a partial meniscectomy had a shorter T1Gd in the ipsilateral knee compartment than patients without ipsilateral meniscectomy (mean ± SD), 296 ± 62 vs 380 ± 49 ms medially ($P = 0.002$), and 368 ± 48 vs 406 ± 44 ms laterally ($P = 0.05$) (Table III, Figs. 2 and 3).

There was a positive correlation (Pearson) between the T1Gd value at follow-up and the time between dGEMRIC examinations, medially: r = 0.41, $P = 0.026$, n = 29 and a trend for a positive correlation laterally: r = 0.35, $P = 0.061$, n = 29 (Figs. 2 respectively 3). However, ACL-injured patients with and without meniscectomy did not have a significant difference in the mean time to follow-up (Table II).

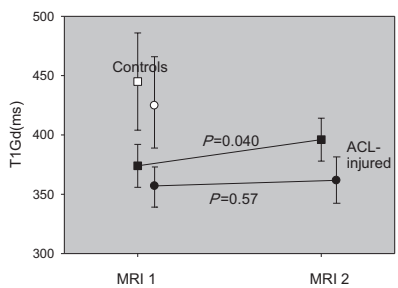

Fig. 1. T1Gd (ms), mean, 95% C.I. in the cartilage of the medial (●) and lateral (■) femoral condyles after ACL injury (n = 29). T1Gd (ms), mean, 95% C.I. in the cartilage of the medial (○) and lateral (□) femoral condyles in reference subjects (n = 24).

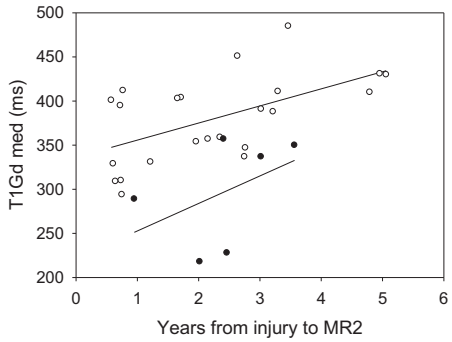

Fig. 2. T1Gd (ms) in the cartilage of the medial femoral condyle after ACL injury (n = 29) at the second MRI in correlation with time from injury to MRI, in subjects (●) (n = 6) who also underwent a medial meniscectomy during follow-up and in subjects (○) who did not (n = 23).

Longitudinal analysis (change in T1Gd between first and second MRI) showed that the absolute value of the change in T1Gd in the medial femoral cartilage between patients with (n = 6) and without (n = 23) medial meniscectomy was (mean ± SD) −30 ± 53 ms vs +16 ± 56 ms, $P = 0.09$ (Table III). Subjects having had a lateral partial meniscectomy (n = 8) decreased their T1Gd in the lateral femoral cartilage by in average 12 ± 42 ms, whereas subjects without meniscectomy (n = 21) increased their T1Gd by in average 35 ± 55 ms (independent samples *t* test between the two groups, $P = 0.04$, Table III).

T1Gd and MRI-verified meniscal injuries

Cross-sectional T1Gd analysis at the second MRI showed that ACL-injured patients who had been diagnosed with a medial meniscal tear at the first or second MRI had a shorter T1Gd in the ipsilateral knee compartment than patients without ipsilateral medial meniscal tear (mean ± SD), 325 ± 68 ms (n = 10) vs 382 ± 49 ms (n = 19) ($P = 0.014$). Corresponding values for ACL-injured patients who had been diagnosed with or without a lateral meniscal tear was 381 ± 39 ms (n = 13) vs 408 ± 52 ms (n = 16) laterally ($P = 0.13$).

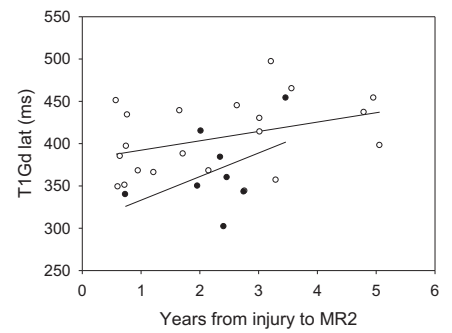

Fig. 3. T1Gd (ms) in the cartilage of the lateral femoral condyle after ACL injury (n = 29) at the second MRI in correlation with time from injury to MRI, in subjects (●) (n = 8) who also underwent a lateral meniscectomy during follow-up and in subjects (○) who did not (n = 21).

T1Gd and patient characteristics

Only patients with normal weight (BMI < 25), increased their T1Gd medially between the two dGEMRIC investigations, $P = 0.013$ (Table III). Moreover, patients with normal weight (BMI < 25) had longer T1Gd values medially than over-weight patients (BMI > 25), at the second MRI investigation, $P = 0.045$ (Table III).

T1Gd was not related to age or sex (data not shown) and did not differ between patients with an ACL reconstruction vs non-ACL reconstruction, at baseline or the follow-up examination (Table III).

