Human studies evaluating dGEMRIC as a prognostic tool for knee osteoarthritis

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2014

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AKADEMISK AVHANDLING
som med vederbörligt tillstånd av Medicinska fakulteten vid Lunds universitet för avläggande av doktorsexamen i medicinsk vetenskap kommer att offentligen försvaras i ortopediska klinikens föreläsningssal, Skånes universitetssjukhus, Malmö, fredagen den 28 februari 2014, kl. 13.00.

Fakultetsopponent

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Organization LUND UNIVERSITY Department of Clinical Sciences, Malmö	Document name DOCTORAL DISSERTATION
	Date of issue 2014-02-06
Author(s) Henrik Owman	Sponsoring organization

Title and subtitle
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Early in the progression of the disease, the articular cartilage is depleted of glycosaminoglycans (GAGs), which are responsible for cartilage load distribution and compressive stiffness. Delayed gadolinium-enhanced magnetic resonance imaging of cartilage, abbreviated dGEMRIC, can be used to estimate the GAG content of cartilage. A contrast medium is used, the concentration of which in the cartilage is inversely proportional to the amount of GAG. The purpose of this work was to evaluate dGEMRIC as a prognostic tool for knee cartilage changes and knee OA in humans.

It was found that dGEMRIC could be used to predict the development of radiographic knee OA in patients at risk of developing OA. An association was also found between dGEMRIC values and important features of knee OA, such as joint space narrowing and osteophytes (bony changes).

Unloading of joints has previously been shown to affect the constituents of cartilage. The knees of patients with ankle fractures, prescribed unloading of the injured leg for six weeks, were investigated. Unloading resulted in a measurable effect on the constituents of the knee cartilage, seen as a decrease in GAG content and an increase in the range of dGEMRIC values. These findings should be taken into account when considering treatment of patients involving an unloading regimen.

Anterior cruciate ligament (ACL) injury has previously been shown to be an important risk factor for the development of OA. Patients who had sustained an ACL injury 20 years earlier, who had not undergone ACL reconstruction, were investigated. Notably, these patients showed good cartilage quality and subjective knee function, similar to that in healthy reference groups. This is an important finding and should be considered when recommending treatment for patients with ACL injuries.

Key words
Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC), knee, cartilage, glycosaminoglycan, anterior cruciate ligament injury, meniscal injury, osteoarthritis, joint space narrowing, osteophyte

Classification system and/or index terms (if any)

Supplementary bibliographical information Faculty of Medicine Doctoral Dissertation Series 2014:22		Language English
ISSN and key title 1652-8220		ISBN 978-91-87651-46-5
Recipient's notes	Number of pages 120	Price
	Security classification	

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Lund University, Faculty of Medicine Doctoral Dissertation Series 2014:22
ISBN 978-91-87651-46-5
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2014

To my relief

Contents

Abstract

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Populärvetenskaplig sammanfattning

Artros är den vanligaste ledsjukdomen i världen, och orsakar ledvärk och stelhet. Artros leder till försvagning av brosket och sedermera förlust av allt större mängder belastat ledbrosk. För närvarande är vanlig röntgen den gyllene standarden för att diagnostisera knäartros. Dock är sjukdomsförloppet ofta väldigt långt framskridet när broskförändringar börjar synas på röntgen. Behandlingsalternativen i detta skede sträcker sig från åtgärder för att behålla det brosk som finns kvar till operation med knäledsprotes. Att upptäcka artrosförändringar i ett tidigare stadium av sjukdomsförloppet skulle göra det möjligt att inleda botande eller förebyggande behandling långt innan vad som är möjligt idag.

Tidigt i sjukdomsprocessen förlorar ledbrosket glykosaminoglykaner (GAG), som ansvarar för fördelning av broskets belastning och broskets styvhet när det trycks samman. Kontrastförstärkt magnetkameraundersökning av brosk, eller helt kort dGEMRIC, är en metod för att uppskatta mängden GAG i brosk. Vid dGEMRIC används ett kontrastmedium som tas upp i brosket i ett omvänt förhållande till mängden GAG, vilket innebär att en låg mängd GAG ger upphov till en stor mängd av kontrastmedium i brosket. Syftet med denna avhandling var att undersöka hur dGEMRIC-tekniken kan fungera som ett prognostiskt verktyg för broskförändringar och artros i knäet.

I delarbete I studerades patienter som löpte risk att utveckla artros. Denna studie visade att dGEMRIC-metoden kunde förutsäga utvecklingen av knäartros sex år senare. I delarbete II blev ännu en grupp av patienter med risk för artros undersökta. Den här gången fanns ett samband mellan dGEMRIC-värdena och viktiga artrosfynd i knäet, nämligen ledbrosksänkning och osteofyter (beniga förändringar). Resultaten från delarbete I och II antyder att dGEMRIC-metoden har förmåga att förutsäga framtida artrosutveckling i knäet.

Avlastning av brosk har tidigare visat sig påverka dess beståndsdelar. I delarbete III undersöktes knäna hos patienter med fotledsfraktur som ordinerats avlastning av det skadade benet i sex veckor. Avlastning av knäbrosket i sex veckor resulterade i en mätbar effekt på knäbroskets beståndsdelar som kom till uttryck genom ett minskat GAG-innehåll och ett ökat spann av dGEMRIC-värden. Dessa fynd måste beaktas när man överväger behandling av patienter som omfattar avlastning.

Främre korsbandsskada har tidigare visat sig vara en riskfaktor för artrosutveckling. I delarbete IV undersöktes patienter som hade ådragit sig en främre korsbandsskada 20 år tidigare, och som inte hade genomgått korsbandsrekonstruktion. Till vår förvåning hade dessa patienter bra broskkvalitet och bra knäfunktion, samma som friska kontrollgrupper. Detta är ett viktigt fynd att beakta vid behandling av korsbandsskadade patienter.

Abbreviations

ACL	Anterior cruciate ligament
ACLR	ACL reconstruction
ADL	Activities of daily living
BMI	Body mass index
dGEMRIC	Delayed gadolinium-enhanced MRI of cartilage
GAG	Glycosaminoglycan
Gd-DTPA^{2-}	Gadolinium diethylene triamine pentaacetic acid
JSN	Joint space narrowing
KOOS	Knee injury and osteoarthritis outcome score
MRI	Magnetic resonance imaging
OA	Osteoarthritis
QOL	Quality of life
ROI	Region of interest
Sport/rec	Subscale describing the ability to take part in sports and recreational activities
T1	Spin-lattice relaxation time
T1Gd	T1 of cartilage after saturation with Gd-DTPA^{2-}

List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals. The papers are appended at the end of the thesis.

I. **Association between findings on delayed gadolinium-enhanced magnetic resonance imaging of cartilage and future knee osteoarthritis**
Owman H, Tiderius CJ, Neuman P, Nyquist F, Dahlberg LE
Arthritis Rheum 2008;58:1727-1730

II. **Association between delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) and joint space narrowing and osteophytes: a cohort study in patients with partial meniscectomy with 11 years of follow-up**
Owman H, Ericsson YB, Englund M, Tiderius CJ, Tjörnstrand J, Roos EM, Dahlberg LE
Submitted

III. **Long-term effect of removal of knee joint loading on cartilage quality evaluated by delayed gadolinium-enhanced magnetic resonance imaging of cartilage**
Owman H, Tiderius CJ, Ericsson YB, Dahlberg LE
Submitted

IV. **Knee cartilage assessment with MRI (dGEMRIC) and subjective knee function in ACL injured copers: a cohort study with a 20 year follow-up**
Neuman P, Owman H, Müller G, Englund M, Tiderius CJ, Dahlberg LE
Article in press in *Osteoarthritis Cartilage*

Introduction

Articular cartilage

Articular cartilage is a layer of low-friction, load-bearing soft tissue that overlies the articulating bony ends in diarthrodial joints. It provides the joint with essential biomechanical properties, such as wear resistance, load bearing, shock absorption, and lubrication. Cartilage is remarkable in its ability to withstand high compressive, tensile, and shearing stresses. The viscoelastic properties that make this possible depend on its unique composition and structure (Mow 2003).

The major structural element of articular cartilage is collagen II, accounting for 75% of the tissue dry weight (Mow 2003). These fibers, which are specific for cartilage, form the bulk of the stable collagen triple helix structure, made up of three polypeptide strands. When a load is applied to the cartilage, the collagen II fibers respond to the tension and shear by stretching (Heinegaard 2003, Mow 2003).

Aggrecan is a large molecule (> 2,500 kDa) and the most common of the proteoglycan family. It consists of a central core protein to which numerous covalently bound GAG chains are attached. Aggrecan molecules, in turn, attach to hyaluronan, forming even larger proteoglycan aggregates (Heinegaard 2003). The large size and complex structure of the proteoglycans means that they are trapped within the collagen network, forming the solid matrix of articular cartilage (Mow 2003).

GAGs are second only to collagen II among the constituents of cartilage, accounting for 20–30% of the dry weight. GAGs consist of repeating sugar units containing at least one negatively charged group (COO^- or $SO3^-$), and provide the cartilage with a negative fixed charge density. This negative fixed charge density attracts positive counter-ions, mostly sodium (Na^+), to the cartilage, thus maintaining electroneutrality (Mow 2003). This results in an osmotic imbalance of mobile ions between the cartilage and the synovial fluid, causing water to be drawn into the cartilage according to the Donnan effect (Maroudas 1970). As the Donnan effect only accounts for approximately 50% of the swelling pressure (Mow 2003), a triphasic model for articular cartilage has been proposed, where a phase represents all of the chemical compositions with similar physical properties (Lai 1991, Lu 2008). According to this model, the compressive stiffness of

cartilage depends on three load-supporting mechanisms: 1) a solid phase, composed predominantly of the collagen II network with intertwined proteoglycan macromolecules; 2) a fluid phase (water); and 3) an ionic phase, comprised of various dissolved electrolytes with positive and negative charges.

The response of cartilage to loading is an increase in the amount of GAG. In humans, exercise has been shown to have a positive effect on cartilage composition, improving cartilage quality, allowing it to withstand daily wear. Individuals taking regular exercise have a higher content of GAG in knee cartilage than non-exercising individuals (Tiderius 2004a, Van Ginckel 2010). However, changes in cartilage quality and GAG content as a result of joint immobilization and unloading have mostly been studied in animals due to the lack of non-invasive methods. A decrease in cartilage quality and GAG content has been reported after immobilization of the temporomandibular joint in primates (Glineburg 1982), and of the canine stifle joint (Palmoski 1979, Jurvelin 1986, Kiviranta 1987, Saamanen 1990, Haapala 2000). The effect has been found to be partially reversed after remobilization (Haapala 1999). A decrease in knee cartilage proteoglycans, similar to changes found in canine knee cartilage after cast immobilization, was described by Palmoski *et al.* in dogs with ipsilateral paw transection (Palmoski 1980). Contrary to these findings, an increase in cartilage proteoglycans was found in the knee cartilage of guinea-pigs three months after ipsilateral below-knee amputation (Wei 2001). The diversity of these findings makes it difficult to interpret and assess the relevance of animal studies in the clinical setting.

