Elevated adiabatic $T_{1\rho}$ and $T_{2\rho}$ in articular cartilage are associated with cartilage and bone lesions in early osteoarthritis: a preliminary study

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Acknowledgments: The Center for Magnetic Resonance Research, University of Minnesota is gratefully acknowledged for providing the adiabatic T1ρ and T2ρ sequences.

Grant Support: This research was supported by the Academy of Finland (grants 260321, 285909, 268378, 293970) and by the Medical Research Center Oulu, University of Oulu and Oulu University Hospital, Oulu, Finland.

Running title: Elevated adiabatic $T_{1\rho}$ and $T_{2\rho}$ in cartilage are associated with cartilage and bone lesions in early OA

Abstract

Purpose: To evaluate adiabatic $T_{1\rho}$ and $T_{2\rho}$ of articular cartilage in symptomatic osteoarthritis (OA) patients and asymptomatic volunteers; to determine their association with MRI-based structural abnormalities in cartilage and bone.

Materials and Methods: A total of 24 subjects (age range: 50-68 years; 12 female) were enrolled, including 12 early OA patients and 12 volunteers with normal joint function. Patients and volunteers underwent 3 T MRI. T_2, adiabatic $T_{1\rho}$ and $T_{2\rho}$ relaxation times of knee articular cartilage were measured. Clinical MR image series were separately evaluated for pathological changes using the MRI OA Knee Scoring (MOAKS) system. Comparisons using the Mann-Whitney nonparametric test were performed after dividing the study participants according to physical symptoms or presence of cartilage lesions, bone marrow lesions or osteophytes.

Results: Elevated adiabatic $T_{1\rho}$ and $T_{2\rho}$ relaxation times of articular cartilage were associated with different OA signs including cartilage loss (p-values = 0.024-0.047), physical symptoms (0.0068-0.035) and osteophytes (0.0039-0.027). Elevated adiabatic $T_{1\rho}$ was also associated with bone marrow lesions (0.033).

Conclusions: Preliminary data suggest that elevated adiabatic $T_{1\rho}$ and $T_{2\rho}$ of cartilage are associated with morphological abnormalities of cartilage and bone, and thus may be applicable for *in vivo* OA research and diagnostics.

Key words: articular cartilage; relaxation; T1ρ; T2ρ; spin-lock; in vivo

Introduction

Osteoarthritis (OA) of the knee is commonly associated with disabling symptoms such as pain and joint dysfunction, and displays morphological alterations in multiple joint structures. Knee radiographs are traditionally used as instrumental investigation to support the clinical diagnosis, although radiographic features such as osteophytes and joint space narrowing become useful criteria to assess severity of OA mainly when the disease is relatively advanced. The poor sensitivity and reliability of the radiographic assessment of the knee [1, 2] is the principal reason for the discordance between clinical and radiographic OA in the early stages of the disease. Plain X-ray images lack the ability to directly visualize soft tissue and have shown weak associations with physical symptoms [3]. To improve the accuracy of early OA diagnosis, an ideal biomarker should be associated with both morphological changes in the joint structures and symptoms. Furthermore, structural abnormalities are accompanied and preceded by biochemical changes in articular cartilage (AC) [4], therefore the ideal biomarker for early OA should also reflect such changes. Quantitative MRI (qMRI) parameters, such as T_2 and $T_{1\rho}$ relaxation time, have shown their ability to detect pathological changes in AC composition [5]. T_2 and $T_{1\rho}$ provide information about slow interactions of water molecules with their local macromolecular environment in biological tissues [6]. Both techniques, however, have some drawbacks for clinical application. T_2 maps can be confounded by magic angle effect, while $T_{1\rho}$ is particularly susceptible to field inhomogeneities. Moreover, conventional continuous wave (CW) spin-lock results in relatively high RF power deposition in tissues, hence pulse amplitudes *in vivo* are limited to a maximum of few hundred Hz. Adiabatic $T_{1\rho}$ ($AdT_{1\rho}$) and $T_{2\rho}$ ($AdT_{2\rho}$) relaxation time techniques use adiabatic spin-lock pulses, which are particularly advantageous in the clinical domain. During an adiabatic pulse, both amplitude and frequency are varied in time [7], therefore $AdT_{1\rho}$ and $AdT_{2\rho}$ are sensitive over a wide range of slow molecular interactions [8, 9]. $AdT_{1\rho}$ and $AdT_{2\rho}$ mapping have demonstrated high sensitivity to cartilage degeneration in several preclinical studies [10-13] and degenerated human cartilage specimens [14]. They have been shown to strongly correlate with OA histopathology and AC biomechanical parameters [14], and have better sensitivity to OA cartilage changes as compared to CW-$T_{1\rho}$ or T_2 relaxation time [10, 11]. Moreover, the use of adiabatic RF pulses for spin-locking is advantageous in reduction of the effects of magnetic field inhomogeneities and mitigation of specific absorption rate (SAR) [15-17]. Reduced sensitivity to the magic angle effect of adiabatic $T_{1\rho}$ has also been reported compared to T_2 and conventional CW-$T_{1\rho}$ [18]. $AdT_{1\rho}$ and $AdT_{2\rho}$ have already been optimized for AC imaging in the clinical environment and have shown good to excellent reproducibility [19]. Therefore, the techniques are promising candidates as quantitative non-invasive biomarkers for early OA.

The aims of the present study were (1) to evaluate $AdT_{1\rho}$ and $AdT_{2\rho}$ in symptomatic OA patients and asymptomatic volunteers, and (2) to determine the association of $AdT_{1\rho}$ and $AdT_{2\rho}$ with MRI-based structural abnormalities in cartilage and bone.