ANCOVA

To compensate for potential confounding by BMI and follow-up time, we performed an ANCOVA. T1Gd at follow-up (cross-sectional analysis) and change in T1Gd between first and second MRI (longitudinal analysis), respectively, were used as the continuous outcome variable, and if a meniscectomy had been performed during follow-up or not as a fixed factor. The time between dGEMRIC examinations and BMI at index were used as covariates.

Cross-sectional analysis. The ANCOVA did not change the association between meniscectomy and having a shorter T1Gd value at follow-up, $P = 0.002$ medially and $P = 0.042$ laterally. The association between the T1Gd value and the time between dGEMRIC examinations persisted in the ANCOVA model ($P = 0.006$ medially and $P = 0.046$ laterally). No significant associations persisted between T1Gd values and BMI in the ANCOVA model ($P = 0.19$ medially and $P = 0.43$ laterally).

Longitudinal analysis. The ANCOVA did not substantially change the association between meniscectomy and having a reduction in T1Gd value ($P = 0.040$ laterally). Also for the medial side, the ANCOVA did not essentially change the result with a non-significant reduction in T1Gd value ($P = 0.14$ for meniscectomy). There were no significant associations between the change in T1Gd and the time between dGEMRIC examinations in the ANCOVA model ($P = 0.09$ medially and $P = 0.34$ laterally). No significant associations existed between change in T1Gd and BMI in the ANCOVA model ($P = 0.47$ medially and $P = 0.97$ laterally).

Discussion

We have previously shown a decreased T1Gd already 3 weeks after the injury in this ACL-injured cohort[8]. In this follow-up study in the chronic phase after the ACL injury, our results indicate that the estimated cartilage GAG content (T1Gd) was still lower than reference values in medial and lateral compartments. However, laterally T1Gd was partly regained. It is well known that the lateral tibiofemoral cartilage more often suffers a greater direct blunt trauma than the medial condyle, due to the axial/rotational mechanism of an ACL injury. This often results in a lateral compartment bone marrow lesion that eventually disappears as shown in the present study (Table I) and by others[20]. In this respect, our finding that lateral cartilage T1Gd to some extent normalized over time may suggest improved healing capacity laterally. It may also imply differences in OA pathogenesis between lateral and medial compartments since T1Gd medially, where OA most often develops, was still low at follow-up. Similarly, Fleming *et al.* recently published a dGEMRIC study in ACL-injured patients reporting a decreased mean T1Gd value from the medial tibiofemoral cartilage of the injured knee compared to the uninjured knee[21].

To further explore a possible relation between T1Gd and important risk factors for developing knee OA after an ACL injury[7] we

Table II
Time from injury to surgery and to the second MRI in the included ACL-injured study subjects, n = 29

	Time from injury to surgery	Time from injury to MRI 2
	Mean ± SD (range) months	Mean ± SD (range) months
ACL reconstructed, n = 14	4.8 ± 3.6 (0.8–13.1)	31.2 ± 14.4 (11.4–59.4)
Not ACL reconstructed	9.6 ± 9.6 (1.4–25.6), n = 5	24.0 ± 16.8 (6.8–60.6), n = 15
Medial meniscectomy, n = 6	8.4 ± 8.4 (1.2–25.6)	28.8 ± 10.8 (11.4–42.7)
Lateral meniscectomy, n = 8	7.2 ± 8.4 (0.8–25.6)	27.6 ± 9.6 (8.8–41.5)
No medial or lateral meniscectomy	4.8 ± 3.6 (1.4–13.1), n = 8	26.4 ± 19.2 (6.8–60.6), n = 18
All	6.0 ± 6.0 (0.8–25.6), n = 19	27.6 ± 15.6 (6.8–60.6), n = 29

examined patients according to meniscus injuries and BMI. We have recently in another ACL-injured cohort shown that only patients who had sustained a meniscectomy developed radiographic tibiofemoral OA 15 years after the ACL injury[4]. Hence, we calculated the longitudinal changes and the cross-sectional differences in cartilage T1Gd in ACL-injured patients who had or had not been meniscectomized. Patients with meniscectomy had a shorter T1Gd in the ipsilateral compartment in the chronic phase, after the ACL injury. Further post-hoc analysis suggest a trend for a negative correlation between BMI and T1Gd in the medial femoral cartilage, in agreement with previous studies examining OA risk factors and T1Gd[22,23]. However, the ANCOVA did not indicate BMI to be a statistically significant factor, although the limited sample size makes interpretation difficult (type II error). Still, in the perspective that OA commonly develops medially, in ACL-injured patients, in those meniscectomized and in overweight subjects suggest that a shorter T1Gd, indicative of GAG loss, may be an early feature of developing knee OA.

The finding that there was no difference in T1Gd between ACL reconstructed and non-ACL reconstructed patients, further supports that dGEMRIC is able to predict risk for developing OA since several radiographic studies have shown that an ACL reconstruction does not decrease the risk of post-traumatic OA development[2,3,24–26].

