Is T1rho-mapping an alternative to delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) in assessing sulphated glycosaminoglycan content in human osteoarthritic knees? An *in vivo* validation study

Type of manuscript: original research

Advances in Knowledge

1. dGEMRIC $T1_{GD}$ relaxation times acquired at 3T correlate strongly with cartilage sulphated glycosaminoglycan (sGAG) content measured with dimethylmethylene blue assay (r=0.73, 95% credible interval (95%CI) 0.60 to 0.83) and weakly with cartilage collagen content measured with hydroxyproline assay (r=0.40, 95%CI 0.18 to 0.58).
2. Although sGAG is the most important component of cartilage that influences contrast distribution throughout the articular cartilage *in vivo* in human osteoarthritic knees, our results suggest that dGEMRIC measurements may also depend on other composites of the cartilage.
3. T1rho relaxation times acquired at 3T correlate neither with cartilage sGAG content nor with cartilage collagen content.
4. T1rho-mapping cannot be regarded as an alternative for dGEMRIC to measure cartilage sGAG content in clinical OA research.

Implications for Patient Care

1. dGEMRIC is a validated and robust method for quantifying cartilage sulphated glycosaminoglycan (sGAG) content in human osteoarthritis (OA) subjects.

Summary Statement

Although the technique is not completely sGAG specific and requires a contrast agent, dGEMRIC is a validated and robust method for quantifying cartilage sGAG content in human OA subjects whereas T1rho-mapping appears not suitable for this purpose.

Abstract

Purpose

The aim of our study was to determine if T1rho-mapping can be used as an alternative to dGEMRIC for quantifying cartilage biochemical composition *in vivo* in human OA knees.

Materials and Methods

Institutional review board study approval and written informed consent from participants was obtained. Twelve knee OA patients underwent dGEMRIC and T1rho-mapping at 3T before total knee replacement (TKR). Outcomes of dGEMRIC and T1rho-mapping were calculated in 6 cartilage regions of interest (ROI). Femoral and tibial cartilage were harvested during TKR. Cartilage sGAG and collagen content were assessed with dimethylmethylene blue and hydroxyproline assays, respectively. A four-dimensional multivariate mixed-effects model was used to simultaneously assess the correlation between outcomes of dGEMRIC, outcomes of T1rho-mapping, sGAG, and collagen content of the articular cartilage.

Results

DGEMRIC T1 relaxation times correlated strongly with cartilage sGAG (r=0.73, 95% credible interval (95%CI) 0.60-0.83) and weakly with cartilage collagen content (r=0.40, 95%CI 0.18-0.58). T1rho relaxation times did not correlate with cartilage sGAG (r=0.04, 95%CI -0.21-0.28), nor with cartilage collagen content (r=-0.05, 95%CI -0.31-0.20).

Conclusion

dGEMRIC can accurately measure cartilage sGAG content *in vivo* in human knee OA subjects whereas T1rho-mapping appears not suitable for this purpose. Although the technique is not completely sGAG specific and requires a contrast agent, dGEMRIC is a

validated and robust method for quantifying cartilage sGAG content in human OA subjects in clinical research.

Word count abstract: 224

Introduction

Knee osteoarthritis (OA) is the most common joint disease in middle-aged and elderly, causing serious morbidity and large socio-economic impact (1). Since no definitive treatment options other than joint replacement surgery in end stage OA are available, research mainly focuses on novel interventions such as disease modifying OA drugs. These should be effective in the early stages of OA by modifying the course of the disease, for example by improving cartilage biochemical composition (2, 3). To monitor the structural effectiveness of such novel interventions in early OA, accurate *in vivo* imaging biomarkers are essential. Therefore, quantitative biomarkers that measure cartilage biochemical composites, e.g. sulphated glycosaminoglycan (sGAG) content, have become of interest during the last decade (4).