Material and Methods

Study Participants

This prospective case-control study was approved by the local IRB on human research ethics. Participants were selected from the Oulu Knee Osteoarthritis Study cohort [20] and recruited between February and November 2014. A total of 24 eligible subjects (12 female, mean age 60.1 years, range: 50-68; 12 male, mean age 58.8 years, range: 50-65) were identified, including 12 early OA patients, who fulfilled the American College of Rheumatology (ACR) criteria for classification of idiopathic OA [21], and 12 asymptomatic volunteers matched for age and sex. As the real effect size cannot be estimated using previously published data for $AdT_{1\rho}$ and $AdT_{2\rho}$, we calculated *a priori* sample size on the basis of the average T_2 and CW $T_{1\rho}$ values reported for OA patients and controls by Wang and Regatte [22]. For a power of 80% with the non-parametric Mann Whitney test at alpha = 0.05, sample size required for T_2 and $T_{1\rho}$ is 12 and 8, respectively. The exclusion criteria were as follows: for patients, age below 50 years, total or partial prosthesis or knees with radiographic Kellgren and Lawrence (KL) grade $\geq$ 3; for volunteers, previous knee surgery, any recent traumatic knee injury (fractures, sprains or torsions in the past 15 years), and functional impairment or moderate to severe physical symptoms in the past six months in any knee joint. All the subjects provided informed consent for participation in the study and completed the Western Ontario and McMaster Universities questionnaire (WOMAC) [23] for assessment of pain, stiffness, and physical dysfunction perceived in the last week. All the 24 parameters in the questionnaire were graded with a 100 mm-visual analogue score (VAS). The average WOMAC index (the total score divided by the number of parameters in the questionnaire) was used to classify the overall severity of the symptoms [23].

Radiograph acquisition and interpretation

Weight-bearing postero-anterior non-fluoroscopic radiography of the symptomatic knees of patients was undertaken before their enrollment in the present study, within a maximum of six months before the MRI examination. The X-ray beam was angulated 10° caudally and the patient's feet were positioned in 5° of external rotation with the knees at fixed 20° flexion. Radiographic severity of the knee OA was scored by a single observer (J.M.K. 30 years of experience) using the KL grading system. Since the primary focus of this preliminary study were symptomatic patients at an initial stage (i.e. pre- and early radiographic stage) of OA disease process, radiographic readings were used to exclude subjects with definite joint space narrowing (KL 3 or above).

MRI acquisition

MRI data of patients and volunteers were acquired on a 3T scanner (Skyra, Siemens Healthcare, Erlangen, Germany) using a dedicated 15-channel transmit/receive coil (QED, Mayfield Village, OH, USA). In patients the knee with clinical signs of OA (or with greater KL score in case of subjects with symptoms in both knees) was imaged whilst in volunteers the knee was chosen at random. The protocol included six sequences: PD-weighted (PD-w) Turbo Spin Echo (TSE), Fat-Suppressed (FS) PD-w SPACE, T_1-weighted TSE, T_2 mapping, $AdT_{1\rho}$ and $AdT_{2\rho}$ mapping [19]. The imaging parameters of each sequence are listed in Table 1.

MRI interpretation

Semi-quantitative MRI OA Knee Score (MOAKS) [24] was used to assess the knee images (interpreted by A.G., 17 years of experience with semi-quantitative MRI analysis of knee OA) for cartilage loss and bone marrow lesions (BMLs) in 12 anatomical regions (six femoral and six tibial, Fig. 1): lateral/medial trochlea (Tr), lateral/medial central femur (CF), lateral/medial posterior femur (PF), lateral/medial anterior tibia (AT), lateral/medial central tibia (CT) and lateral/medial posterior tibia (PT). For each region, the relative size of cartilage loss and the relative volume of BML were separately scored with a four-grade scale (0-3, 0 for intact tissue) [24]. Similarly, the presence and extent of marginal osteophytes was evaluated with the MOAKS (0-3, 0 for no osteophytes) in lateral/medial Tr, CF and PF, and in lateral/medial peripheral tibial plateau [24].

MRI quantitative analysis

Cartilage $AdT_{1\rho}$, $AdT_{2\rho}$ [8, 9, 19, 25] and T_2 maps were obtained by mono-exponential fitting of the signal intensity decays on a pixel-by-pixel basis. Lateral and medial cartilage compartments were manually segmented on T_2- and $AdT_{1\rho}$-weighted images by a single reader blinded to morphological findings (V.C., three years of experience) in a manner analogous to the MOAKS anatomical regions, which were further sub-divided into half for superficial and deep cartilage layers (Fig. 1). Finally, the average values for the three quantitative MRI parameters were calculated in all regions of interest (ROIs), not including pixels showing a clear partial volume effect with fluids or other structures, i.e., hyperintense pixels on T_2-weighted images with relaxation values out of physiological range for articular cartilage. ROIs containing less than 10 pixels were excluded from the analysis. All the calculations were performed using MATLAB in-house software (MathWorks, Natick, Massachusetts).

Statistical analysis

For each ROI, non-parametric Mann-Whitney tests were performed to compare $AdT_{1\rho}$, $AdT_{2\rho}$ and T_2 values between: (i) symptomatic patients (average WOMAC index > 0) and asymptomatic volunteers (average WOMAC index = 0); (ii) subjects with intact cartilage (MOAKS = 0) and subjects with any cartilage loss (MOAKS greater than 0) in the anatomical region of the considered ROI; (iii) subjects with normal bone marrow (MOAKS = 0) and subjects with BML (MOAKS > 0) in the anatomical region of the considered ROI; (iv) subjects with no osteophytes (MOAKS = 0) and subjects with osteophytes (MOAKS > 0) in the anatomical region of the considered ROI. Based on former $T_{1\rho}$ data [22], the minimum group size was set equal to four ROIs. Pearson's correlation coefficients (r) were calculated to assess the relationship between $AdT_{1\rho}$, $AdT_{2\rho}$ and T_2 averaged across the whole femoral or tibial compartment. All statistical analyses were performed using SPSS software (IBM SPSS 22.0, Armonk, NY USA). Differences at level $p < 0.05$ were considered significant.