Few attempts have been made to study the long-term effect of changes in load bearing on cartilage in vivo in humans. A post-ankle fracture model was used by Hinterwimmer *et al.* to investigate the effect of partial load bearing on cartilage thickness, volume, and surface area (Hinterwimmer 2004). A significant decrease in cartilage thickness and volume was found after seven weeks of partial load bearing. However, changes in the structural matrix on the molecular level were not studied. In a study by Souza *et al.*, using T1rho and T2 mapping (two MRI techniques for the assessment of cartilage composition) and a follow-up period of 10–12 weeks, a broad distribution of cartilage composition was observed after 6–8 weeks of knee joint unloading (Souza 2010). This was attributed to a decrease in proteoglycan content and disorganization of the collagen network. After four weeks of remobilization, the relaxation times returned to baseline levels, demonstrating reversibility in compositional fluctuations.

Osteoarthritis

OA is the most common joint disorder throughout the world (Arden 2006), with a prevalence of 10% in people older than 55 years, and 30% in people older than 65

years (Felson 1987, Peat 2001). According to the World Health Organization, OA is ranked 11th regarding the number of years lived with disability (Vos 2012). It has a greater impact on everyday activities, such as climbing the stairs, walking, and housekeeping, in the elderly than other severe conditions such as hip fracture, stroke, heart disease, and chronic obstructive pulmonary disease (Guccione 1994). In a recent Swedish population-based cohort study, subjects with clinically diagnosed knee OA had twice the risk of being on sick leave and a 40–50% increased risk of being granted disability pension, compared to the general population. It was also found that about 2% of all days of sick leave in society was related to OA in the knee (Hubertsson 2013).

Pathologically, OA involves the entire joint (Felson 2000, Guermazi 2003, Poole 2012). It is believed to be the result of an imbalance between the biosynthesis and degradation of cartilage constituents, in which degradative processes outpace compensatory repair (Rizkalla 1992, Ishiguro 1999, Dahlberg 2000, Heathfield 2004). The depletion of GAGs, responsible for load distribution and compressive stiffness, from articular cartilage is considered to be an early event in the progression of OA (Rizkalla 1992, Heinegaard 2003). During a two-year period, Boegard *et al.* observed the appearance, increase, decrease, and disappearance of cartilage defects in knees with OA changes (Boegard 2001). This indicates that the progress of the disease is not continuous, and that cartilage repair is possible in the early stages of OA. A crucial point of no return is considered to be molecular damage to the collagen II molecules constituting the fiber network of the cartilage matrix (Heathfield 2004). Early macroscopic findings include cartilage swelling, fibrillation, and fissuring. Finally, cartilage loss gives rise to JSN, together with changes in the underlying bone, causing bone cysts, subchondral sclerosis, and marginal outgrowths, or osteophytes (Felson 2000, Lohmander 2007).

The symptoms associated with OA are pain, joint stiffness, and functional impairment (Dieppe 2005, Lohmander 2007). However, symptoms can occur in patients showing no overt OA changes on plain radiographs. In a study by Hannan *et al.*, radiographic OA changes were seen in only 15% of patients reporting knee pain, while only 47% of patients with radiographic OA changes reported knee pain (Hannan 2000). Thus, there is only a weak correlation between radiographic evidence of OA and symptoms (Dieppe 2005).

Risk factors for knee OA are clearly multifactorial (Nuki 1999). As a consequence, the emphasis in OA epidemiology has shifted towards the identification of risk factors for the development and progression of OA, rather than the incidence in the population (Sharma 2006). ACL injury is among the best documented risk factors for the development of knee OA (Lohmander 2007, Neuman 2008, Oiestad 2009, Friel 2013). Isolated meniscal injury and meniscectomy in the ACL-injured knee, as well as muscle weakness, are other well-known risk factors (Slemenda 1997, Roos 1998b, Lohmander 2007, Neuman 2008, Oiestad 2009, Keays 2010). Obesity is not only a risk factor for the

development of knee OA (Felson 1988, Englund 2004), but also accelerates the progression of the disease (Felson 1997). Age, family history, developmental conditions that affect joint growth or shape, joint injuries, as well as certain work or leisure activities, are other contributing elements (Felson 1998, Felson 2000, Lohmander 2007).

Diagnostic tools in OA

Radiography

The gold standard for diagnosing OA is currently weight-bearing radiography with the knee in a semiflexed position, which has high reproducibility (Peterfy 2003). The most frequently used grading systems are those of Kellgren and Lawrence (Kellgren 1957), and Ahlbäck (Ahlback 1968). The radiographic changes that are graded comprise JSN, the occurrence of osteophytes (Boegard 1998), and subchondral sclerosis. As in the case of JSN, Boegard *et al.* proposed that a narrowing of 3 mm or less should be used for the diagnosis of OA (Boegard 1997). The Kellgren and Lawrence scale is mostly used for screening patients for radiographic diagnosis in the clinical setting. Several new grading systems have emerged for OA staging in clinical trials, one being the Atlas of the Osteoarthritis Research Society International, which was first published in 1995 and revised in 2007 (Altman 1995, Altman 2007).

Knee radiography has several limitations, such as discrepancies in knee positioning (Buckland-Wright 1999, Davies 1999, Vignon 2003). Furthermore, there is only a weak association between radiographic signs of knee OA and symptoms (Lawrence 1966, Hannan 2000). But most importantly, radiography is unable to detect OA at an early stage, meaning that minor changes in cartilage are only visible on plain radiographs several years or decades after the actual onset of cartilage loss (Lysholm 1987, Brandt 1991, Jones 2004). Despite the development of more sophisticated imaging techniques, plain radiography remains the least expensive and most easily available knee joint imaging modality.

Radiography alone may be used for OA diagnosis for research purposes, although it does not provide any information on cartilage structure. Clinical diagnosis of OA depends on symptoms such as pain, decreased joint function, and joint stiffness after inactivity. Radiographic OA is of less importance in the early phases of patient counseling, which focus on patient information and education, training, and weight loss. Clinical findings such as joint crepitus, decreased range of motion, and joint swelling also contribute to the clinical diagnosis of OA. Only at a later stage – when knee surgery may be considered – is radiographic OA, in

combination with the symptoms and clinical findings described above, of importance.

Arthroscopy

Arthroscopy makes it possible to visually inspect and, to some extent, mechanically investigate all the cartilage surfaces in the knee. This procedure is more sensitive – i.e. it was useful in identifying the presence of an abnormality – than radiography (Lysholm 1987, Brandt 1991) and MRI in detecting cartilage lesions consistent with OA (Blackburn 1994). A major problem associated with arthroscopy is the difficulty in classifying the observed cartilage lesions (Brismar 2002). This has resulted in a profusion of classification systems over the years. Another shortcoming is that arthroscopy primarily provides information on the cartilage surface. Furthermore, it is an invasive method associated with a risk of complications, although this has recently been reported to be as low as 0.6% (Bohensky 2013).

Magnetic resonance imaging

MRI provides non-invasive, high-resolution visualization of all the structures of the knee, including the cartilage, subchondral bone, menisci, ligaments, and muscle. In a meta-analysis by Crawford *et al.* comparing MRI to arthroscopy, MRI was found to have an overall higher specificity – i.e. it was useful in identifying the absence of an abnormality – than sensitivity for the lateral meniscus and ACL (Crawford 2007). For the medial meniscus, the findings were the opposite. Regarding ACL injury, another study reported a very low sensitivity (44%), concluding that in ACL injury, MRI does not provide any valuable information over and above that obtained by clinical examination (Munk 1998). In the case of cartilage lesions, MRI has been reported to underestimate the extent of cartilage pathology compared to arthroscopy (Blackburn 1994, Munk 1998). However, MRI has been shown to provide accurate values of knee cartilage volume and thickness (Eckstein 1998).

Technique

All atomic nuclei apart from hydrogen consist of positively charged protons and neutral neutrons (nucleons). These possess an intrinsic quantum-mechanical property called spin. The number of protons and neutrons determine whether the nucleus itself will have an overall spin or not, as the spins of the nucleons sometimes cancel out. The spin of the nucleus results in a net magnetic moment causing the nucleus to function like a dipole, resembling a miniature compass

needle. When no external magnetic field is present, the nuclei in tissue are randomly arranged, and the magnetic moments cancel each other out.

The hydrogen consists of only a single proton and, consequently, has an overall spin. This, together with the fact that the body contains a large quantity of hydrogen, as it is found in both water and fat, makes hydrogen especially interesting for MRI purposes. When placed in a strong magnetic field, the magnetic vectors of the hydrogen nuclei align in the direction of the static magnetic field to create a net magnetization. The vectors themselves are not strictly parallel to the applied magnetic field, but rotate, or precess, at a certain frequency around an axis that is parallel to the applied magnetic field.

A radio frequency magnetic pulse of a specific frequency is briefly applied, which tilts the magnetization away from the direction of the magnetic field. When it is turned off, the magnetization vectors of the nuclei realign with the static magnetic field, i.e. return to equilibrium. The time taken for 63% of the nuclei to realign is called the relaxation time, and is denoted T1. T1 depends not only on the tissue, but also on the magnetic field strength, temperature, and the presence of paramagnetic ions. Paramagnetic ions are substances with unpaired electrons. They have small magnetic fields that shorten T1. Gadolinium ions ($Gd3^{+}$) have seven unpaired electrons and are therefore a potent shortener of T1. However, gadolinium is toxic and must be bound to a carrier molecule for use in the clinical setting. Therefore, Gd-$DTPA^{2-}$ is used.

The value of T1 can be measured using the inversion recovery technique. T1 measurements are performed by disrupting the magnetization with a 180° inversion pulse. The nuclei are then allowed to recover (relax) for a specific inversion time, after which the amplitude of the magnetization is read out. This procedure is repeated for different inversion times, chosen to correspond to a recovery varying from a few percent to more than 70%. The amplitudes are then fitted to a known recovery curve, giving the value of T1.