An example of such a quantitative imaging biomarker to measure articular cartilage sGAG content is delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) (5). This technique utilizes the inverse relation between the amount of sGAG in cartilage and an intravenously administered negatively charged contrast agent. Although dGEMRIC is an established imaging biomarker for quantitative imaging of articular cartilage, the technique has disadvantages. These are mainly related to the contrast administration that increases costs and is potentially harmful for patients with impaired renal function, and the long delay between contrast administration and MR acquisition. Because of these drawbacks, T1rho-mapping was suggested as a non-contrast-enhanced alternative to dGEMRIC to measure cartilage sGAG content (3, 4, 6). T1rho-mapping quantifies the spin relaxation in the rotating frame by using a constant radiofrequency field referred to as a “spin lock” pulse to change relaxation rates of water associated with large macromolecules in cartilage such as sGAG (7, 8).

Although both dGEMRIC and T1rho-mapping are increasingly used as outcome measures for cartilage biochemical composition in clinical OA research, they have been validated mainly *in vitro* (9, 10) or *ex vivo* (11, 12) using bovine and cadaveric human cartilage. *In vivo* validation was performed in only one study for dGEMRIC (13) and only two studies for T1rho-mapping (14, 15). Besides, no study applied both imaging biomarkers in humans *in vivo* and compared the outcomes with a reference standard for cartilage sGAG content to validate and compare their performance. Finally, the influence of the cartilage extracellular matrix integrity, mainly provided by the collagen network, has not yet been studied in detail for dGEMRIC and T1rho-mapping.

The aim of our study was to determine if T1rho-mapping can be used as an alternative to dGEMRIC for quantifying cartilage biochemical composition *in vivo* in human OA knees.

Materials and Methods

Study design and participants

For our prospective observational study, conducted between October 2012 and December 2013, all consecutive patients scheduled for total knee replacement (TKR) at our institution were approached. Our study was approved by the Medical Ethical Committee of Erasmus MC (MEC-2012-218) and written informed consent was obtained from all participants

The inclusion criteria were: age ≥ 18 years and radiographic knee OA with asymmetric distribution and a maximum of grade 1-2 (doubtful or definite osteophyte formation without definite joint space narrowing) according to Kellgren & Lawrence (KL) grading (16) in the least affected tibiofemoral compartment. Exclusion criteria

were: renal insufficiency (glomerular filtration rate < 60 ml/min), history of previous reactions to contrast agent, or significant co-morbidities in the ipsilateral lower extremity (e.g. severe hip OA, neurologic or muscular diseases causing hip or knee disability), which prohibit exercising after contrast administration for dGEMRIC.

MRI acquisition

One day before TKR, MRI was performed using a 3T MRI scanner (Discovery MR750, General Electric Healthcare, Milwaukee, USA) using a dedicated 8-channel knee coil (Invivo Inc., Gainesville, USA).

The MRI protocol included the following three pulse sequences, all acquired in the sagittal plane (specific imaging parameters shown in **Table 1**): (I) a 3D high resolution fat-saturated spoiled gradient-echo (SPGR) sequence, (II) a 3D fast spin-echo (FSE) T1rho-mapping sequence with five different spin lock times (TSL) (17) and (III) a 3D inversion recovery (IR) non-fat-saturated SPGR sequence with five different inversion times (TI) for dGEMRIC (18).

Before dGEMRIC acquisition, a double dose (0.2 mmol per kg body weight) of gadopentetate dimeglumine (Magnevist®, Bayer Schering AG, Berlin, Germany) was injected intravenously as advocated previously (19). Subsequently, participants cycled for 10 minutes on a home trainer at constant speed to promote contrast distribution into and throughout the knee. After a delay of 90 minutes, the IR SPGR sequence was acquired (20).

MRI analysis

Using Matlab (R2011a, The MathWorks, Natick, MA, USA), three cartilage regions of interest (ROIs) in both tibiofemoral compartments were drawn: weight-

bearing cartilage of the femoral condyles (wbFC), posterior non weight-bearing cartilage of the femoral condyles (pFC) and weight-bearing cartilage of the tibial plateaus (wbTP) (**Figure 1**). All ROIs consisted of 15 consecutive slices: the most central slice through the medial or lateral tibiofemoral compartment (defined as the sagittal section depicting the most caudal point of the femoral condyle identified on multiplanar reconstructions of the 3D high resolution fat-saturated SPGR sequence) along with the neighboring seven slices medially and laterally. All ROIs were drawn on the high resolution SPGR images by a researcher with a medical degree and 4 years' experience in musculoskeletal research (JvT).