Regarding a possible correlation between a short T1Gd and an increased risk of developing OA, it is important to acknowledge that several studies have shown good agreement between cartilage GAG content and T1Gd[12,27,28]and that a short T1Gd is a feature of early-stage knee and hip OA[15,29–32]. Indeed, several factors may be involved in OA pathogenesis in the ACL-injured joint. An ACL deficient or reconstructed knee will have an altered gait pattern[33]. A joint lacking meniscus function has a higher compressive contact stress during gait[34]. Together with cartilage matrix GAG changes, an ACL-injured knee is also more susceptible to fatigue and progressive destruction of the collagen fibril network[35]. The cumulative effect of the above mentioned factors: (1) altered knee loading pattern due to meniscal tear, high BMI and instability and (2) cartilage matrix changes may result in increased mechanical shearing of the collagen network with subsequent fibrillations and overt OA changes.

The present study was designed to observe cartilage matrix changes by dGEMRIC in a cohort at risk for OA. An additional objective was to explore relationships between T1Gd and known exogenous variables involved in OA development. It may be argued that the study sample is too small. However, previous studies showing that activity level, quadriceps strength and BMI, but not gender and age, affect T1Gd, suggest that only a limited number of patients are needed to detect statistically and clinically significant differences using dGEMRIC (15–20 subjects). This is mainly due to a low variability in T1Gd values in examined cohorts. Furthermore, it is not feasible to include patients that make multivariable modeling reasonable in dGEMRIC studies due to costs, shortage of MRI capacity and difficulties to schedule patients. Unfortunately, we had to exclude more patients than expected because of motion artifacts in the MR images.

Between reference subjects and ACL-injured patients, we had a power of 1.0 respectively 0.94, with alpha set at 0.05, to detect a difference (65 ms medially and 49 ms laterally) in T1Gd values from the medial and lateral femoral cartilage respectively, at follow-up. However, the power to detect a difference (38 ms laterally and 84 ms medially) in T1Gd between meniscectomized and not meniscectomized patients were lower: 0.58 for the lateral and 0.42 for the medial compartment with alpha set at 0.05.

In the ideal study design, the T1Gd value *before* the actual ACL injury is known (and not as in the present study 3 weeks after the injury). In this study, the same T1Gd value 3 weeks after injury could, for example, be present in; (1) a subject whose knee sustained a relatively low-energy ACL trauma-mechanism and who had an inferior knee cartilage quality already before the ACL injury, (2) a subject who had a superior knee cartilage quality (well-trained athlete) before the injury and whose knee sustained a relatively high-energy ACL trauma-mechanism. The development of OA is slow with changes appearing gradually during decades

Table III
Medial (Med) and lateral (Lat) femoral condyles cartilage T1Gd values for the ACL-injured subjects of first (MRI 1) and second (MRI 2) MRI scan performed approximately 3 weeks and 2 years after the ACL injury

	MRI 1 (Mean ± SD)		MRI 2 (Mean ± SD)		*P*-value
	Med T1Gd (ms)	Lat T1Gd (ms)	Med T1Gd (ms)	Lat T1Gd (ms)	
All knees (n = 29)	357 ± 50[1,3]	374 ± 48[2,3]	363 ± 61[1,4]	396 ± 48[2,4]	0.57[1], 0.040[2], 0.006[3], 0.006[4]
MM (n = 6)	326 ± 47	369 ± 53	296 ± 62[1]	390 ± 58	0.002[1]
No MM (n = 23)	364 ± 48	375 ± 47	380 ± 49[1]	398 ± 46	
LM (n = 8)	347 ± 53	381 ± 34	331 ± 84	368 ± 48[1]	0.053[1]
No LM (n = 21)	360 ± 49	371 ± 52	375 ± 47	406 ± 44[1]	
ACLR (n = 14)	350 ± 52[1]	373 ± 45	368 ± 60	397 ± 54	0.56[1]
NACLR (n = 15)	362 ± 48[1]	375 ± 51	363 ± 65	395 ± 42	
Activity level 1–2 (n = 6)	321 ± 48[1]	354 ± 32[2]	354 ± 83	403 ± 49	0.047[1], 0.24[2]
Activity level 3–4 (n = 23)	366 ± 47[1]	379 ± 50[2]	365 ± 56	394 ± 48	
BMI < 25 (n = 19)	352 ± 46[3]	360 ± 45[1,5]	379 ± 50[2,3]	394 ± 49[5]	0.026[1], 0.045[2], 0.013[3], 0.12[4], 0.024[5]
BMI > 25 (n = 10)	365 ± 57[4]	401 ± 42[1]	332 ± 70[2,4]	400 ± 48	

MM = medial meniscectomy during follow-up. LM = lateral meniscectomy during follow-up. ACLR = ACL reconstructed knee. NACLR = non-ACL reconstructed knee. Activity level (1–4), see Method section.
Superscript numerals 1 to 5 represents the *P*-values

before clinically and radiographically OA occurs. The fact that our study participants are in the very beginning of eventual OA development suggests small individual T1Gd differences within the first 1–5 years after the injury Therefore, we judge that T1Gd values from the second MRI investigation are probably more reliable in the analysis than longitudinal data on the change of T1Gd between the first and the second MRI. Once the initial post-traumatic values have stabilized, it may even be that the injured cartilage slowly restitutes within the first years after the injury, as indicated by the correlation between T1Gd at follow-up and follow-up time (Figs. 2 and 3). However, discrepancies in time from injury to the second follow-up did not substantially affect T1Gd in the ipsilateral femoral cartilage after meniscectomy according to the ANCOVA model.