Results

Participants

Table 2 presents the demographic characteristics of the participants. Body mass index was significantly different between the volunteer and patient groups. Two volunteers reported mild physical symptoms in the WOMAC questionnaire. Five symptomatic patients had radiographic OA (KL = 2). The majority of the subjects presented signs of morphological changes with substantial overlap between patients and volunteers. Only five subjects did not present any MRI OA features, specifically three asymptomatic subjects and two patients. The number of subjects with cartilage damage, BMLs and osteophytes for each anatomical region are reported in Table 3. Figure 2 shows an example of cartilage $AdT_{1\rho}$, $AdT_{2\rho}$ and T_2 maps.

Asymptomatic volunteers and symptomatic patients

The two volunteers who reported mild physical symptoms and the matching subjects in the patient cohort were excluded from this comparison. Due to the slice orientation, Tr and AT regions in the medial compartment were only partially visible and, after correction to minimize partial volume effect from surrounding tissues and synovial fluids, some ROIs became too small (< 10 pixels) and therefore were excluded from the analysis. As a result, there were not enough valid cases in the two groups to perform the comparisons in medial deep Tr and medial superficial and deep AT. In symptomatic patients, compared to asymptomatic subjects, elevated $AdT_{1\rho}$ and $AdT_{2\rho}$ were found in deep PT in medial ($p < 0.01$ and $p = 0.035$, respectively) and lateral compartment ($p = 0.029$ and $p = 0.035$, respectively) (Table 4). Compared to asymptomatic subjects, patients showed significantly longer T_2 ($p = 0.018$) in medial deep PT.

Cartilage loss

There were not enough subjects in the AC lesion group in lateral Tr and PF, lateral and medial AT and medial PT (Table 3), therefore no comparisons were performed in those regions. Significant differences were found in relaxation times between subjects with intact cartilage and subjects with cartilage loss in multiple sites (Table 5). Specifically, elevated $AdT_{1\rho}$, $AdT_{2\rho}$ and T_2 associated with cartilage loss were observed in two, three and one ROIs, respectively, with similar significance levels ($p = 0.023$-0.047). Furthermore, at most ROIs for which the differences were not significant, a trend towards prolonged relaxation times was observed in presence of cartilage damage. Considering only the ROIs showing significant differences, the relative differences in means were greater in T_2 (39%) as compared with $AdT_{1\rho}$ (16-21%) and $AdT_{2\rho}$ (17-20%). $AdT_{1\rho}$ and $AdT_{2\rho}$ had both larger values in superficial and deep layers of lateral CT. At the same site, prolonged T_2 relaxation time was observed in the superficial and deep layers,

although the difference did not reach statistical significance (p = 0.075-0.055). Significantly elevated T_2 was found in lateral deep CF. In the medial compartment, elevated $AdT_{2\rho}$ in subjects with damaged cartilage were found in deep PF.

Bone marrow lesions

Among the considered regions, only in medial CF the number of subjects with BMLs was enough to perform the comparison (Table 3). Significantly longer $AdT_{1\rho}$ was found in the superficial layer in the lesion group (p = 0.033), with similar relative differences in mean relaxation times (13 and 15%) (Table 6). In the same ROI, although not significant, T_2 showed a trend towards elevated values in presence of BMLs (p = 0.056).

Osteophytes

Lateral compartment regions were excluded from analyses since there were not enough subjects in the group with osteophytes (Table 3). In the medial compartment, all ROIs had enough valid cases except superficial and deep AT for all the three parameters ROIs and superficial PT for $AdT_{2\rho}$. Relaxation times showed significant differences in PT in subjects with osteophytes (Table 7). $AdT_{1\rho}$, $AdT_{2\rho}$ and T_2 were simultaneously elevated in deep PT. Differences in $AdT_{1\rho}$ and T_2 showed a stronger level of significance ($p \leq 0.01$) as compared to $AdT_{2\rho}$. In the same site, $AdT_{1\rho}$ and T_2 were significantly elevated also in the superficial layer. The relative differences in means were greater in $AdT_{1\rho}$ (19-35%) as compared to $AdT_{2\rho}$ (24%) and T_2 (23-26%). Elevated relaxation times were also observed in presence of osteophytes in most of the ROIs that showed no significant differences.

Correlation

The T_2 averaged across the whole of femoral cartilage was moderately correlated with $AdT_{1\rho}$ ($r = 0.48$; $p = 0.00054$) and $AdT_{2\rho}$ ($r = 0.40$; $p = 0.0045$). The correlations were stronger in tibia ($r = 0.77$ and 0.76, respectively; $p < 0.0001$). Cartilage $AdT_{1\rho}$ and $AdT_{2\rho}$ were strongly correlated, and the correlation was stronger in tibia ($r = 0.86$; $p < 0.0001$) than in femur ($r = 0.75$; $p < 0.0001$).

Discussion

The most important findings of this study are the significant increases in $AdT_{1\rho}$, $AdT_{2\rho}$ and T_2 with physical symptoms and structural changes in cartilage and bone.