Image analysis was performed with Software for Post-processing And Registration of Cartilage of the Knee (21, 22). The image analysis pipeline included registration to correct for patient motion and performed fitting of dGEMRIC post-contrast T1 ($T1_{GD}$) and T1rho relaxation times. First, all images of the T1rho-mapping and dGEMRIC sequence with different TSL and TI values were registered to the TSL=1 ms and TI=2100 ms images. The femoral condyle and tibial plateau were registered separately. The images were registered using a 3D rigid transformation model by maximization of localized mutual information (23). To minimize the blurring of the registered images, cubic interpolation was used. All registrations were performed using open source registration software (Elastix, http://elastix.isi.uu.nl/) (24). Second, both registered T1rho-mapping and dGEMRIC datasets were registered to the high resolution SPGR images. This registration was based on the TSL=1 ms and TI=2100 ms images; the other TSL and TI images were transformed accordingly. This second registration step allows analyzing matching cartilage ROIs on matching slices in both sequences.

After registration, T1rho- and dGEMRIC $T1_{GD}$ maps were estimated using a maximum likelihood fit. Before fitting, partial volume voxels for cortical bone within the cartilage ROIs were excluded by using a threshold. Next, weighted T1rho and $T1_{GD}$ relaxation times were calculated using the reciprocal of the uncertainty of the estimated T1rho and $T1_{GD}$ relaxation time in each voxel (21). This uncertainty was measured by the square root of the Cramér-Rao Lower Bound, which gives a lower bound for the standard deviation of the estimated T1rho or $T1_{GD}$ (25-27). If after registration T1rho- and $T1_{GD}$-weighted images are not yet perfectly aligned, this might result in implausible T1rho and $T1_{GD}$ relaxation times especially at tissue boundaries. Using the weighted mean, these implausible T1rho and $T1_{GD}$ relaxation times will not heavily influence the results of the analyses (21). Finally, as proposed by Tiderius *et al.*, $T1_{GD}$ relaxation times were corrected for the participants' body mass index (BMI) (28).

The weighted T1rho and $T1_{GD}$ relaxation times for each anatomical cartilage ROI were averaged over the 15 consecutive MR images. Thus, for each patient six mean T1rho and $T1_{GD}$ relaxation times from six cartilage ROIs were obtained.

Harvesting of cartilage and biochemical cartilage analyses

During TKR, weight-bearing and non-weight-bearing femoral cartilage and weight-bearing tibial cartilage were harvested and stored in saline for 30 minutes to one hour before further processing in the laboratory. Depending on size of the specimen, four (posterior femoral cartilage), six or eight (weight-bearing femoral and plateau cartilage, number of explants depending on specimen size) full thickness cartilage explants of 6 mm diameter were taken using a biopsy punch, corresponding

with cartilage of the ROIs analyzed with dGEMRIC and T1rho-mapping. All explants were cut in halves and stored separately in airtight tubes at -20 °C.

Before biochemical analysis, explants were thawed at room temperature. One half was digested in a papain solution overnight and used to quantify sGAG content with the dimethylmethylene blue (DMMB) assay as described by Farndale *et al.* (29). The other half of each explant was not digested and used to quantify collagen content based on the hydroxyproline content as described by Bank *et al.* (30). This assay quantifies the degraded as well as the intact collagen content. The outcomes of both measures were summed together resulting in the total collagen content per explant. For each cartilage ROI, the mean sGAG or collagen content was calculated by adding up the sGAG or collagen content of each explant analyzed and dividing this by the numbers of explants taken from that specific ROI.

Statistical analysis

To assess the correlation between dGEMRIC or T1rho-mapping and reference tests (sGAG content and collagen content), a four-dimensional multivariate mixed-effects model was applied. In this model, it is assumed that dGEMRIC, T1rho-mapping, sGAG and collagen content are multivariately normally distributed (i.e. $Y \sim N_4(\mu,\Sigma)$, where Y = (dGEMRIC, T1rho-mapping, sGAG content, collagen content); μ and Σ are the mean vector (i.e. μ = (μ_1 = dGEMRIC, μ_2 = T1rho-mapping, μ_3 = sGAG content, μ_4 = collagen content)) and covariance matrix of these variables, respectively.