We have chosen to examine the central weight-bearing cartilage of the medial and lateral femoral condyles because this cartilage volume is most commonly affected by early degenerative cartilage changes[36]. In longitudinal cohort analysis it is necessary to use same MRI-protocol as previously[8,10,16,23,32,37]. Arguably more information may be achieved if the total knee cartilage volume (tibia and femur) is used[38]. Fleming *et al.* show similar T1Gd values in femoral and tibial cartilage[21].

In summary, the general decrease in cartilage T1Gd in ACL-injured patients compared with references provide evidence for structural matrix GAG changes that seem more pronounced if a concomitant meniscal injury is present. The fact that post-traumatic OA commonly develops in ACL-injured patients, in particularly those with meniscectomy, suggests that shorter T1Gd may be an early biomarker for OA.

Authors' contribution

Paul Neuman: contributed to and critically commented on the design of the study; collected and assembled data; analyzed and interpreted data; critically revised the article and wrote the manuscript.

Jon Tjörnstrand: contributed to conception and design; critically revised the article; collected and assembled data; and recruited patients to the study.

Jonas Svensson: contributed to conception and design; collected and assembled data; critically revised the article; and provided technical MRI support.

Charlotte Ragnarsson: collected and assembled data; interpreted data; and critically revised the article.

Harald Roos: commented on the study design; analyzed and interpreted data; and critically revised the article.

Martin Englund: commented on the study design; analyzed and interpreted data; critically revised the article; and contributed to the writing of the manuscript.

Carl Johan Tiderius: contributed to conception and design of the study; analyzed and interpreted data; critically revised the article; recruited patients to the study; collected and assembled data; and contributed to the writing of the manuscript.

Leif Dahlberg: contributed to conception and design of the study; analyzed and interpreted data; critically revised the article; and contributed to the writing of the manuscript.

Conflict of interest

The authors have no conflict of interest and this manuscript has not been submitted elsewhere.

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Paper IV

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Osteoarthritis and Cartilage

Osteoarthritis development related to cartilage quality-the prognostic value of dGEMRIC after anterior cruciate ligament injury

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SUMMARY

Objective: Rupture of the anterior cruciate ligament (ACL) increases the risk of developing osteoarthritis (OA). Delayed Gadolinium enhanced magnetic resonance imaging (MRI) of cartilage (dGEMRIC) investigates cartilage integrity through T1-analysis after intravenous contrast injection. A high dGEMRIC index represents good cartilage quality. The main purpose of this prospective cohort study was to investigate the prognostic value of the dGEMRIC index regarding future knee OA.

Method: 31 patients with ACL injury (mean age 27 ± 6.7 (±SD) years, 19 males) were examined after 2 years with 1.5T dGEMRIC of femoral cartilage. Re-examination 14 years post-injury included weight-bearing knee radiographs, Lysholm and Knee Osteoarthritis Outcome Score (KOOS).

Results: At the 14-year follow up radiographic OA (ROA) was present in 68% and OA symptoms (SOA) in 42% of the injured knees. The dGEMRIC index of the medial compartment was lower in knees that developed medial ROA, 325 ± 68 (ms±SD) vs 376 ± 47 (51 (7–94)) (difference of means (95% confidence interval (CI))), in patients that developed symptomatic OA (SOA), 327 ± 61 vs 399 ± 42 (52 (11–93)), and poor knee function 337 ± 54 vs 381 ± 52 (48 (7–89)) compared to those that did not develop ROA, SOA or poor function. The dGEMRIC index correlated negatively with the OARSI osteophyte score in medial ($r = -0.44$, $P = 0.01$) and lateral ($r = -0.38$, $P = 0.03$) compartments.

Conclusion: The associations between a low dGEMRIC index and future ROA, as well as SOA, are in agreement with previous studies and indicate that dGEMRIC has a prognostic value for future knee OA.

Introduction

The incidence of injury to the Anterior Cruciate Ligament (ACL) has been estimated at 0.81/1000 in ages 18–65 years[1], with the highest risk in younger active patients. Approximately 50% (15–84%) of ACL injured knees have radiographic osteoarthritis (ROA) in the femorotibial joint 10–20 years after the injury[2]. ACL reconstruction (ACLR) improves knee stability without decreasing the risk of developing ROA[2,3]. Young age at injury, in combination with a high risk of post-traumatic osteoarthritis (OA), implies that many patients will already suffer from OA in their 4th decade of life, an age where many treatment options, such as total joint replacement, are controversial.