Physical symptoms are considered an important feature for clinical diagnosis of OA. Previous studies have reported elevation of $T_{1\rho}$ and T_2 with symptoms [26-28]. In the presentstudy $AdT_{1\rho}$, $AdT_{2\rho}$ and T_2 were able to differentiate the symptomatic patients from the asymptomatic volunteer group with statistical significance in one ROI. Interestingly, the region is characterized by the low prevalence of BMLs and cartilage loss (only two subjects had cartilage and/or bone marrow lesions in medial PT), whilst more than half of the symptomatic medial tibial compartments presented osteophytes**.** The association between osteophytes and knee pain is well known [29]. In some anatomical sites the association between elevated cartilage relaxation times and physical symptoms may have been masked by the overlap between the two groups in terms of cartilage and/or bone lesions. Since OA is asymptomatic in the earliest stages [3], it was considered important in this study to evaluate the ability of the quantitative MRI parameters to reflect relevant features connected with the disease, which are elusive to radiographic criteria in the initial stages of the disease. The presence of prolonged relaxation time foci, confirmed by three different quantitative MRI parameters, in association with pain and definite osteophytes might be already a signature of early OA events and could help the clinician in the diagnosis especially in context of early OA where radiographic features alone are not contributive.

As per definition, OA disease process is characterized by areas of cartilage loss associated with alterations, such as lesions and osteophytes in the periarticular bones. Such OA signs are not all directly detectable by plain radiography, yet these are important features to consider for differential diagnosis. Previous studies have demonstrated an association of such OA features with quantitative MRI parameters. Specifically, elevated T_2 values have been determined in patients with cartilage lesions [27, 30, 31] and alteration of subchondral bone [27], whilst increased $T_{1\rho}$ of cartilage have been observed in subjects with AC lesions [32, 33]. $AdT_{1\rho}$ and $AdT_{2\rho}$ relaxation time constants have been shown to increase with AC degeneration *in vitro* [10-12, 14]. In this study, $AdT_{1\rho}$ and $AdT_{2\rho}$ and T_2 were significantly increased in multiple cartilage sites presenting lesions. $AdT_{2\rho}$ relaxation time was the most sensitive to morphological changes in cartilage, with significantly increased values observed altogether in three ROIs. Surprisingly, while the medial compartment was the one with the highest prevalence of lesions, differences in quantitative parameters associated with cartilage defects were significant mostly in the lateral compartment. This result confirms that the link between morphological and biochemical changes may not always be obvious in articular cartilage, particularly in early OA [34]. Among the three parameters, only $AdT_{2\rho}$ showed significant

difference in the medial compartment. In the lateral compartment, increased values of $AdT_{1\rho}$ and $AdT_{2\rho}$ were associated with cartilage defects in tibial load-bearing area (CT), while on the contrary T_2 was significantly elevated in femoral load-bearing region (CF). These dissimilarities may depend on diverse sensitivities of effective relaxation mechanisms to different cartilage components as well as the higher sensitivity of T_2 relaxation time on the magic angle effect [18]. Therefore, the adiabatic rotating frame of reference (RFR) techniques may provide complementary information regarding changes in the cartilage tissue.

Bone marrow alterations can occur at very early stages of OA and are thought to be functional adaptation attempts in response to abnormal loading, hence, being proportional to the applied load magnitude [35]. The findings of this study seem to be consistent with this paradigm and showed the highest prevalence of BMLs in the most critical area for the load-bearing function of the knee joint, the central regions of medial compartment. The highest prevalence of BMLs (central femur region) was also associated with significantly longer $AdT_{1\rho}$ values in cartilage and, although not significant, trend for elevated $AdT_{2\rho}$ and T_2. The specific physiological interactions occurring between articular cartilage and subchondral bone in the different OA stages are currently not completely understood. Nonetheless, it is likely that any pathological change in either tissue would eventually affect the structural and compositional organization of the other. Since the compositional changes are often accompanied by elongation of cartilage relaxation times, reflective of biochemical alterations, the elevated values found in this study are likely reflecting those changes, which may be either cause or consequence of the bone marrow lesions.

Previously, it has been shown that a relationship exists between elevated T_2 relaxation values in cartilage and BMLs [27]. In this study, differences in T_2 were close to significance ($p = 0.056$) and might have reached significance with a larger sample size. Nevertheless, differences in $AdT_{1\rho}$ were statistically significant, suggesting better sensitivity to BMLs as compared to T_2.

At different significance levels, all studied quantitative MRI parameters showed association with osteophytes. The presence of osteophytes is a hallmark of OA [36] and is more predictive of symptomatic knee OA as compared with joint space narrowing [29]. The ROI analysis found significantly elevated $AdT_{1\rho}$, $AdT_{2\rho}$ and T_2 in an equal number of cartilage sites. Particularly $AdT_{1\rho}$ presented greater discrimination power for the presence of osteophytes and higher significance as compared to $AdT_{2\rho}$ and T_2.

The rotating frame techniques are able to detect changes in the slow molecular motion which reflect extracellular matrix alterations [6]. During an adiabatic RF pulse, both amplitude and frequency vary in time, and the pulse frequency is off-resonance for a significant part of the pulse [7]. Therefore $AdT_{1\rho}$ and $AdT_{2\rho}$ are sensitive to an extended range of slow motional correlation times [8, 9]. At higher field strengths

elevated $AdT_{1\rho}$ and $AdT_{2\rho}$ have been observed in human degenerated cartilage and showed better accuracy for discriminating mild and advanced cartilage degeneration as compared to T_2 and $T_{1\rho}$ relaxation measurements [14]. The association of $AdT_{1\rho}$ and $AdT_{2\rho}$ with clinical relevant OA features has not been studied *in vivo* yet. In this study, different levels of significance were found for associations of adiabatic $T_{1\rho}$ and $T_{2\rho}$ with various OA features, also as compared with T_2, suggesting different biochemical pathways in AC and different sensitivity of the quantitative MRI parameters for OA features. RFR techniques could be used in combination with other quantitative tools to improve the accuracy of OA diagnostics.