Delayed gadolinium-enhanced MRI of cartilage

dGEMRIC can be used to estimate the GAG content in cartilage, thereby allowing the quality of cartilage to be determined in vivo since the concentration of the contrast agent is inversely proportional to the concentration of GAGs in the cartilage (Bashir 1996). When the negatively charged contrast medium, Gd-$DTPA^{2-}$, is administered intravenously, it collects in the cartilage via the synovium. In normal cartilage, the high concentration of negatively charged GAG molecules repels the likewise negatively charged Gd-$DTPA^{2-}$, thus giving rise to a low concentration of contrast medium in the cartilage. If, on the other hand, the concentration of GAGs in cartilage is low, as in the early stages of OA, the concentration of Gd-$DTPA^{2-}$ in the cartilage will be higher.

The pioneers of the dGEMRIC method were Adil Bashir, Deborah Burstein and Martha Gray, in collaboration with Alice Maroudas (Bashir 1996, Burstein 2001, Gray 2008). In the first in vitro study, a strong correlation was observed between T1Gd and the GAG concentration (Bashir 1996). The dGEMRIC technique has since been validated both in vitro and in vivo (Bashir 1999, Mlynarik 1999, Trattnig 1999, Nissi 2004). Low intra- and interobserver variability has also been reported (Tiderius 2004b, Bittersohl 2009), as well as a high repeatability (Bittersohl 2009, Multanen 2009, Siversson 2010) in both the knee and hip.

Results from dGEMRIC have already made valuable contributions to clinical research on early changes in the cartilage matrix. For example, it has been shown that it is possible to discriminate between high and low cartilage quality in the knees of different groups of patients. Lower cartilage quality is found in subjects with a lower level of physical activity, following an ACL injury, following a partial meniscectomy, with a higher BMI, and with lower thigh muscle strength (Tiderius 2004a, Tiderius 2005, Ericsson 2009, Fleming 2010).

Aims of the thesis

The general aim of this thesis was to investigate the feasibility of using the dGEMRIC method to determine cartilage quality and its relation to the development of OA in humans. The specific aims were:

- to examine the association between dGEMRIC findings and future appearance of radiographically visible OA in a cohort of patients at risk of developing OA (Paper I);
- to investigate the association between dGEMRIC findings and later radiographic grade of JSN and osteophytes in a cohort of meniscectomized patients (Paper II);
- to investigate how unloading of the knee affects cartilage quality in the short and long term (Paper III);.
- to evaluate knee cartilage quality using dGEMRIC and subjective knee function 20 years after ACL injury in a group of individuals that had not been treated with ACLR and with no radiographic evidence of overt OA (Paper IV).

Methodology

Patients

The Ethics Committee of the Medical Faculty of Lund University approved all the studies, and written informed consent was obtained from all subjects.

The study described in Paper I included 16 out of 17 knees (M:F = 11:4, age 35–70, mean 50 years), originally examined in 1998 using dGEMRIC (Tiderius 2003). The patients had knee pain, normal results of weight-bearing radiography, and arthroscopic cartilage changes ranging from superficial fibrillation to fissuring and softening. The patients were identified at baseline by reviewing surgical reports and clinical records at the Department of Orthopaedics at Malmö, Skåne University Hospital, southern Sweden. None of the patients had palpable or visible subchondral bone. The mean BMI for the group was 28.5 (range 27.5–35.8). It has previously been shown that these patients had variable 1/T1 values (Tiderius 2003).

A group of patients, aged 35–50 years at the time of inclusion, who had undergone arthroscopic partial medial meniscectomy 1–6 years earlier, was identified in a previous study (Roos 2005, Ericsson 2009) through the surgical code system at the Department of Orthopaedics at Malmö, Skåne University Hospital. Patients from this group able and willing to participate in an exercise intervention lasting 4 months – with dGEMRIC and physical tests before and after the intervention – were included. Exclusion criteria were misclassification in the surgical code system (i.e. no meniscectomy), known concomitant ACL injury, cartilage changes defined as deep clefts or visible bone in the arthroscopy report, excessive level of physical activity (e.g., being a competitive athlete), too low a level of activity (only walking indoors), a self-reported limiting comorbid condition, and not living in the geographic area during the whole study period. Eighty-one patients accepted the invitation to participate, and 56 who fulfilled the inclusion criteria were enrolled in the study. Forty-five patients completed the baseline tests and underwent dGEMRIC investigations, during which a lower GAG content was found in the medial meniscectomized compartment than in the lateral reference compartment, as reported previously (Ericsson 2009). Thirty of the 45 patients who completed the exercise intervention program were randomized to a group that underwent supervised exercise three times weekly for four months, or to a non-

intervention control group. These results have been reported earlier, and showed that articular cartilage has the potential to adapt to changes in loading (Roos 2005).

The 45 patients who completed baseline testing in the previous study were invited to participate in the study described in Paper II, and 34 accepted (see the flowchart in Figure 1). Of these patients, 20 were men, and the age of the patients ranged from 50 to 61 years (mean 57) at the time of follow-up radiography. Arthroscopic surgery had been performed at the ages of 33–45 years (mean 41). The patients were included in the study 1–5 years (mean 3.7) after surgery, when they were aged 38–50 years (mean 46). The BMI at follow-up ranged from 20.6–34.1 (mean 26.6).

The loss to follow-up was 11 patients (24%), but they were similar to those included in the study group in terms of age, sex, BMI and T1Gd.

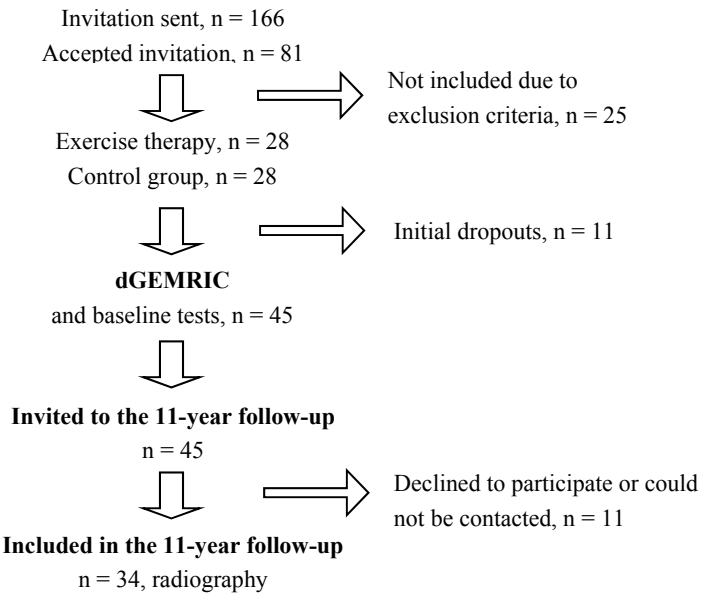

Figure 1. Flowchart showing the inclusion and loss to follow-up for the patients included in the study described in Paper II.

Ten patients (M:F = 5:5) who had sustained ipsilateral ankle fractures that required osteosynthesis were included in the study presented in Paper III. The mean age was 43 years (range 19–67), and the mean BMI for the group was 26.0 (range 22.8–29.7).

Surgery was immediately followed by cast immobilization of the ankle, and unloading of the affected leg for six weeks was prescribed. No restrictions were placed on knee movement. Exclusion criteria for the study were age < 18 or ≥ 70 years, a severe illness/condition making completion of the trial impossible, alcohol or drug abuse, prior knee injury, knee pain, or claustrophobia.

One hundred consecutive patients referred to the Department of Orthopaedics at Lund, Skåne University Hospital, between February 1985 and April 1989 for acute ACL injury, were recruited to the study described in Paper IV. Both short-term and long-term follow-ups were originally planned for this cohort, which was treated with early neuromuscular knee rehabilitation without primary ACLR. It has previously been shown that these patients had a favorable outcome 16 years after injury regarding functional performance and thigh muscle strength (Ageberg 2008), subjective knee function (Kostogiannis 2007), and knee laxity (Neuman 2012). The prevalence of tibiofemoral and/or patellofemoral OA was also low (Neuman 2008, Neuman 2009).

Forty patients of the 100 described above satisfied the inclusion criteria for 20-year post-injury dGEMRIC imaging: no ACLR and no radiographic OA (grade ≤ 1) 16 years post-injury. Of these patients, three suffered from claustrophobia and could not complete the dGEMRIC examination, four patients declined to participate due to lack of time and logistic problems, and one patient could not be contacted. The eight patients who were not included were similar to those included in the study group regarding patient characteristics, concomitant meniscal and chondral knee injuries, and radiographic changes. Thus, 32 participants (M:F = 17:15, aged 35–61, mean 45 years) were examined with dGEMRIC and completed a self-administered questionnaire (see flowchart in Figure 2).

The dGEMRIC values obtained from the participants were compared with those in a healthy reference group described previously (Tiderius 2004a), comprising 24 individuals without any knee symptoms or previous knee injuries, examined with the same dGEMRIC protocol as that used in the present study. The individuals in the reference group were matched with the study participants regarding level of physical activity, and consisted of 14 men and 10 women with a mean age of 25 years and similar BMIs to the group with acute ACL injury at inclusion.

The characteristics of the study group, the healthy reference group, and patients not included in the study described in Paper IV are given in Table 3.

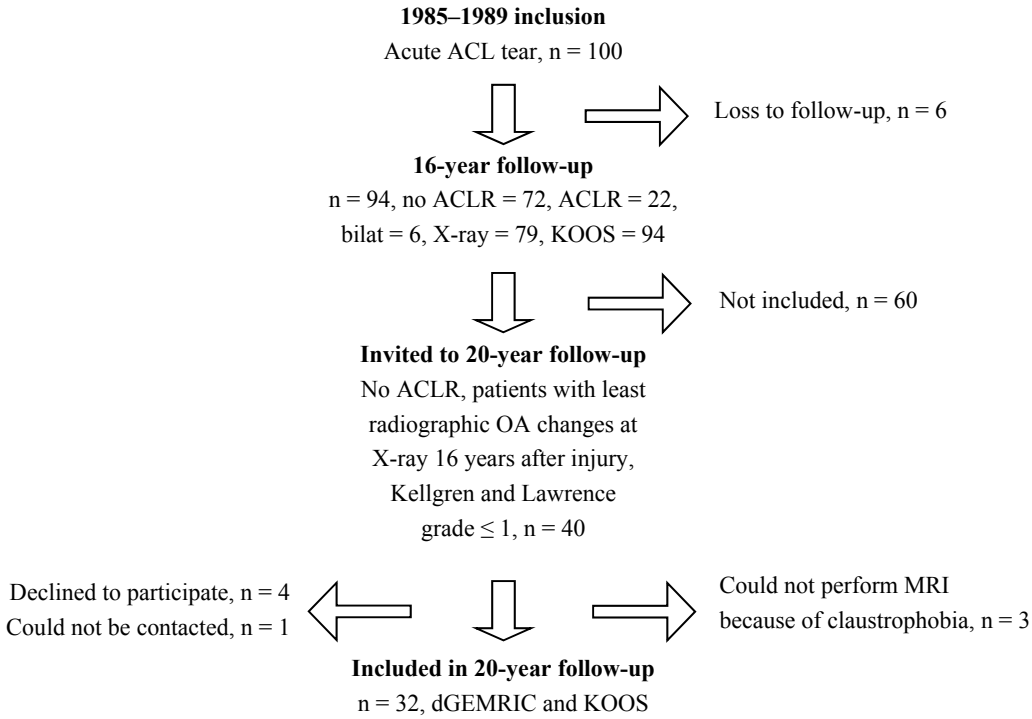

Figure 2. Flowchart showing the inclusion of participants in the study described in Paper IV.