OA development after ACL injury is multifactorial, involving mechanical factors and subsequent complex inflammatory responses depleting cartilage of glycosaminoglycan (GAG) and eventually disruption of the collagen II network[4]. At early stages of OA, the cartilage can still be macroscopically intact with changes undetectable by diagnostic tools such as radiographs, magnetic resonance imaging (MRI) and even arthroscopy.

Delayed Gadolinium enhanced MRI of cartilage (dGEMRIC) estimates the GAG content of hyaline cartilage by quantitative T1 analysis. After an intravenous injection, the negatively charged contrast medium distributes into the cartilage in an inverse relationship to the negatively charged GAG[5]. T1 within a cartilage region (the dGEMRIC index) is therefore a surrogate marker for cartilage quality. A low dGEMRIC index has been associated with an

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increased risk of developing ROA, both in the knee[6–8] and in the hip[9–12].

We have previously reported a low dGEMRIC index (indicating GAG loss) of the femoral knee cartilage in this cohort[13,14].

The aim of the present prospective study was to evaluate the prognostic value of the dGEMRIC index regarding future OA symptoms and ROA.

Methods

The cohort

The initial cohort included 40 patients with no previous knee injury who had sustained an acute ACL tear[13,14]. Patients were recruited from the orthopedic trauma center at Skåne University Hospital, Malmö, Sweden. Inclusion criteria were: age <40 years, closed physes and MRI verified ACL rupture in a previously uninjured knee. All patients were treated according to the standard treatment algorithm at the orthopedic department, which was not changed during the study period. The need for surgical ACL repair was based on the amount of persistent functional knee instability after physiotherapy.

Patients in the present study

For this 14-year follow-up study, all 40 patients from the original cohort were contacted with a letter of consent and patient-reported outcome measurements (PROMS). Despite multiple attempts, two patients never responded and three patients responded but never showed up for radiographs. Of the 35 patients that could be radiographically investigated, two had not completed the dGEMRIC investigation and two had invalid dGEMRIC investigations. Thus, 31 patients (19 men and 12 women) with complete dGEMRIC, radiographs and PROMS data were included, representing a 78% inclusion rate from the original cohort. There was no difference between participants and dropouts regarding sex, body mass index (BMI) or age.

21 of the 31 patients had been operated with ACLR and eight patients had been subject to partial meniscectomy. In one patient, operated with a high tibial valgus osteotomy 11 years after the injury, the immediate preoperative radiographs were used for radiographic scoring and the patient was dichotomized as symptomatic OA (SOA).

dGEMRIC

MRI was performed on a 1.5 T system with a dedicated knee coil (Magnetom Vision/Sonata; Siemens Medical Solutions, Erlangen, Germany). Gd-DTPA^{2-} (Magnevist®, Bayer Schering Pharma AG, Berlin, Germany) at 0.3 mmol/kg body weight was injected intravenously. Post-contrast MRI was performed 2 h after the injection. Regions of interest (ROIs) were drawn in the weight-bearing central parts of the lateral and medial femoral condyle cartilages according to a previously validated protocol[15]. Results are presented as mean T1 (ms) of each ROI (the dGEMRIC index). Average time from ACL injury to dGEMRIC for the 31 patients included was 2 years (median 24, range 7–61 months).

Radiography

Weight-bearing radiographs were taken according to a standardized knee OA protocol of standing antero-posterior radiographs with both knees in 20° of flexion. Using a validated method[16], radiographs were analyzed independently by two of the authors; an orthopedic surgeon specialized in joint replacement (J.T.) and a senior radiologist specialized in skeletal radiology (B.L.). In cases of discrepancy, the images were reassessed by the two investigators together and a consensus was reached. The OARSI atlas[17] was used for the medial and lateral compartments respectively, grading radiographic change on a four point scale for Joint Space Narrowing (JSN) (0–3, 0 = no evidence of JSN) and marginal osteophytes of femoral and tibial condyles (0–3 each, 0 = no bony change). Dichotomization for diagnosis of ROA was defined according to Englund *et al.*[16] as any of the following criteria fulfilled in either of the two femorotibial compartments: JSN grade ≥2, the sum of the marginal osteophyte score in the same compartment ≥2, or JSN grade 1 in combination with osteophyte grade 1 in the same compartment. This definition approximates grade two knee OA based on the Kellgren–Lawrence scale.

The sum of femoral and tibial marginal osteophytes in the OARSI score (grade 0–6) was used to quantify the grade of ROA in the medial and lateral compartments, respectively.

Patient reported outcome measures (PROMs)

The self-administered outcome scales Lysholm[18] and Knee Osteoarthritis Outcome Score (KOOS)[19] were sent to patients to complete and returned by mail. The KOOS data was used to define patients that had SOA according to Englund *et al.*[16]. In summary, this definition of SOA requires that the score for the KOOS subscale of knee-related quality of life *and* at least two of the four additional subscales should be below 86 after conversion to a 0–100 scale.

Similarly, a Lysholm score ≤84 reflects an unsatisfactory knee function and can be regarded as cut-off point for dichotomization to "poor function"[20].