To take into account potential intrinsic correlation between outcomes of different ROIs within one participant, a random intercept was included in the model (e.g. $\mu_{1,i.j.} = \beta_1 + b_{1,I}$; i = 1, … , 12, j; j= 1, …, 63). We also included the participants' BMI as a predictor in our multivariate mixed-effects model. Pearson's correlation

coefficients of CTa and each reference test were extracted from the results of this model. These analyses were performed using a Bayesian approach with Markov chain Monte Carlo (McMC) sampling using WinBugs (31).

In Bayesian statistics a parameter is a stochastic variable and has a distribution. This distribution is called the posterior distribution, which is comprised of your a prior information (your believe) and the likelihood (data). Bayesian approaches can summarize the parameter uncertainty by giving a range of values on the posterior distribution that includes 95% of the probability. This is called a 95% credibility interval (CI). For all Pearson's correlation coefficients the 95% credible interval (95%CI) was calculated. To assess goodness-of-fit, we used an omnibus posterior predictive check (PPC) (32). We computed a Bayesian p-value; extreme values of this p-value (e.g., < 0:05 or > 0:95) indicate a poor fit of the model to the data (32). In our study, we employed relatively non-informative priors for the model parameters, i.e., a normal distribution with mean zero and a large variance for the fixed-effects parameters, an inverse-gamma with small shape and rate values for the variance of the random effects. We also used an inverse-Wishart with identity scale matrix and degree of freedom four for the covariance matrix of the four-dimensional multivariate normal distribution. Finally, to compare age and BMI distributions between male and female individuals, a Mann-Whitney U test was used.

Results

Participants

Fourteen patients participated in our study. Two participants were excluded because their TKR was postponed after inclusion. Therefore, 12 participants (6 women and 6 left knee joints) were analyzed. In one participant T1rho-mapping data

were unavailable since its acquisition failed. Three cartilage specimens (one posterior non weight-bearing cartilage specimen of the lateral femoral condyle and two weight-bearing cartilage specimens of the medial tibial plateau) were severely damaged during surgery and were excluded from the analysis.

Mean age of the participants was 63 (interquartile range 61 - 65) years and their mean body mass index was 32 (28 - 38) kg/m^2. For women the mean age was 64 (63 – 65) and the mean BMI was 33 (30 – 37), whereas for men the mean age was 62 (58 – 66) and the mean BMI was 32 (27 – 36). The p-values for age and BMI were 0.94 and 0.75 between women and men individuals, indicating that both distributions were similar for both sexes.

The KL grades in the medial tibiofemoral compartments were 3 or 4 in 7 participants and 1 or 2 in 4 participants. The KL grades in the lateral tibiofemoral compartments were 1 or 2 in 9 participants and 3 in 2 participants.

Correlation of dGEMRIC and T1rho-mapping with biochemical cartilage analyses

For the applied four-dimensional mixed-effects model, the Bayesian p-value of the PPC was 0.52, which indicates that the model assumptions appear to be satisfied. The effect of BMI as a predictor in our multivariate mixed-effects model was not significant.

$T1_{GD}$ relaxation times for all femoral and tibial cartilage ROIs correlated strongly with cartilage sGAG content measured using the DMMB assay (n=69, r=0.73, 95%CI 0.60 to 0.83; **Figure 2A**) and weakly with cartilage collagen content measured using the hydroxyproline assay (n=69, r=0.40, 95%CI 0.18 to 0.58; **Figure 2B**). When each ROI was analyzed separately, the correlation coefficients between

outcomes of dGEMRIC and sGAG content ranged from 0.70 to 0.80. For the correlation between dGEMRIC and collagen content, the range of correlation coefficients was 0.30 to 0.49.

T1rho relaxation times for all femoral and tibial cartilage ROIs did neither correlate with cartilage sGAG content (n=63, r=0.04, 95%CI -0.21 to 0.28; **Figure 3A**), nor with cartilage collagen content (n=63, r=-0.05, 95%CI -0.31 to 0.20; **Figure 3B**). A range of -0.07 to 0.06 was observed for the correlation coefficients between T1rho relaxation times and sGAG content for all separate cartilage ROIs in both knee compartments. This range was -0.18 to 0.10 for the correlation between T1rho-mapping and collagen content.