The qMRI parameters were significantly positively correlated with each other. Based on the coefficients of non-determination $1 - r^2$, the unexplained variance was moderate to very high between T_2 and RFR parameters (41-81%) and mild to moderate (27-44%) between $AdT_{1\rho}$ and $AdT_{2\rho}$, which further confirms their ability to provide complementary information. The correlation between the parameters was highest in the tibia. This could be explained by the reduced angular dependence of RFR techniques on collagen fibers [18], which is a dominating factor for T_2 in femur.

Interestingly, the $AdT_{1\rho}$ and $AdT_{2\rho}$ values in the superficial layers were constantly longer than those previously reported for younger asymptomatic adults (age range 25–35 years) [19]. On the contrary, values in the deep layers were more consistent between the groups. This is consistent with the hypothesis that senescent changes in cartilage matrix begin from the articular surface, as observed in a previous study [37].

Some limitations of this study can be identified. First, the limited sample size may have prevented significance from being reached in some ROIs. Furthermore, the present study lacks a reference cohort with no symptoms, KL grade 0, and minimal morphological abnormality findings, required to assess diagnostic performance of these techniques. This warrants longitudinal investigations with a larger number of participants, for it is widely known that a significant fraction of the general population above 50 years shows OA signs in otherwise normal knees [38-40]. Finally, while the whole knee joints were evaluated with MOAKS, only one slice per compartment was assessed with quantitative MRI, in order to keep the total acquisition time as well as SAR of the prototype RFR sequences within an acceptable range. Although the centers of the femoral condyles and tibial plateau are considered the most relevant areas, foci of increased relaxation time may have existed also at other cartilage regions, potentially masking some significant findings. Future research will be directed towards different imaging strategies able to cover the whole knee within an acquisition times suitable for clinical use and to overcome the known limitations of the spoiled gradient echo sequences for measurements of MRI parameters [41]. Furthermore, studies are warranted to compare cartilage $T_{1\rho}$ relaxation measured with conventional CW sequences with $AdT_{1\rho}$ and $AdT_{2\rho}$ *in vivo*.

In conclusion, this study provides evidence of the association of quantitative MRI with clinical OA features. Specifically, Ad$T_{2\rho}$ was more closely related to cartilage lesions and Ad$T_{1\rho}$ to osteophytes and BMLs. The findings suggest the potential of adiabatic $T_{1\rho}$ and $T_{2\rho}$ as biomarkers for early OA.

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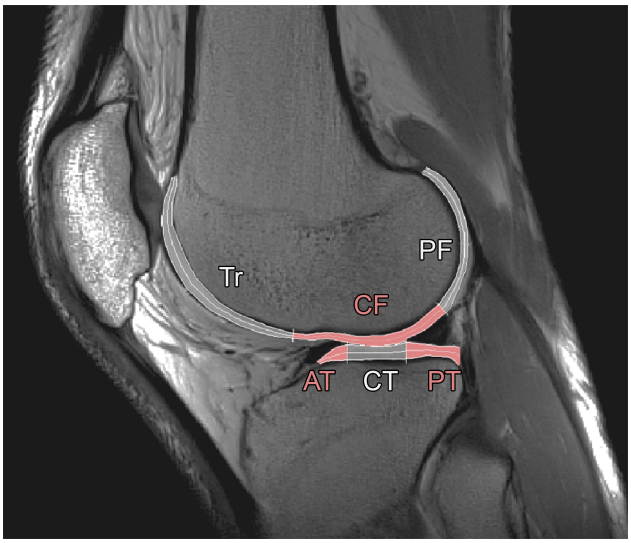

Figure 1. Segmentation of articular cartilage and ROI nomenclature [24] (Tr = Trochlea; CF = Central Femur; PF = Posterior Femur; AT = Anterior Tibia; CT = Central Tibia; PT = Posterior Tibia). Color has been introduced to help distinguish the regions.

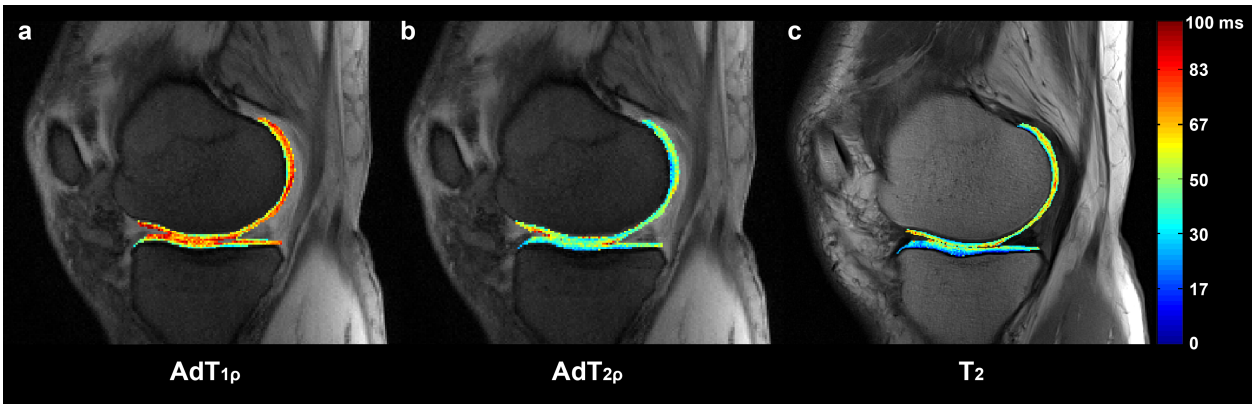

Figure 2. Different regional distributions of adiabatic $T_{1\rho}$ (a) and $T_{2\rho}$ (b) and standard T_2 (c) across tibiofemoral cartilage of a representative subject (65 years,male). The three quantitative MRI parameters can provide complementary information regarding structural abnormalities.