Table 3. Characteristics of the study group, the healthy reference group, and patients not included in the study described in Paper IV

	Study sample at baseline (at injury), n = 32	**Study sample at 20-year follow-up, n = 32**	**Healthy reference group dGEMRIC, n = 24**	**Patients not included at baseline (at injury), n = 68**	**Patients not included in 16-year follow-up, n = 62**
Men, n	17 (53%)	17 (53%)	14 (58%)	41 (60%)	38 (61%)
Age, years (mean ± SD)	25 ± 6.4	45 ± 6.6	25 ± 0	26 ± 8.0	42 ± 8.1
BMI (mean ± SD)	23.2 ± 3.1	25.3 ± 3.5	22.5 ± 2.3	24.5 ± 2.6	26.8 ± 4.5

dGEMRIC

The details of the dGEMRIC studies are specified in Table 1. Standard 1.5 T MRI Siemens systems with dedicated knee coils were used for all investigations. Each patient received an intravenous injection of Gd-DTPA^{2-} (Magnevist®) followed by exercise to optimize contrast medium transport into the cartilage. Post-contrast imaging was performed and quantitative T1 measurements were obtained in two sagittal single slices that were positioned over the central parts of the weight-bearing medial and lateral femoral condyles, respectively. T1 maps were generated for these slices using sets of turbo inversion-recovery images with different inversion times: repetition time = 2,000 ms, echo time = 15 ms, turbofactor 11, field of view 120 x 120 mm^2, matrix 256 x 256. A full-thickness ROI was drawn manually in the central parts of the T1 images of the medial and lateral femoral weight-bearing cartilage between the center of the tibial plateau and the rear insertion of the meniscus, a region where OA lesions usually first appear (Boegard 1997, Tiderius 2004b). The ROI values were subsequently used for the calculation of T1Gd. T1Gd does not seem to correlate with either age or sex, hence no corrections were made for these parameters (Dahlberg 2012). In the study presented in Paper II, T1Gd was corrected for dosing bias resulting from differences in BMI, according to the formula presented by Tiderius *et al.*:

T1Gd (corrected) = T1Gd (measured) + 3(BMI - 20) (Tiderius 2006).

Table 1. Details of the dGEMRIC investigations

	Paper I	**Paper II**	**Paper III**	**Paper IV**
Typer of MRI scanner	Magnetom Vision	Magnetom Vision	Magnetom Sonata	Magnetom Vision
Gd-DTPA^{2-} dose (mmol/kg body weight)	0.3 (triple dose)	0.3 (triple dose)	0.2 (double dose)	0.3 (triple dose)
Type of exercise and time	Walking up and down stairs for 5 minutes	Stationary bicycle for 15 minutes	Stationary bicycle for 10 minutes	Walking up and down stairs for 10 minutes
Post-contrast imaging time	1.5 h	2 h	1.5 h	2 h
Slice thickness	5 mm	3 mm	3 mm	3 mm
Inversion times (ms)	100, 200, 400, 800, 1,600	50, 100, 200, 400, 800, 1,600	50, 100, 200, 400, 800, 1,600	50, 100, 200, 400, 800, 1,600

Radiography

Procedure

Standing posteroanterior radiographs were obtained using a standardized knee position with both knees at 20° flexion and weight bearing, using a General Electric Prestige 2 on a tilt table (film–focus distance 1.5 m) (Papers I and IV). A fluoroscopically positioned X-ray beam was used to optimize medial tibial plateau alignment.

In the study presented in Paper II, standing posteroanterior radiographs were obtained with a standardized knee position with both knees at 20° flexion and weight bearing, using a Siemens Aristos FX on a tilt table (film–focus distance 1.15 m). The X-ray beam was positioned from behind the knee at an angle of 10° from above.

Grading

All radiographs were independently read en masse, and OA scoring was performed by one (P.N. Paper I) or two (H.O. and M.E. Paper II, P.N and M.E. Paper IV) observers blinded to the clinical details. In cases of discrepancy, the radiographs were re-read and consensus was reached. JSN and femoral and tibial osteophytes were individually graded on frontal images using a 4-point scale (0–3, 0 = no evidence of JSN or bony change), according to the Osteoarthritis Research Society International Atlas (Altman 2007). Medial and lateral osteophyte scores were evaluated for each knee, consisting of the sum of the femoral and tibial osteophyte grades in the medial and lateral compartments, respectively (both ranging from 0–6).

Radiographic tibiofemoral OA was considered present if any of the following criteria was fulfilled in either of the two tibiofemoral compartments: JSN $\geq$ 2, osteophyte score $\geq$ 2, or JSN grade 1 in combination with osteophyte grade 1 in the same compartment. This definition approximates grade 2 knee OA based on the Kellgren and Lawrence scale (Kellgren 1957).

Arthroscopy

Arthroscopic findings were graded according to the rating system recommended by the International Cartilage Repair Society (www.cartilage.org) (Brittberg 2003) (Paper I and II). The depth of a lesion was classified as superficial softening based

on indentation or superficial fissures and cracks (ICRS grade 1), lesions extending to less than half of the cartilage thickness (ICRS grade 2), lesions extending to half or more of the cartilage thickness but not into the subchondral bone (ICRS grade 3), or osteochondral lesions (ICRS grade 4).

Self-reported knee function

Knee injury and Osteoarthritis Outcome Score

KOOS is a self-administered questionnaire developed by Roos *et al.* as a tool to assess the patient's opinion of their knee and associated problems (Roos 1998a). It is widely used for research purposes in clinical trials, large-scale databases, and registries. The questionnaire is intended to be used for knee OA and injury that can result in OA, such as injury to the cartilage, ACL or meniscus. KOOS has been shown to be sensitive, valid, reliable, and responsive (www.koos.nu).

The questionnaire consists of five subscales: pain, other symptoms, ADL, sport/rec, and knee-related QOL. The previous week is the time period considered when answering the questions. A normalized score is calculated for each subscale, where 100 indicates no symptoms and 0 indicates extreme symptoms. Each subscale is analyzed and presented separately, unlike other scoring systems where results are pooled to produce a total score. The effect of each subscale is different in different patients groups.

KOOS results were compared with those of a population-based postal survey of randomly selected inhabitants in southern Sweden with a similar age range as the current cohort (n = 158, age 35–54 years, 51% women) (Paper IV) (Paradowski 2006).

Tegner score

The Tegner score (range 0–10) was used to assess the individual's level of physical activity (Tegner 1985). Grade 10 represents highly demanding knee activities and 0 represents no physical activity due to sick leave or disability pension. Grade 4 represents non-competitive activities, such as jogging and bicycling.

Statistical analysis

The Mann-Whitney rank sum test, linear regression analysis, and maximum likelihood estimation using logistic regression were used in the study reported in Paper I.

Test for trend between T1Gd and radiographic changes (JSN grade and osteophyte score) was evaluated using Cuzick's extension of the Kruskal–Wallis test (Cuzick 1985), and the Wilcoxon rank-sum test was used to compare the OA group with the non-OA group described in Paper II.

In the study presented in Paper III, Pitman's *t*-test was used to investigate the variance ratio of T1Gd (Pitman 1939). The paired *t*-test was used to evaluate differences in mean T1Gd for the group. All calculations were performed using STATA (StataCorp LP, College Station, TX, USA).

For comparisons of data involving T1Gd, *p*-values were calculated using Student's *t*-test, and the Pearson correlation was used to test for correlations between KOOS and T1Gd values in Paper IV. No adjustment was made for age for T1Gd as it does not seem to correlate with age (Dahlberg 2012). The statistical analysis was performed with SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA).

In all statistical analyses, a two-tailed p-value ≤ 0.05 was considered to indicate a statistically significant difference.

Results

Relation between dGEMRIC findings and the development of OA (Paper I)

Values of T1Gd in the 16 knees investigated in this study ranged from 194 ms to 471 ms at baseline. Six years later, 9 of the 16 knees showed radiographic OA changes. Mean baseline T1Gd was lower in these 9 knees than that in the knees without radiographic signs of OA (mean ± SD 312 ± 64 ms vs. 383 ± 60 ms, respectively; $p = 0.03$) (Figure 3). The radiographic changes were as follows: 1 knee had JSN grade ≥ 2, 3 knees had an osteophyte score ≥ 2, and 5 knees had grade 1 JSN in combination with grade 1 osteophytes in the same compartment. Two of the knees had undergone joint replacement due to OA (dGEMRIC indices 194 ms and 329 ms, respectively). Figure 4 shows the relationship between values of T1Gd and the probability of development of radiographic OA six years later (odds ratio 0.98, $p = 0.07$).

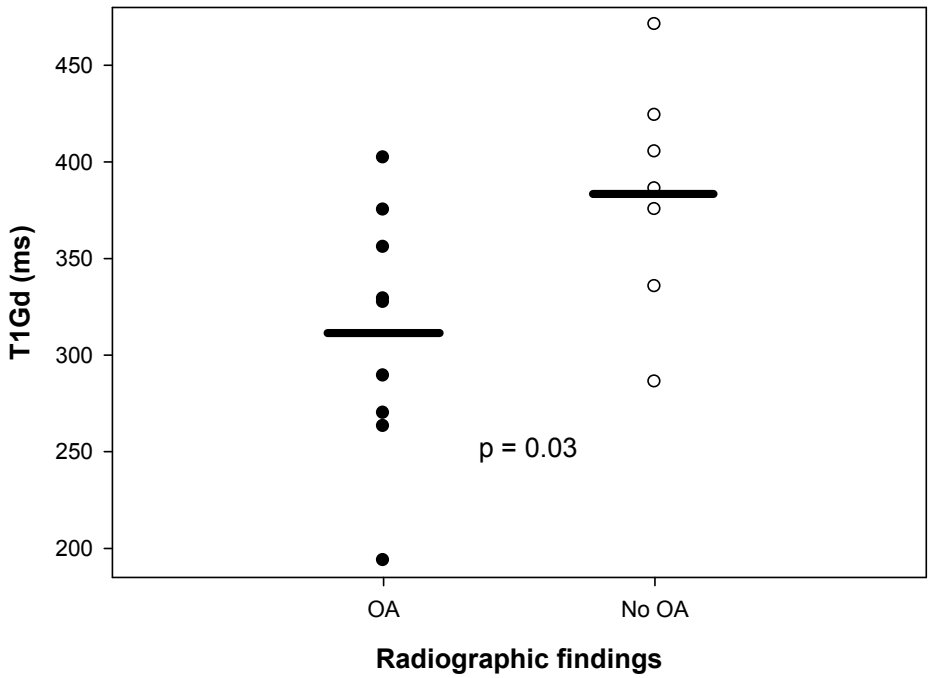

Figure 3. Baseline T1Gd for knees with and without radiographic OA findings at the six-year follow-up. Bars show mean values.