Statistics

Tests for normal distribution, kurtosis and skewness were conducted. Continuous variables (e.g., the dGEMRIC index) are reported as mean values with standard deviation (mean ± SD), Student's *t*-test and 95% confidence interval (95% CI) was used to compare difference of means (MD). The following outcome variables were dichotomized and compared regarding the dGEMRIC index: ROA, SOA and poor knee function (Lysholm). Correlations were evaluated with Spearman rank correlation for ordinal variables (e.g., osteophyte score). Fisher exact test was used for dichotomous variables. Possible confounders (age, sex and BMI) were correlated (Spearman) with the dGEMRIC index. Logistic regression was used to calculate the predicted probability of ROA. A receiver operating characteristics (ROC) curve was used to illustrate the predictive value of the dGEMRIC index on an individual level. SPSS 25 was used for the statistical analysis.

Ethics

The study was approved by the Ethical Review Board at Lund University (Etikprövningsnämnden #EPN:2014/752, LU#73–96 and LU#651-00), the Radiation Protection Committee (Strålskyddskommittén #SSFo2014-050), and the Image Research Committee (BOF053). Patients signed a renewed informed consent before the 14-year follow-up data collection.

Results

Demographics

The median age at injury was 27 years (range 15–40) and at follow-up 40 years (range 26–53). Mean BMI had increased to 26.0 (SD 3.8) kg/m^2, (2.3 (95% CI 1.4–3.1)) from 23.7 (SD 2.7) kg/m^2 at

injury. Median follow-up time was 14 years (range 10.4–16.7) after injury and 12 years (range 9.7–13.6) after dGEMRIC. There were no significant correlations between dGEMRIC values and the possible confounding factors age ($r = -0.23$, $P = 0.92$), sex ($r = 0.07$, $P = 0.71$) or BMI ($r = -0.25$, $P = 0.18$).

Prevalence of OA

ROA was present in 21 of 31 (68%) of ACL-injured knees at follow-up. Of these, seven knees had isolated medial ROA, 11 had isolated lateral ROA and three knees had ROA in both compartments. ROA of the ACL-injured knee was present in 6 of 12 women and 15 of 19 men. OA symptoms (SOA) was present in 13 of 31 patients (42%). Two patients had SOA without radiographic signs of OA. BMI did not differ between patients with and without ROA, or SOA. A subgroup analysis of patients with and without ACL-reconstruction or meniscectomy was not considered reliable in this limited number of individuals.

dGEMRIC in relation to outcome

Knees that developed ROA in the medial compartment 14 years after injury already had a lower dGEMRIC index in the medial femoral cartilage 2 years after injury than knees with no ROA development. The mean difference between the groups was 50.7 ms (95% CI 7.2–94) (Fig. 1, Table I). Fig. 2 illustrates the calculated probability of developing medial compartment ROA. The medial dGEMRIC index (continuous variable) as a marker of medial radiographic OA (dichotomous variable) yielded an area under the ROC curve of 0.70 (95% CI 0.49–0.91) (Fig. 3). The best cut-off, maximizing the Youden index, was 330 ms with a sensitivity of 50% and a specificity of 91%.

In the lateral compartment, the difference between groups was 29 ms (95% CI −3.1–62) (Fig. 1, Table I). The grade of ROA, assessed with osteophyte score, correlated negatively with the dGEMRIC index, both in the medial compartment ($r = -0.44$, $P = 0.01$) and in the lateral compartment ($r = -0.38$, $P = 0.03$) (Fig. 4).

Patients with SOA at follow-up had a lower dGEMRIC index medially than patients without SOA (MD 52.4 (95% CI 11–93)) (Fig. 1, Table I). Patients with poor/fair knee function had lower dGEMRIC values in the medial compartment vs patients with good/excellent knee function at follow-up (MD 48.0 (95% CI 7.2–89)) (Fig. 1, Table I).

Discussion

Patients with an acute ACL-injury are suitable for the study of post-traumatic OA development since approximately half will have the disease within 10–15 years. The main result of the present study is very encouraging, i.e., that both clinical and ROA are associated with a low dGEMRIC index as soon as 2 years after the initial injury (Fig. 1). In addition, the dGEMRIC index had a negative correlation with the grade of ROA as assessed with the osteophyte score, suggesting a dose–response effect (Fig. 4). At an individual level, the dGEMRIC index has a limited predictive value, as illustrated by the large confidence interval in Fig. 2. The low dGEMRIC index in the cartilage of knees that eventually develop OA indicates a decreased GAG content in that cartilage, which in turn reflects impaired cartilage quality. GAG depletion is generally regarded as a very early event in the molecular pathway of OA progression[4]. There are clinical data to support the idea that GAGs can be replenished by intervention, such as physical exercise[21], osteotomy[9] and patella stabilizing surgery[22]. To determine the optimal treatment of an acute ACL-injury, whether this may be surgical or non-surgical, randomized controlled studies (RCT) are needed. One major issue with RCTs in OA is the long timespan needed for the ROA changes to occur. Instead, most researchers agree that we need early and sensitive markers for cartilage quality that ideally predict OA development.