ble 1. MRI protocol and sequence parameters

Parameters	PD-w	3D PD-w	T_1-w	T_2-mapping	$AdT_{1\rho}$-mapping
Pulse sequence	TSE	TSE FS SPACE	TSE	MESE	HS4-AFP pulse trains[a] followed by *FL*
TR [ms]	2800	1200	650	1680	
TE [ms]	33	26	18	n*13.8 (n = 1-5)	
Flip Angle [deg]	150	120	150	180	
ETL	7	49	2	5	23 1
FOV [mm²]	140x140	160x160	130x130	160x160	
Matrix [px²]	384x384	256x256	320x320	384x384	
Plane	sagittal	sagittal	coronal	sagittal	
Slices [n] (Thk [mm])	35 (3.0)	176 (0.6)	25 (0.6)	18 (3.0)	
Acquisition Time [min:s]	4:09	8:48	1:56	5:41	4:42

PD-w = Proton Density-weighted; T_1-w = T_1-weighted; Ad = Adiabatic; TSE = Turbo Spin-Echo; FS = Fat-Suppressed; MESE = Multi-Echo Sp
AFP = Adiabatic Full Passage; HS*n* = hyperbolic secant (*n* = 4, stretching factor); AHP = Adiabatic Half Passage
TR = Repetition Time; TE = Time Echo; ETL = Echo Train Length; FOV = Field of View; Thk = slice thickness
[a] Trains of 4*n pulses (n = 0-4, 6 ms/pulse, maximum pulse amplitude $\omega_{max}/2\pi$ = 800 Hz)
[b] located at the center of each femoral condyle

ble 2. Demographic characteristics of patients (N = 12) and volunteers (N = 12)

Variable	Volunteers	Patients	P-value	All subjects
Female/Male, n (%)	6/6 (50.0/50.0)	6/6 (50.0/50.0)	> 0.99[a]	12/12 (50.0/50.0)
Age, mean (range) [years]	59.8 (52-68)	59.1 (50-67)	0.77[b]	59.4 (50-68)
BMI, mean (SD) [kg/m^2]	24.8 (3.2)	30.4 (6.9)	0.018[b]	27.6 (5.9)
KL grade, n (%)				
1		7 (58.3)		
2		5 (41.7)		
Symptomatic[c], n (%)	2 (16.7)	12 (100.0)	< 0.001[d]	14 (58.3)
AC lesions[e], n (%)	9 (75.0)	10 (83.3)	> 0.99[d]	19 (79.2)
BMLs[e], n (%)	4 (33.3)	7 (58.3)	0.41[a]	11 (45.8)
Osteophytes[e], n (%)	2 (16.7)	7 (58.3)	0.089[d]	9 (37.5)

BMI = Body Mass Index; AC = Articular Cartilage; BMLs = Bone Marrow Lesions
[a] Chi-square test
[b] Independent samples t-test
[c] WOMAC index > 0
[d] Fisher exact test
[e] MOAKS > 0 in any knee joint anatomical region

ble 3. Total number of subjects with cartilage lesions, bone marrow lesions and osteophytes in lateral and medial femoral and tibia

ROI		AC lesions	BMLs	Osteophytes
Lateral compartment				
FEMUR	**Tr**	3 (2)	2 (1)	2 (2)
	CF	4 (2)	1 (1)	1 (1)
	PF	1 (1)	1 (1)	1 (1)
TIBIA	**AT**			
	CT	7 (3)		1 (1)[a]
	PT	6 (5)		
Medial compartment				
FEMUR	**Tr**	7 (4)	3 (2)	4 (4)
	CF	15 (9)	6 (4)	6 (5)
	PF	6 (4)	3 (3)	6 (6)
TIBIA	**AT**	1 (1)	1 (1)	
	CT	8 (7)	2 (2)	5 (5)[a]
	PT	2 (2)		

ROI = Region Of Interest
AC = Articular Cartilage; BMLs = Bone Marrow Lesions
Tr = Trochlea; CF = Central Femur; PF = Posterior Femur; AT = Anterior Tibia; CT = Central Tibia; PT = Posterior Tibia
[a] Number of osteophytes in the whole lateral and medial tibia plateaus

ble 4. Means for adiabatic $T_{1\rho}$ ($AdT_{1\rho}$), adiabatic $T_{2\rho}$ ($AdT_{2\rho}$) and T_2 relaxation time values (ms) in lateral and medial ROIs of arti
= 10) and asymptomatic volunteers (Asympt, n = 10)