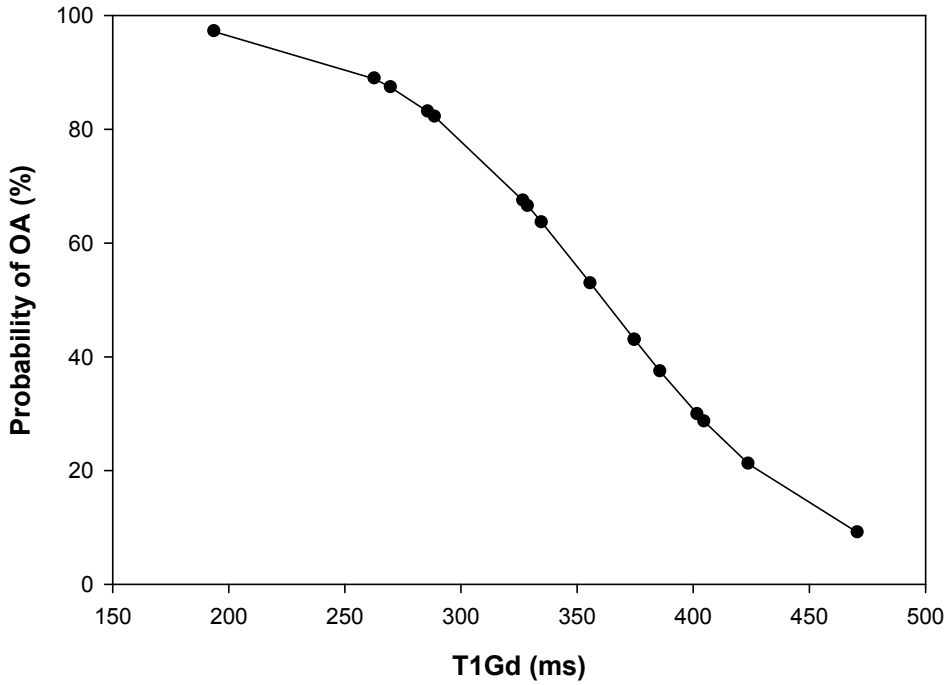

Figure 4. Probability of radiographic OA at the six-year follow-up vs. baseline T1Gd.

Relation between dGEMRIC findings and JSN and osteophytes (Paper II)

T1Gd values and radiographic changes

At baseline, the T1Gd values for the knees in this group of patients ranged from 231 to 562 ms in the medial compartment, and from 313 to 542 ms in the lateral compartment. The numbers of knees with the various grades of JSN and osteophytes 11 years later (12–16 years after surgery) are presented in Table 2.

Table 2. Number of knees with each grade of JSN and osteophytes

Grade	JSN medial, n	JSN lateral, n	Osteophytes medial, n	Osteophytes lateral, n
0	1	31	7	22
1	21	3	19	11
2	9	0	2	0
3	3	0	2	0
4	-	-	3	1
5	-	-	1	0
6	-	-	0	0

JSN

The baseline T1Gd values (mean ± SD) were significantly different between the groups of patients with different medial compartment JSN grades: grade 0 (351 ms), grade 1 (386 ± 48 ms), grade 2 (342 ± 85 ms), and grade 3 (259 ± 24 ms), p for trend < 0.001. A statistically significant difference in T1Gd was also found between lateral compartment JSN grades 0 (436 ± 51 ms) and 1 (346 ± 32 ms), p for trend = 0.026. None of the patients had JSN grade 2 or 3 in the lateral compartment. The results are shown in Figure 5.

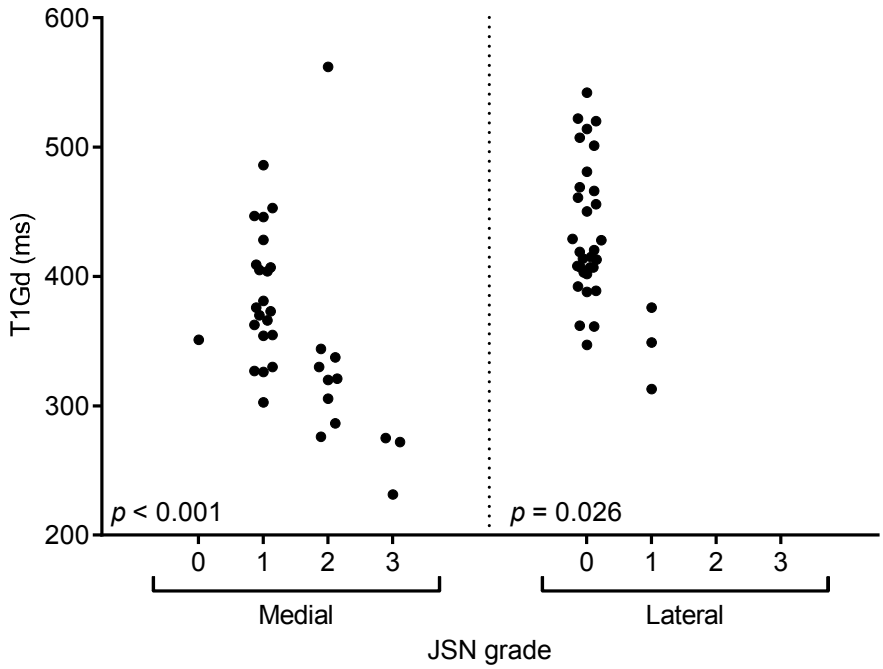

Figure 5. T1Gd (ms) vs. grade of JSN in the medial and lateral knee compartments.

Osteophytes

The baseline T1Gd values (mean ± SD) were significantly different between the groups with different osteophyte scores in the medial compartment: score 0 (371 ± 34 ms), score 1 (389 ± 67 ms), score 2 (354 ± 23 ms), score 3 (289 ± 24 ms), score 4 (265 ± 29 ms), and score 5 (275 ms), p for trend = 0.001. No significant difference in T1Gd was found between the groups with different osteophyte scores

in the lateral compartment: score 0 (432 ± 63 ms), score 1 (423 ± 43 ms), and score 4 (362 ms), p for trend = 0.16. None of the patients had osteophyte score 2, 3 or 5 in the lateral compartment, or score 6 in either compartment. The results can be seen in Figure 6.

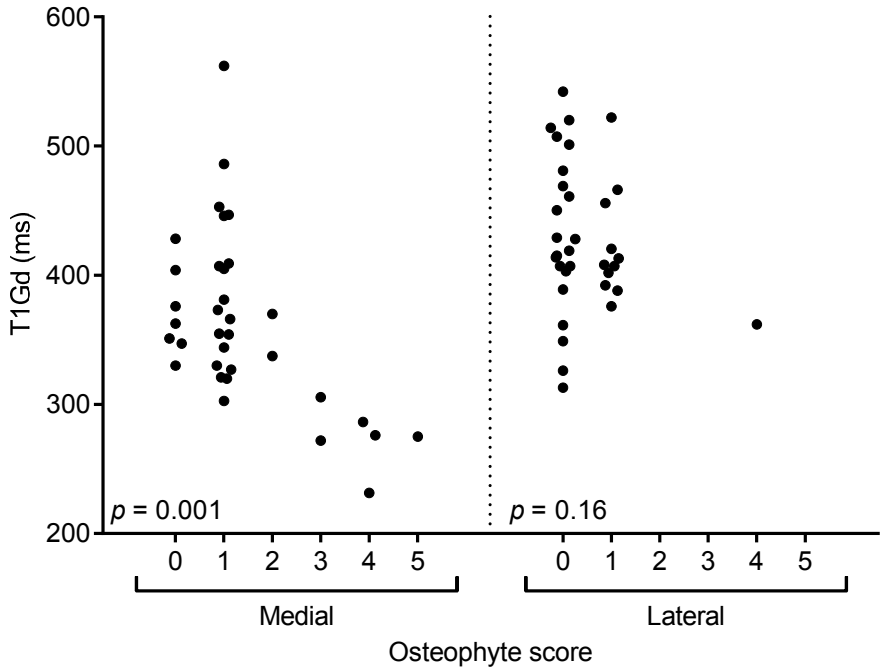

Figure 6. T1Gd (ms) vs. osteophyte score for the medial and lateral knee compartments.

OA

Radiographic OA was observed in 27 knees, according to our definition, corresponding to Kellgren and Lawrence grade 2 or worse. In these knees, the baseline T1Gd values (mean [95% CI]) for the medial (361 ms [331–390]) and lateral (434 ms [414–455]) compartments were not significantly different from the values in the medial (368 ms [333–403]) and lateral (401 ms [335–467]) compartments in the 7 knees without radiographic signs of OA (p = 0.61 medial and 0.26 lateral).

Effect of removing knee joint loading on cartilage quality (Paper III)

T1Gd values

The mean baseline value of T1Gd (mean ± 1 SD [95% CI]) for the whole group of patients was 567 ± 19 (553–580) ms. Figure 7 shows the mean T1Gd values with their confidence intervals at various times. After six weeks of prescribed unloading, T1Gd had increased slightly to 582 ± 71 (479–689) ms, but the change was not statistically significant (p = 0.5). Four months after remobilization, the mean T1Gd for the group was 541 ± 43 (454–591) ms, which was lower than the baseline value (p = 0.05), and after six weeks of prescribed unloading (p = 0.04). At the one-year follow-up, the mean T1Gd was 540 ± 60 (447–619) ms, showing no statistical difference between the value four months after remobilization (p = 0.8).