Our results are in line with several previous dGEMRIC studies in other cohorts[6–12]. A low preoperative dGEMRIC index of hip cartilage was found to be the strongest predictor for a bad clinical outcome (OA progression) after periacetabular osteotomy in patients with hip dysplasia[9,12]. Similarly, a high preoperative dGEMRIC index before hip arthroscopy was correlated to a favorable clinical outcome 2 years postoperatively[11]. In hips with femoroacetabular impingement, baseline dGEMRIC predicted the ROA development at the 5-year follow up[10]. Regarding knee OA, Owman *et al.* have presented two different cohorts of middle-aged patients at risk of developing OA. In patients with early cartilage degeneration found at arthroscopy, a low dGEMRIC index was associated with ROA development 6 years later[8]. In patients who had been

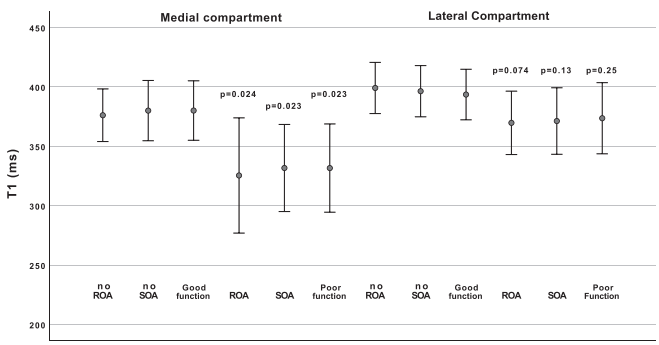

Fig. 1. The Delayed Gadolinium enhanced MRI of cartilage (dGEMRIC) index (ms) in medial and lateral femoral cartilage 2 years after anterior cruciate ligament (ACL) injury related to radiographic OA (ROA) and symptomatic OA (SOA) at the 14-year follow-up. Knees that developed ROA, SOA or poor knee function (see methods for details) had lower dGEMRIC index medially than knees that did not. "•" represents mean value with 95% CI as error bars. Difference of means and absolute values are presented in Table I.

Table I
The Delayed Gadolinium enhanced MRI of cartilage (dGEMRIC) index (**bold**) 2 years after anterior cruciate ligament (ACL) injury related to ipsicompartmental radiographic OA (**ROA**, Yes/No), OA symptoms of the whole knee (**SOA**, Yes/No) and knee function (**Lysholm <84**, poor/good) 14 years after the ACL injury. Results are presented for medial and lateral femoral cartilage in separate columns

Outcome in respective compartment	ROA medial	ROA lateral	SOA medial	SOA lateral	Lysholm <84 medial	Lysholm <84 lateral
Yes						
mean (±SD) T1 ms	**325**(68)	**370**(46)	**332**(61)	**371**(46)	**333**(59)	**375**(45)
n=	*10*	*14*	*13*	*13*	*14*	*14*
No						
mean (±SD) T1 ms	**376**(47)	**399**(42)	**380**(51)	**396**(43)	**381**(52)	**394**(46)
n=	*21*	*17*	*18*	*18*	*17*	*17*
Student *t*-test	*.024*	*.074*	*.023*	*.13*	*.023*	*.25*
p=						
difference of means	50.7	29.3	52.4	25.1	48.0	19.2
95% CI	7.2–94	−3.1–62	11–93	−8.1–58	7.2–89	−14–53

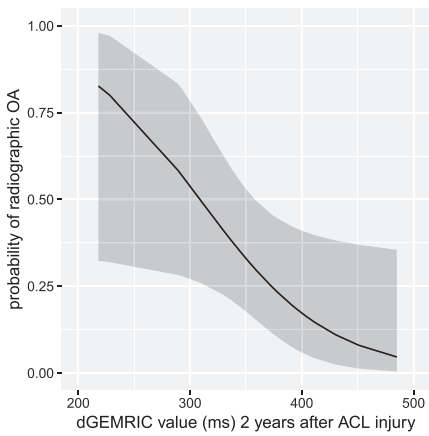

Fig. 2. dGEMRIC index of medial femoral cartilage vs calculated probability of medial ROA at the 14-year follow-up, the shaded area represents 95% CI.

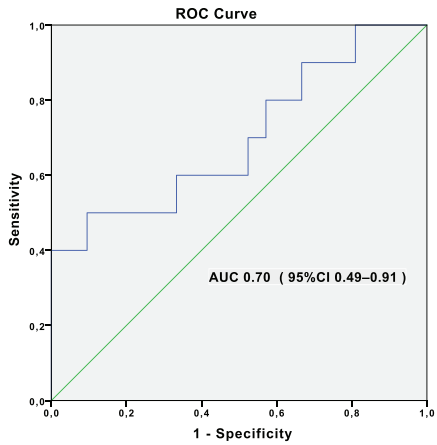

Fig. 3. ROC curve of medial dGEMRIC index as a marker of ROA outcome. The area under the curve was 0.70 (95% CI 0.49–0.91). The maximal Youden index was at 330 ms with a sensitivity of 50% and a specificity of 91%.

operated with a partial medial meniscectomy, the dGEMRIC index correlated negatively with the amount of ROA 11 years later[6]. More recently, we have shown a negative correlation between the dGEMRIC index in the adjacent cartilage after surgical cartilage repair and future OA, again suggesting a clinical relevance of dGEMRIC[7].