ROI		$AdT_{1\rho}$			$AdT_{2\rho}$			T_2		
		Asympt	**Sympt**	**P-value**	**Asympt**	**Sympt**	**P-value**	**Asympt**	**Sympt**	**P-val**
Lateral compartment										
SUP FEMUR	**Tr**	86.3 (8.8)	82.8 (5.1)	0.53	53.5 (5.2)	52.4 (6.1)	0.53	59.9 (8.1)	66.9 (11.1)	0.12
	CF	79.6 (7.6)	80.1 (7.8)	> 0.90	53.1 (6.7)	54.7 (5.4)	0.80	56.8 (8.5)	57.5 (6.0)	> 0.9
	PF	71.5 (9.2)	75.1 (8.0)	0.28	45.2 (6.2)	49.2 (5.3)	0.19	56.0 (8.8)	61.6 (10.4)	0.20
DEEP FEMUR	**Tr**	61.6 (4.7)	60.0 (4.7)	0.58	42.0 (2.9)	40.7 (4.6)	0.44	39.9 (10.4)	48.4 (8.8)	0.11
	CF	61.2 (8.5)	61.4 (5.2)	0.74	43.4 (7.1)	44.0 (5.1)	0.74	35.7 (10.3)	43.6 (12.9)	0.19
	PF	58.7 (4.1)	57.9 (6.3)	0.35	38.7 (1.8)	41.8 (4.3)	0.08	43.4 (6.2)	45.3 (7.4)	0.48
SUP TIBIA	**AT**	78.0 (16.6)	73.1 (7.3)	0.73	47.6 (8.7)	45.9 (2.2)	0.48	53.4 (12.9)	51.4 (9.1)	0.85
	CT	70.4 (11.5)	73.7 (16.9)	> 0.90	43.9 (7.5)	44.8 (10.5)	> 0.90	42.8 (8.2)	47.7 (12.4)	0.39
	PT	75.6 (12.3)	78.0 (12.3)	0.66	47.5 (7.0)	49.7 (6.7)	0.48	56.0 (9.9)	57.5 (8.3)	0.58
DEEP TIBIA	**AT**	48.8 (10.2)	58.4 (17.6)	0.28	34.3 (9.2)	40.5 (9.3)	0.30	36.7 (9.8)	34.0 (8.5)	0.62
	CT	43.9 (7.4)	44.5 (7.5)	0.63	29.2 (5.9)	31.5 (6.5)	0.63	26.3 (5.3)	26.9 (5.6)	0.85
	PT	**55.3 (4.7)**	**64.0 (11.7)**	**0.029**	**37.6 (3.9)**	**43.8 (6.1)**	**0.035**	34.4 (3.0)	37.4 (8.9)	0.80
Medial compartment										
SUP FEMUR	**Tr***	93.2 (18.4)	83.2 (11.2)	0.53	58.9 (12.7)	50.0 (8.0)	0.23	56.1 (8.3)	66.7 (13.5)	0.11
	CF	81.5 (11.4)	81.1 (8.0)	0.85	53.1 (7.2)	54.2 (3.6)	0.80	59.4 (7.6)	59.8 (5.0)	> 0.9
	PF	76.3 (5.2)	77.4 (9.1)	> 0.90	49.5 (3.1)	48.6 (5.5)	> 0.90	58.0 (5.4)	60.6 (6.6)	0.17
DEEP FEMUR	**CF**	62.0 (6.4)	62.7 (6.3)	0.63	45.1 (6.8)	46.6 (5.5)	0.48	38.5 (10.1)	41.5 (10.3)	0.39
	PF	62.1 (3.1)	63.4 (8.3)	0.80	38.7 (3.3)	41.0 (6.0)	0.63	44.2 (6.9)	42.8 (6.4)	0.74
SUP TIBIA	**CT**	78.5 (5.9)	77.7 (9.6)	> 0.90	52.3 (4.7)	49.6 (7.5)	0.59	54.0 (8.2)	54.1 (9.1)	> 0.9
	PT	74.9 (9.3)	74.2 (7.8)	0.55	46.5 (7.4)	45.9 (8.8)	> 0.90	51.7 (9.2)	52.6 (5.8)	0.25
DEEP TIBIA	**CT**	43.4 (4.6)	46.9 (9.3)	0.28	31.5 (4.8)	36.3 (9.4)	0.14	24.4 (3.3)	26.5 (3.9)	0.39
	PT	**51.5 (6.1)**	**60.9 (7.3)**	**0.0068**	**38.1 (5.1)**	**45.3 (7.7)**	**0.035**	**32.1 (4.9)**	**38.6 (5.3)**	**0.018**

ROI = Region Of Interest; SD = Standard Deviation
SUP = Superficial; Tr = Trochlea; CF = Central Femur; PF = Posterior Femur; AT = Anterior Tibia; CT = Central Tibia; PT = Posterior Tibia
*In medial superficial Tr, n was four and seven for Asympt and Sympt group, respectively
P-values from Mann-Whitney nonparametric test, significant differences are shown in bold with gray background

ble 5. Means for adiabatic $T_{1\rho}$ ($AdT_{1\rho}$), adiabatic $T_{2\rho}$ ($AdT_{2\rho}$) and T_2 relaxation time values (ms) of articular cartilage of subjects w
ions (independently of their symptomatic/asymptomatic status) in the considered anatomical region