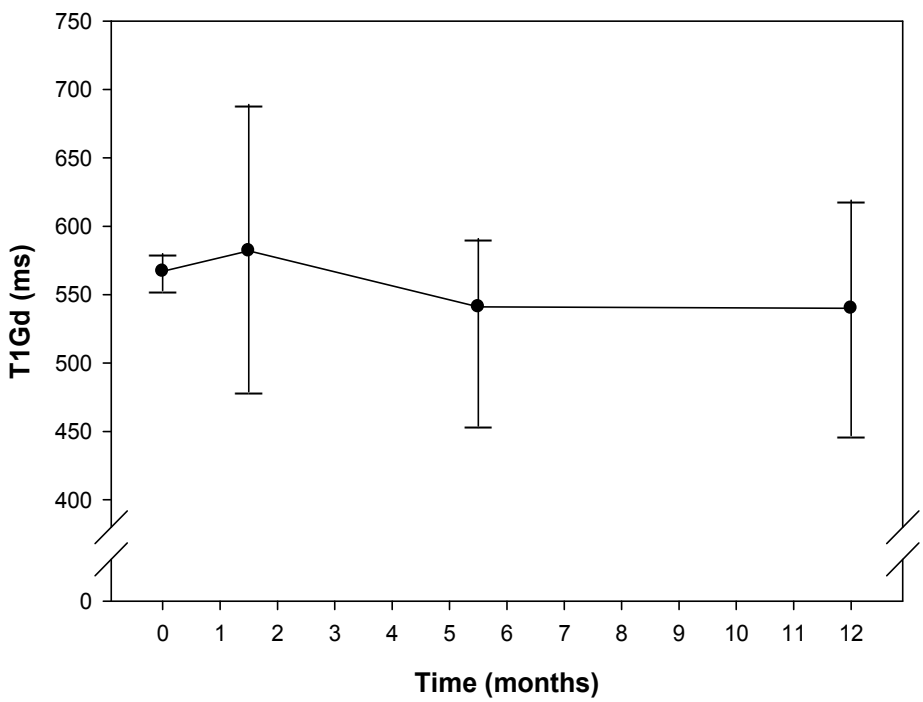

Figure 7. Mean T1Gd values (with 95% confidence intervals) at baseline, after six weeks of prescribed unloading, four months after remobilization, and one year after injury.

Range of T1Gd values

The range in baseline T1Gd values was 542–607 ms (Figure 8). After six weeks of prescribed unloading, this had increased significantly, to 479–689 ms (p = 0.002, ratio of standard deviations = 0.28, 95% CI = 0.14–0.58). The range of T1Gd four months after remobilization was 454–591 ms, and was significantly broader than at baseline (p = 0.012, ratio of standard deviations = 0.44, 95% CI = 0.24–0.82). At the one-year follow-up, the range was 447–619 ms, showing a persisting increase in range compared to the baseline value (p = 0.008, ratio of standard deviations = 0.33, 95% CI = 0.15–0.73).

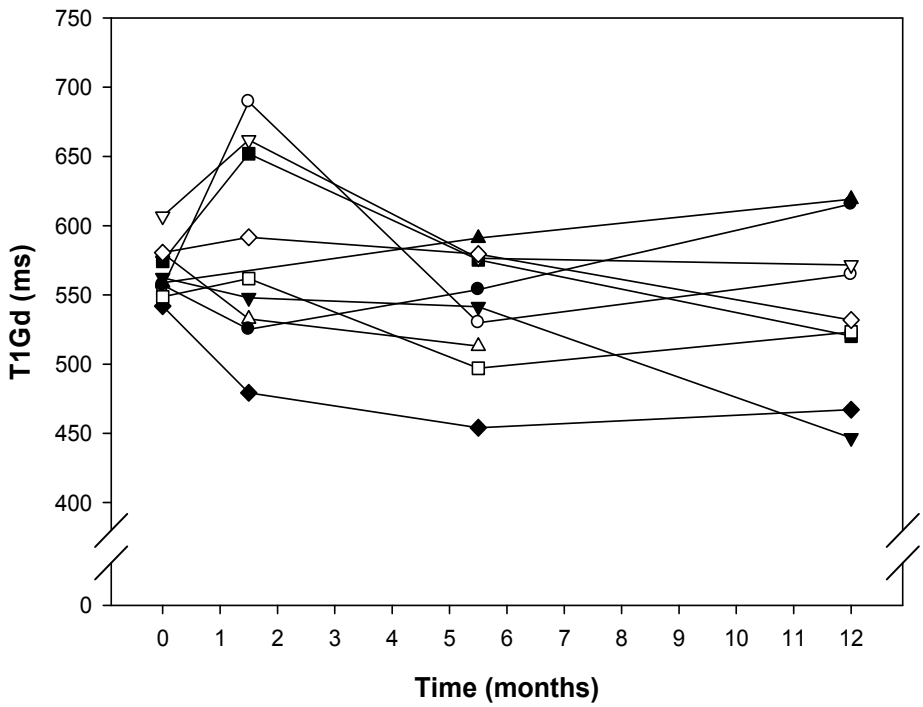

Figure 8. Individual T1Gd values at baseline, after six weeks of prescribed unloading, four months after remobilization, and one year after injury.

Long-term cartilage quality in ACL-injured copers (Paper IV)

dGEMRIC findings

The values of T1Gd in the group that had suffered an ACL injury were not significantly different from those in the healthy reference group, medially or laterally. The T1Gd value (mean ± 1 SD [95% CI]) in the medial compartment in the injured group was 404 ± 53 (385–423) ms, vs. 428 ± 38 (412–444) ms in the reference group ($p = 0.065$). The corresponding values in the lateral compartment were 427 ± 79 (399–455) vs. 445 ± 41 (428–462) ms ($p = 0.31$) (Figure 9).

No difference was observed in T1Gd values obtained when combining the values for the medial and lateral femoral cartilage (bulk mean) in a subgroup analysis comparing patients with radiographic signs of OA (grade 1 osteophyte or grade 1 JSN) and patients without osteophytes or JSN; the values being 415 ± 70 (380–450) ms and 412 ± 38 (392–432) ms, respectively ($p = 0.85$). All patients included in this study had a Kellgren and Lawrence grade ≤ 1.

The values of T1Gd in medial femoral cartilage in patients with (n = 3) and without (n = 29) a major medial meniscal injury (partial meniscectomy) were 384 ± 48 (329–439) ms and 407 ± 53 (387–427) ms, respectively ($p = 0.48$). The values in lateral femoral cartilage in patients with (n = 10) and without (n = 22) a major lateral meniscal injury were 410 ± 105 (344–476) ms and 435 ± 65 (407–463) ms, respectively ($p = 0.41$). T1Gd was not related to BMI, to the Tegner activity score, or to age or sex (data not shown).

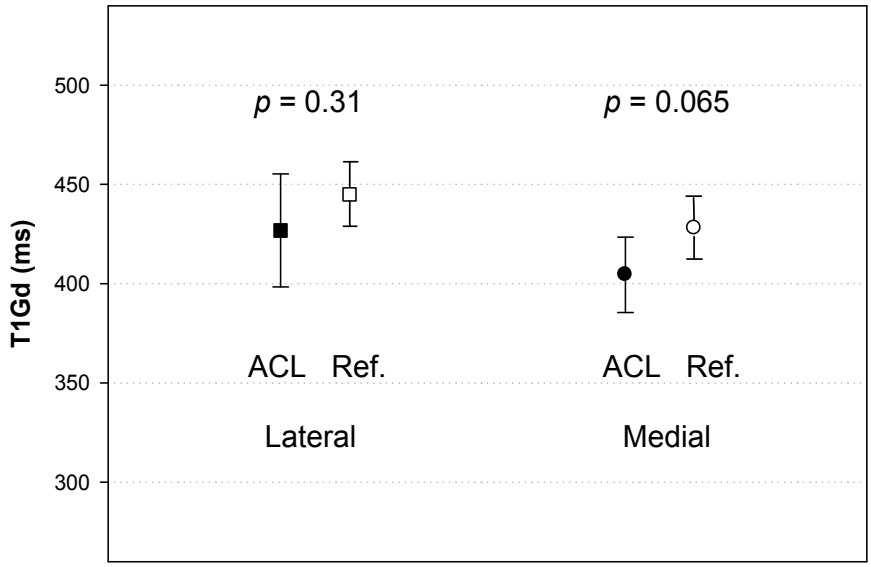

Figure 9. Mean T1Gd values (with 95% confidence intervals) for the lateral and medial femoral cartilage in the study cohort (ACL) (n = 32), and the healthy reference group (Ref.) (n = 24).

Self-reported knee function

The results obtained from the KOOS questionnaire for the study group, consisting of patients who had not undergone ACLR, were for most subscales better than those previously reported for the reference group by Paradowski *et al.* (Paradowski 2006). The difference was statistically significant for the pain and ADL subscales.

The scores obtained for the reference group were (mean ± SD): pain 88 ± 18, symptoms 88 ± 16, ADL 89 ± 19, sport/rec 78 ± 29, and QOL 81 ± 24. The corresponding scores for the study group (n = 32) were: pain 95 ± 10, symptoms 92 ± 11, ADL 98 ± 4, sport/rec 86 ± 20, and QOL 81 ± 20 (Figure 10). The *p*-values obtained for the five subscales were: 0.034 (pain), 0.18 (symptoms), 0.0084 (ADL), 0.14 (sport/rec), and 1.0 (QOL).

KOOS increased with improving knee cartilage quality estimated with dGEMRIC, although the only association that was statistically significant was that between the QOL subscale and the quality of the medial femoral cartilage. Pearson correlation *p*-values between the five KOOS subgroups and values of T1Gd obtained from the medial femoral cartilage were: $p = 0.090$ (pain), $p = 0.17$ (symptoms), $p = 0.058$ (ADL), $p = 0.35$ (sport/rec), and $p = 0.021$ (QOL). The corresponding Pearson correlation *p*-values for the lateral femoral cartilage were: $p = 0.72$ (pain), $p = 0.74$ (symptoms), $p = 0.90$ (ADL), $p = 0.19$ (sport/rec), and $p = 0.40$ (QOL).