There are issues with the dGEMRIC technique in addition to the inherent complexity with intravenous contrast injection 1–2 h before imaging. Emerging safety concerns have restricted[23,24] the use of Gd-DTPA^{2-} since the contrast agent has been associated with both nephrogenic systemic fibrosis (NSF) in patients with severe renal impairment[25], and accumulation in the brain after repeat investigations[26]. Such concerns limit the future use of Gd-DTPA^{2-} for *in vivo* application. However, macrocyclic gadolinium chelates of higher molecular stability, such as gadoteric acid (Gd-DOTA^{1-})[27] have been tried as substitution for Gd-DTPA^{2-} in dGEMRIC of hip, wrist and knee cartilage with comparable results[28]. Ultimately, national authorities must determine which contrast agents can be safely used in patients as well as in healthy subjects.

Much research has also been focused on MRI techniques that do not require contrast enhancement, such as T2-mapping, gagCEST[29], Ultrashort echo-time T2* (UTE-T2*)[30] and sodium MRI[31]. For example, data from the Osteoarthritis Initiative cohort has shown that long T2 values of tibiofemoral cartilage may predict ROA over a 4-year period[32]. In a recent study of patients 2 years after ACLR, UTE-T2* profiles from both the reconstructed and the contralateral knees differed from that of uninjured controls[33].

In the present study we found a high rate of ROA (68%) 14 years after an acute ACL injury. A direct comparison of our results with other studies is hampered by many factors, such as different criteria for OA diagnosis, age at injury, duration of follow-up, gender, mechanism of injury, treatment, rate of loss to follow-up, etcetera. The heterogeneity of these factors is illustrated by the fact that reported rates of ROA after ACL injury varies between 10% and 90% with an average of 50% after 10–20 years[2]. The subjects in our study represent a cross sectional selection as they were prospectively and consecutively recruited from the ER department of one single hospital.

The main limitation of our study is the small number of patients, which disenables a multivariate analysis. It is also important to point out that the clinical course of each patient varies considerably, despite the strict inclusion criteria. Some patients need ACLR and some become subject to additional meniscus surgery. From the present data, we cannot evaluate the impact of those individual variables. Considering such and other patient related factors, the

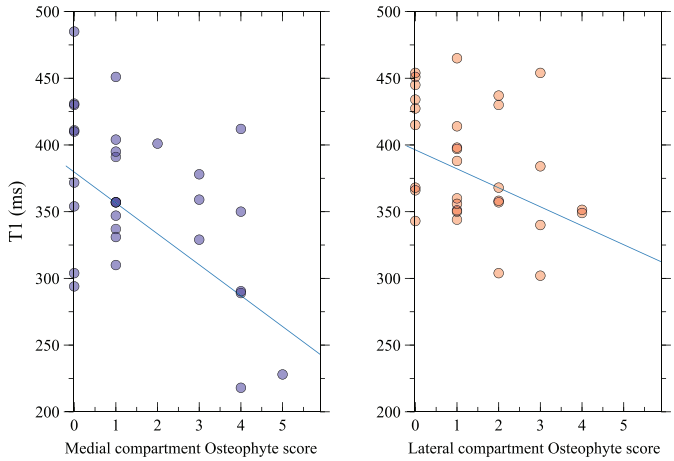

Fig. 4. There was a negative correlation between the dGEMRIC index 2 years after injury and grade of OA, assessed with the OARSI osteophyte score, 14 years after injury (medial compartment $r = -0.44$ ($P = 0.01$, $n = 31$), lateral compartment $r = -0.38$ ($P = 0.03$, $n = 31$)).

prognostic level of the dGEMRIC index on an individual level may be limited. Despite these limitations it is intriguing that the cartilage quality, represented by the dGEMRIC index, seems to influence the long-term outcome after ACL-injury.

In summary, the associations between a low dGEMRIC index and future ROA, as well as SOA, are in agreement with previous studies and indicate that dGEMRIC has a prognostic value for future knee OA.

Author contributions

JT: Study initiation and design, recruitment of patients, radiographic analysis, MRI analysis, data collection and analysis, and writing of manuscript. PN: recruitment of patients, MRI analysis, data collection and manuscript revisions. JS: MRI analysis and manuscript revision. BL: radiographic analysis and manuscript revision. LD: Study design and manuscript revision. CT: Study initiation and design, recruitment of patients, critical revision of the manuscript.

Conflict of interest

All authors state no conflicting interest. None of the funding sources had any involvement in study design, collection, analysis and interpretation of data; in writing of the manuscript; or in the decision to submit the manuscript for publication.

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