ROI		$AdT_{1\rho}$					$AdT_{2\rho}$					
		Intact		Lesion		P-value	Intact		Lesion		P-value	
		n	Mean (SD)	n	Mean (SD)		n	Mean (SD)	n	Mean (SD)		n
Lateral compartment												
SUP FEMUR	**CF**	20	79.2 (7.6)	4	79.8 (6.7)	> 0.90	20	53.8 (6.0)	4	54.5 (1.7)	> 0.90	20
DEEP FEMUR	**CF**	20	62.0 (7.6)	4	66.9 (3.0)	0.11	20	43.4 (6.2)	4	49.0 (2.3)	0.12	**20**
SUP TIBIA	**CT**	**17**	**69.6 (11.9)**	**7**	**84.5 (14.0)**	**0.024**	**17**	**43.6 (8.4)**	**7**	**51.8 (8.2)**	**0.024**	17
	PT	18	77.0 (11.1)	6	80.0 (13.0)	0.61	18	48.7 (6.7)	6	51.0 (7.8)	0.63	18
DEEP TIBIA	**CT**	**17**	**42.6 (5.9)**	**7**	**49.8 (9.1)**	**0.047**	**17**	**29.2 (4.7)**	**7**	**35.0 (7.7)**	**0.040**	17
	PT	18	58.6 (7.7)	6	65.4 (13.2)	0.22	18	40.8 (5.7)	6	43.5 (6.2)	0.38	18
Medial compartment												
SUP FEMUR	**CF**	18	80.1 (11.1)	6	85.1 (11.2)	0.41	18	52.2 (7.7)	6	55.5 (6.0)	0.24	18
	PF	9	77.2 (6.8)	15	78.6 (11.1)	0.87	9	49.5 (4.7)	15	49.7 (8.5)	> 0.90	9
DEEP FEMUR	**CF**	18	60.8 (7.9)	6	64.7 (5.2)	0.29	18	46.5 (5.9)	6	47.7 (8.0)	> 0.90	18
	PF	9	61.6 (4.9)	15	65.9 (8.7)	0.18	**9**	**38.1 (3.5)**	**15**	**44.5 (6.5)**	**0.047**	9
SUP TIBIA	**CT**	16	78.4 (9.1)	8	81.6 (12.9)	0.70	16	51.4 (5.6)	8	50.7 (7.4)	> 0.90	16
DEEP TIBIA	**CT**	16	45.8 (6.6)	8	45.0 (7.8)	0.83	16	33.3 (7.2)	8	34.7 (6.9)	0.35	16

ROI = Region Of Interest; SD = Standard Deviation
SUP = Superficial; CF = Central Femur; PF = Posterior Femur; CT = Central Tibia; PT = Posterior Tibia
P-values from Mann-Whitney nonparametric test, significant differences are shown in bold with gray background

ble 6. Means for adiabatic $T_{1\rho}$ ($AdT_{1\rho}$), adiabatic $T_{2\rho}$ ($AdT_{2\rho}$) and T_2 relaxation time values (ms) ROIs of articular cartilage of subj
8) and subjects with bone marrow lesions (BML, n = 6), independently of their symptomatic/asymptomatic status

ROI		$AdT_{1\rho}$			$AdT_{2\rho}$			T_2	
		No BML	**BML**	**P-value**	**No BML**	**BML**	**P-value**	**No BML**	**BML**
Medial compartment									
SUP FEMUR	**CF**	**80.1 (8.9)**	**92.7 (12.6)**	**0.033**	52.8 (6.5)	58.4 (5.8)	0.16	58.8 (6.8)	65.4 (5.
DEEP FEMUR	**CF**	62.0 (6.1)	67.0 (6.7)	0.14	47.0 (6.9)	47.9 (8.8)	> 0.90	39.1 (8.0)	45.0 (1

ROI = Region Of Interest; SD = Standard Deviation
SUP = Superficial; CF = Central Femur
P-values from Mann-Whitney nonparametric test, significant differences are shown in bold with gray background

ble 7. Means for adiabatic $T_{1\rho}$ ($AdT_{1\rho}$), adiabatic $T_{2\rho}$ ($AdT_{2\rho}$) and T_2 relaxation time values (ms) ROIs of articular cartilage of subj
eophytes (independently of their symptomatic/asymptomatic status) in the considered anatomical region

ROI		$AdT_{1\rho}$					$AdT_{2\rho}$				
		No Osteophytes		Osteophytes		P-value	No Osteophytes		Osteophytes		P-value
		n	Mean (SD)	n	Mean (SD)		n	Mean (SD)	n	Mean (SD)	
Medial compartment											
SUP FEMUR	**Tr**	10	84.5 (13.7)	4	101.9 (29.3)	0.30					
	CF	11	81.1 (8.7)	4	89.5 (15.9)	0.25	9	53.4 (6.3)	4	56.8 (7.9)	0.41
	PF	18	76.8 (6.9)	6	79.8 (10.6)	0.28	18	48.9 (4.8)	6	51.4 (7.8)	0.25
DEEP FEMUR	**Tr**	18	66.3 (11.0)	6	72.5 (9.7)	0.28	18	47.2 (9.2)	6	46.3 (11.3)	0.83
	CF	18	62.8 (6.2)	6	64.6 (7.5)	0.72	18	46.3 (6.0)	6	50.2 (10.1)	0.58
	PF	18	61.8 (4.9)	6	65.4 (9.0)	0.45	18	38.4 (3.4)	6	43.7 (7.4)	0.18
SUP TIBIA	**CT**	19	78.4 (8.4)	5	83.4 (16.5)	0.58	19	51.2 (5.7)	5	51.4 (8.3)	0.83
	PT	**19**	**73.9 (8.7)**	4	**87.8 (16.1)**	**0.027**					
DEEP TIBIA	**CT**	19	45.5 (7.3)	5	45.6 (5.6)	> 0.90	19	33.1 (7.5)	5	36.1 (4.7)	0.30
	PT	**19**	**54.5 (6.5)**	5	**73.4 (19.6)**	**0.0039**	**19**	**40.0 (7.6)**	**5**	**49.6 (8.3)**	**0.015**

ROI = Region Of Interest; SD = Standard Deviation
SUP = Superficial; Tr = Trochlea; CF = Central Femur; PF = Posterior Femur; AT = Anterior Tibia; CT = Central Tibia; PT = Posterior Tibia
P-values from Mann-Whitney nonparametric test, significant differences are shown in bold with gray background