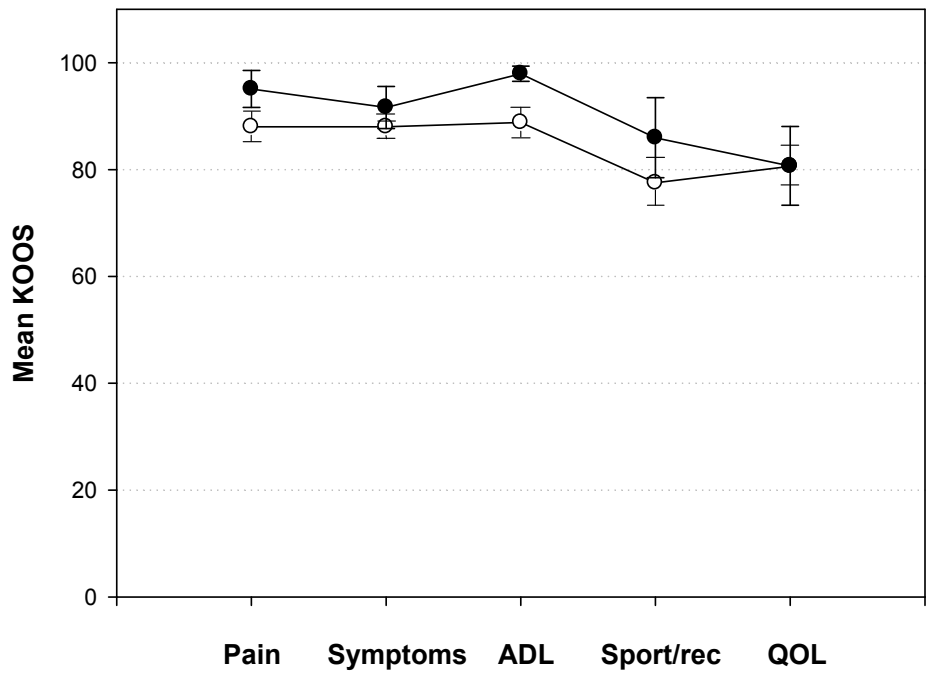

Figure 10. Mean KOOS scores for each subscale (with 95% confidence intervals) for the study sample (ACL injury) -●- (n = 32), and a random population-based reference group -○- (n = 158). The ACL injury group had significantly higher (better) scores for the pain and ADL subscales than the reference group, $p = 0.03$ and $p = 0.008$, respectively.

Discussion

Can T1Gd obtained using dGEMRIC predict future OA, JSN and osteophytosis in the knee?

The ability of dGEMRIC to predict future OA

Only one study has previously been performed to investigate the ability of dGEMRIC to predict the development of OA in the future. Cunningham *et al.* conducted a longitudinal study on patients with acetabular dysplasia of the hip, and found that a low value of T1Gd before correction by periacetabular osteotomy was the strongest predictor of joint failure three years later (Cunningham 2006). This finding indicates that the outcome of osteotomy may depend on the quality of hip cartilage, as assessed by dGEMRIC. The most important aim of the present work was to determine whether dGEMRIC could also predict future development of OA in the knee.

Patients with knee pain, whose weight-bearing radiographs showed normal results, and who exhibited arthroscopic cartilage changes ranging from superficial fibrillation to fissuring and softening were chosen for the first study (Paper I), as they may be at risk of developing OA. Although the sample studied was small it was demonstrated that the value of T1Gd obtained with dGEMRIC was associated with radiographic OA six years later. The radiographic diagnosis of OA applies to the whole knee, regardless of the compartment in which the radiographic findings occur, and for this reason the mean value of T1Gd from the medial and lateral compartments was used in this study.

The ability of dGEMRIC to predict future JSN and osteophytosis

Isolated meniscal injury and meniscectomy in patients who have suffered an ACL injury are well-known risk factors for the development of OA (Roos 1998b, Lohmander 2007, Neuman 2008, Oiestad 2009, Keays 2010). Partial meniscectomy in subjects without an ACL injury also appears to be a substantial risk factor for the development of OA, as radiographically identified OA is seen in 20–60% of patients 8–16 years after arthroscopic partial meniscectomy (Englund

2003, Petty 2011). In this cohort of partially meniscectomized patients it was found that lower values of T1Gd in the medial compartment were associated with higher grades of radiographic JSN and more osteophytosis medially 11 years later. Similarly, lower values of T1Gd in the lateral compartment were associated with higher grades of lateral radiographic JSN, although only two JSN grades (0 and 1) were found in this compartment. As JSN and osteophytosis are key features of radiographic OA, the findings of this study support those in the previous study, that dGEMRIC has the potential to predict the development of knee OA.

Although JSN and osteophytes are important features of OA, they only seem to be coupled early in the development of OA. In a longitudinal study of OA, Wolfe *et al.* reported that osteophytes were associated with the progression of JSN, but only when the JSN grade was 0. When JSN exists, osteophytes do not seem to contribute additionally to the risk of JSN progression (Wolfe 2002). Radiographic progression of JSN has been shown to be related to cartilage loss, evaluated with conventional MRI (Amin 2005), and cartilage loss escalates with increasing grade of JSN (Eckstein 2009). This is in line with the association between T1Gd and future grade of JSN reported in Paper II, as dGEMRIC is able to estimate cartilage GAG content.

The mechanism governing the association between low T1Gd values and osteophytes is somewhat unclear. Hart and Spector showed that a doubtful osteophyte at baseline had developed into a definite osteophyte in 37% of the knees studied after five years, and in 51% after ten years (Hart 2003), demonstrating that osteophytes have the potential to increase in size over time.

It has been suggested that osteophytes do not affect the risk of structural OA progression, but are rather strongly associated with knee malalignment. Any relation between osteophytes and OA progression is partly explained by the association between malalignment and OA progression (Felson 2005). Williams *et al.* found low dGEMRIC T1Gd values medially in varus-aligned knees and laterally in valgus-aligned knees in patients with established OA, confirming a relationship between knee alignment and T1Gd. Later studies have demonstrated that malalignment itself is not a risk factor, but mediates the effects of other risk factors, such as obesity and poor quadriceps strength, and is influenced by structural changes within the joint (Hunter 2007, Hunter 2009). Hence, osteophytes seem to mediate other risk factors and serve as markers for pre-existing cartilage loss, predicting future loss.

Probability of developing OA

The relationship between baseline T1Gd and the probability of developing radiographic OA at the six-year follow-up is presented in Paper I (and in Figure 4 above). The probability of developing OA seemed to be low in patients with T1Gd

> 450 ms, and high in patients with T1Gd < 350 ms. This interval served as an important reference when evaluating the cartilage of the patients at risk of developing OA throughout the work presented in this thesis. For example, the values of T1Gd for all the individuals in the study described in Paper III were in the vicinity of 450 ms and above. It thus appears that removal of knee joint loading for relatively short periods of time is not harmful to the cartilage, and will not give rise to future OA. Furthermore, to the best of the author's knowledge, OA is not more common in patients having sustained an ankle fracture.

The long-term effect of cartilage unloading on cartilage quality

No change was found in the mean value of T1Gd after six weeks of prescribed joint unloading, but a decrease in T1Gd was observed four months after remobilization. This finding suggests that changes in the structural matrix at the molecular level, resulting in deterioration of cartilage quality, may occur more slowly than previously reported regarding cartilage thickness and volume (Hinterwimmer 2004). The mean T1Gd values four months after remobilization and one year after injury were not different, indicating that a longer follow-up time would have been needed to detect any restoration of cartilage quality in this cohort.

Notable changes in the range of T1Gd values were also found in this study. At the time of injury, the values were confined to a relatively narrow range. This has also been observed in a study by Tiderius *et al.*, in which subjects with a moderate level of physical activity exhibited T1Gd values with a narrower range than both non-exercising individuals and elite athletes (Tiderius 2004a). In the present study, removal of knee loading for six weeks resulted in a measurable effect on the cartilage matrix, as evidenced by a broader range of T1Gd values. This broader distribution of T1Gd values in the knee cartilage matrix persisted for four months after remobilization and at follow-up, one year after the injury.

Data presented by our group, as well as those from others, indicate that factors other than cartilage GAG concentration – such as collagen content, cartilage thickness and permeability, macromolecular content, and variations in diffusion – may influence T1Gd (Stanisz 2000, Li 2010, Hawezi 2011, Salo 2012, Stubendorff 2012). It could be speculated that such non-GAG-related factors may contribute to the spread in values seen after the removal of knee joint loading.

Cartilage quality in ACL injured copers

Good cartilage quality after 20 years

Based on previous findings, lower cartilage quality (i.e., lower T1Gd values) could have been expected in the patients described in Paper IV. However, it was found that the values did not differ significantly from those in a healthy reference group. One reason for this may be the small number of patients that had undergone medial meniscectomy, a well-known risk factor for the development of OA (Fairbank 1948, Englund 2003, Englund 2004, Neuman 2008).

Another reason why these copers – i.e., individuals who are hardly affected by their ACL injury and can continue with the same activities as before the injury without any knee symptoms – appeared to have good knee cartilage quality 20 years after their ACL injury is believed to be the individual treatment they received in the original study (Zatterstrom 1998), where:

- all patients were identified early after their knee injury
- the individual pattern of knee injury was established and respected during the rehabilitation process
- meniscus lesions were sparingly treated with meniscectomy
- patients were closely monitored, treated and educated by a competent physiotherapist to achieve functional knee stability, increased neuromuscular function, and to avoid giving-way of the knee
- patients initially (first year after injury) lowered their activity level.

This treatment differs from that normally offered to patients suffering an acute ACL injury.

Particularly low values of T1Gd have been observed in patients suffering an ACL injury with a concomitant meniscus injury (Neuman 2011). In that study, the average follow-up time after ACL tear was two years, and half of the study subjects underwent ACL reconstruction. Although it is difficult to draw any firm conclusions from a comparison between the findings from the present 20-year study and a two-year follow-up study in two different cohorts, it is notable that higher values of T1Gd were observed in the present study. This may indicate that, if treated correctly, knee cartilage may have the potential to improve slowly over a period of several years after a severe knee injury, which may have significant impact on the relatively large number of individuals sustaining an ACL injury.

Thirdly, a selection bias could also partly explain why these copers appeared to have good knee cartilage quality 20 years after their ACL injury, since non-copers who subsequently underwent ACL reconstruction (25%) were not included.

Twenty years after their ACL injury, the patients studied had T1Gd values similar to those in a healthy reference group. This finding is in line with other aspects of knee function reported in previous studies on this cohort of well-functioning ACL-deficient copers (Kostogiannis 2007, Ageberg 2008, Neuman 2008, Neuman 2009, Neuman 2012).

To ensure group homogeneity, subgroup analysis was used in the study in which patients with no radiographic OA findings were compared to patients with discrete radiographic OA changes (grade 1 osteophytes or grade 1 JSN). No correlation was found between the two subgroups, which seems to contradict the findings of the previous study (Paper II). However, the primary objective of the study described in Paper IV was not to investigate the association between radiographic and dGEMRIC findings. The radiographic investigations were actually performed 4–5 years prior to the dGEMRIC investigations, and were only used to identify appropriate candidates for the study.

Findings from self-reported questionnaires

At group level, the KOOS values for the ACL-injured patients were, in general, somewhat higher than those for the reference group. This suggests that the patients as a whole seem to be content with their ACL injured knee. On the subscale level, an association was found between QOL and T1Gd. This is notable as QOL and sport/rec seem to be the most sensitive subscales with regard to ACL injury, according to the Swedish ACL Register (www.aclregister.nu). All other subscales show results in line with numerous previous studies, in which the association between structural cartilage changes and symptoms has been weak.

Limitations of these studies

Sample size

It could be argued that the groups studied in this work are too small for any reliable conclusions to be drawn. However, the results of previous studies performed by our group suggest that only a limited number of subjects is needed to detect statistically and clinically significant differences using dGEMRIC (Tiderius 2001, Tiderius 2003, Tiderius 2004a, Tiderius 2004b, Tiderius 2005,

Tiderius 2006, Ericsson 2009, Neuman 2011). This is mainly due to the low variability of the T1Gd values measured in the examined cohorts.

Patient compliance

There is no way of knowing whether the patients included in the study on the effects of unloading (Paper III) followed the treatment prescribed, since the actual load was not continually monitored. It is unlikely, however, that patients were able to fully load the injured ankle. In addition, the ankle fractures were not of the same type and fixation techniques varied, which could have affected the course of recovery. Little is known about this, since physical activity was monitored only at baseline and after one year, not periodically.

Lack of initial radiographs

No radiographic examinations were performed at the time of inclusion of the patients described in Paper IV, as cartilage status was graded based on arthroscopic findings. However, the status of the cartilage in the medial femoral condyle was stated as being normal in 23 cases, shallow lesions in 19 cases, and localized full-thickness lesions in four cases. This makes the likelihood of patients having radiographic OA changes minimal. In addition, all lateral compartments were reported to be normal.

Methodological considerations

Pharmacokinetics

In recent years, the important question of what is actually being measured with dGEMRIC has been raised. Li *et al.* compared a non-ionic contrast agent (Gd-DTPA-BMA) to Gd-$DTPA^{2-}$ in subjects with OA and a control group, using a standard dGEMRIC protocol. They unexpectedly found that T1(Gd-DTPA-BMA) was not constant across individuals in either group, suggesting that Gd-$DTPA^{2-}$ uptake in cartilage depends not only on the cartilage fixed charge density (i.e. the GAG content), but on other charge-independent factors, such as tissue transport properties (Li 2010). It has previously been noted that factors other than cartilage GAG concentration may influence T1Gd (Silvast 2009, Hawezi 2011, Salo 2012, Stubendorff 2012). In light of this, it may perhaps be more correct to say that values of T1Gd obtained with dGEMRIC estimate cartilage quality in general, not the GAG content in particular. Although the relationship between GAG content

and T1Gd may be of concern, it appears that cartilage degeneration can be visualized shortly after contrast agent administration (Salo 2012).

Cartilage thickness

Nieminen *et al.* expressed a concern over ten years ago that the relation between T1Gd and GAG concentration was not linear in deep cartilage tissue, as the GAG content was overestimated (Nieminen 2002). This finding has also been reported in more recent studies, where incomplete penetration of the contrast medium into deeper parts of the cartilage resulted in falsely high values of T1Gd in full-thickness cartilage analysis (Hawezi 2011, Salo 2012). Full-thickness cartilage analysis was used throughout the work presented in this thesis, making this another potential source of error.

BMI

BMI can be a source of dosing bias in dGEMRIC as the dose of Gd-DTPA^{2-} is administered according to body weight. Gd-DTPA^{2-} only distributes in the extracellular water and hence not into adipose cells. An obese person has relatively less extracellular water content than a lean person, i.e., a smaller distribution volume for the contrast agent. This leads to a higher concentration of Gd-DTPA^{2-} in the cartilage, and consequently shorter T1Gd. Consequently, a correction factor has been recommended in cross-sectional studies with a large range of BMI (Tiderius 2006). In this thesis, the BMI range was substantial only in Paper II, and in that study the correction factor was used.

Investigation of femoral cartilage vs. whole-knee cartilage

The central weight-bearing cartilage of the medial and lateral femoral condyles was investigated because this is most commonly affected by early degenerative changes (Boegard 1997). It may be argued that more information about the knee can be obtained by studying the whole volume of knee cartilage (McKenzie 2006). However, it was considered advantageous to use the same MRI protocol as in previous studies by our group (Tiderius 2001, Tiderius 2003, Tiderius 2004a, Tiderius 2004b, Tiderius 2005, Ericsson 2009, Neuman 2011). This decision is supported by the previous finding of similar T1Gd values in femoral and tibial cartilage in subjects who had sustained ACL injuries (Fleming 2010).

Repeated dGEMRIC investigations in longitudinal studies

The positioning of the ROI slices by the MRI operator may introduce a source of methodological error in the dGEMRIC technique. The slices will inevitably be placed somewhat differently in different investigations, meaning that the ROIs included in the measurements at different time points will cover slightly different areas of the cartilage (Siversson 2010).

Clinical implications

Although dGEMRIC has not found its way into routine clinical use, it may in the future prove to be a valuable tool when advising and treating patients believed to be at risk of developing OA. Identifying these patients at an early stage of joint disease would make it possible to initiate curative or prophylactic treatment much earlier than is possible today. dGEMRIC may also prove useful when evaluating the results of clinical trials on treatments or drugs affecting the joint cartilage and its constituents. A few centers, in Sweden (Lund/Malmö), the USA (Boston), and Germany (Düsseldorf), are already using the dGEMRIC method in the clinical evaluation of hip dysplasia. dGEMRIC has the potential to reveal how molecular changes are related to exogenous factors, which will increase our understanding of joint health and the pathogenesis of OA.

Unloading of joints is common in the treatment of several medical conditions, including fractures and infections. Knee joint unloading appears to have a measurable effect on cartilage quality, and this must therefore be taken into account when considering treatment involving an unloading regimen. However, no T1Gd values below the limit found to be associated with an increased risk of developing OA were observed in the patients in this work. This leads to the conclusion that unloading of a knee joint for a shorter period of time at least does not lead to an increased risk of developing OA in the future.

It appears to be possible to maintain a good subjective knee function and good cartilage quality up to 20 years after an ACL injury treated without ACLR. This is an encouraging finding that is important when advising recently injured patients who are prepared to abstain from pivoting sports, and who are willing to undergo neuromuscular knee rehabilitation without ACL surgery.

Conclusions

- Values of T1Gd obtained using dGEMRIC predicted the development of knee OA in a six-year follow-up study of patients with knee pain and arthroscopic cartilage aberrations.
- dGEMRIC could be used to predict future knee JSN and osteophytosis, which are important features of OA, in a cohort of patients at risk of developing OA.
- Unloading of the knee for six weeks resulted in a measurable effect on the knee cartilage matrix, expressed as a lower mean value of T1Gd – suggesting a decrease in cartilage GAG content – and an increase in the range of T1Gd values.
- It may be possible to maintain good cartilage quality and good subjective knee function 20 years after an ACL injury in knees treated without ACLR, as ACL-injured patients demonstrated T1Gd values and KOOS scores similar to those of healthy reference groups.

Summary

Osteoarthritis (OA) is the most common joint disorder worldwide, and causes joint pain and stiffness. Currently, the gold standard for diagnosing knee OA is radiography. However, the disease has often progressed well beyond the point of no return once radiographic cartilage changes are visible. Treatment options at this stage range from various measures to maintain whatever cartilage is left, to the insertion of a knee prosthesis. Revealing changes in cartilage at an early stage of OA development would make it possible to initiate curative or prophylactic treatment much earlier than is possible today.

Early in the course of the disease, the articular cartilage is depleted of glycosaminoglycans (GAGs), which are responsible for cartilage load distribution and compressive stiffness. Delayed gadolinium-enhanced magnetic resonance imaging of cartilage, abbreviated dGEMRIC, is a method that can be used to estimate the GAG content of cartilage. In dGEMRIC, a contrast medium is injected, which enters the cartilage. The concentration of this contrast medium is inversely proportional to the amount of GAG in the cartilage, thus a low amount of contrast medium indicates a high amount of GAGs. The purpose of the work presented in this thesis was to investigate if and how dGEMRIC can be used as a prognostic tool for knee cartilage changes and knee OA.

Patients at risk of developing OA were studied in Paper I. From the results of this investigation, it was found that the dGEMRIC method could predict the development of knee OA six years later. In Paper II, another group of patients at risk of developing OA was examined, and an association was found between dGEMRIC and important features of knee OA – joint space narrowing and osteophytes (bony changes). The findings in Papers I and II indicate that dGEMRIC has the potential to predict future development of knee OA.

Unloading of joints has previously been shown to affect the constituents of cartilage. In Paper III, the knees of patients with ankle fractures, prescribed unloading of the injured leg for six weeks, were investigated. Unloading the knee resulted in a measurable effect on the constituents of cartilage, expressed as a decrease in GAG content and an increase in the range of dGEMRIC values. These findings should be taken into account when considering the treatment of patients involving an unloading regimen.

Anterior cruciate ligament (ACL) injury has previously been shown to be an important risk factor for the development of OA. In Paper IV, patients who had sustained an ACL injury 20 years earlier, but who had not undergone ACL reconstruction, were investigated. Notably, these patients showed good cartilage quality and subjective knee function, similar to that of healthy reference groups. This is an important finding when advising and treating patients who have sustained an ACL injury.

Acknowledgments

I want to begin by expressing my sincere gratitude to everyone who has supported, helped, and encouraged me over the years, and thus contributed to making this thesis possible. In addition, I would especially like to thank the following:

Leif Dahlberg, my main supervisor, who committed himself to guiding me through the complicated world of research, and who showed a great deal of patience when it became clear that this research project would take a little longer than expected to complete.

Carl Johan Tiderius, my co-supervisor, for his encouragement and help during setbacks. But most importantly, you and your wonderful wife have become dear friends to me and my family.

Jeanette Nilsson, for always being a wonderfully cheerful and positive person, and for facilitating my work over the years.

Ylva Ericsson, for excellent collaboration on the study that led to Paper II.

Jon Tjörnstrand, for allowing me to use his dGEMRIC results in Paper II.

Paul Neuman, for excellent collaboration on the study that led to Paper IV.

Martin Englund and Ewa Roos, for their constructive criticism during the drafting of Paper II.

Pernilla Carlsson, for her invaluable input and feedback during her time with our research team.

Jonas Svensson, Carl Siversson, and Gunilla Müller, for helping me to better understand the MRI technique and for answering all my questions on it. Thanks also to Anetta Bolejko for organizing my dGEMRIC examinations.

Jonas Ranstam, for crucial statistical assistance.

Eveliina Lammentausta, for letting me use her Mokkula software.

All the patients for being just that – patient.

My extended family, for their invaluable support during the writing of this thesis.

Christer and Elin, my parents, for always giving me endless love and support. I love you.

Ulrika, my wife, for steadfastly enduring the burden of my writing this thesis. You are my true love and best friend. I love you with all my heart.

Thea, Walter och Ebba, mina underbara barn som är mitt allt. Förlåt för att pappa varit stressad och frånvarande under denna tid. Jag älskar er massor.

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