Figure 4 shows images representative for cartilage with relatively high and low sGAG content measured using dGEMRIC, T1rho-mapping, equilibrium partitioning of an ionic contrast agent using micro-CT (EPIC-μCT: visual representation of sGAG content) and histology (visual representation of sGAG content using Safranin-O staining). These images confirm the strong correlation between dGEMRIC and cartilage sGAG.

Discussion

Our results showed that outcomes of *in vivo* dGEMRIC in OA patients correlated strongly with cartilage sGAG content measured using the DMMB assay. This finding indicates that dGEMRIC acquired *in vivo* accurately measures sGAG content in OA patients. These results are consistent with previous research showing a strong correlation between $T1_{GD}$ relaxation times acquired *in vitro* and ex *vivo* in cadaveric animal cartilage post microfracture treatment and human OA cartilage (5, 33). Our results are also in agreement with the only *in vivo* validation study of dGEMRIC

283 performed by Watanabe *et al*. in 2006 reporting a strong correlation (r=0.82) between
284 outcomes of dGEMRIC after treatment of focal cartilage defects and cartilage sGAG
285 content measured using high-performance liquid chromatography in nine cartilage
286 explants (13).

287 We found a weak correlation between outcomes of dGEMRIC and the amount
288 of collagen in the articular cartilage (correlation with intact collagen content measured
289 using the hydroxyproline assay was comparable, no correlation between dGEMRIC
290 and degraded collagen content: data not shown). Despite the weak correlation, this
291 finding suggests that in addition to sGAG content, the integrity of cartilage
292 extracellular matrix also influences contrast influx into cartilage. Therefore,
293 dGEMRIC outcomes appear not only dependent on sGAG content, which was
294 recently also suggested by others (34, 35). The difference between the strength in
295 correlation between the outcomes of dGEMRIC and cartilage sGAG and collagen
296 content, however, suggests that sGAG is the composite that influences contrast
297 distribution throughout articular cartilage most.

298 We did not observe any correlation between T1rho relaxation times and
299 cartilage sGAG content. These results are surprising when compared to previous *in*
300 *vitro* and *ex vivo* research in which T1rho relaxation times correlated moderately to
301 strongly with sGAG amount in bovine and human cartilage (9, 15). Our results,
302 however, are more consistent with one of the two previous *in vivo* validation studies
303 of T1rho-mapping which showed only a weak correlation (r=0.44) between T1rho
304 relaxation times and sGAG content in the lateral tibial plateau cartilage of 20 OA
305 patients (14). A possible explanation for the difference in strength of reported
306 correlation values between *in vivo* and *in vitro* or *ex vivo* acquired T1rho-mapping and
307 cartilage sGAG content may be the differences in specific acquisition parameters. For

example, number and duration of TSLs, field of view and in-plane image matrix are usually different for *in vitro* or *ex vivo* (9-11) compared to *in vivo* experiments (7, 14, 15). Optimizing these parameters might improve the ability of T1rho-mapping to assess cartilage sGAG content, but will likely increase acquisition time. Moreover, the spin lock frequency (FSL) was usually higher *in vitro* or *ex vivo* (8, 10) compared to *in vivo* (14, 15). Higher FSL causes less B0 inhomogeneity, possibly improving accuracy of T1rho-mapping, but increased FSL is a limiting factor *in vivo* since it induces higher specific absorption rate (4). T1rho-mapping acquired with an FSL higher than 500 Hz has been described to be safe (8), but we applied a 500 Hz FSL since this is most commonly used *in vivo*, enabling us to compare our results with previous literature. Another option to improve T1rho-mapping would be to acquire a B0 map to correct for B0 inhomogeneity. Thus, different results may be obtained if the acquisition is optimized in future research.

T1rho relaxation times also did not correlate with cartilage collagen content (also no correlation with intact and degraded collagen content measured using the hydroxyproline assay: data not shown). While this finding is consistent with previous research in human cartilage after TKR (12), it suggests that T1rho-mapping measures other elements of cartilage, e.g. water content or a combination of composites of the cartilage extracellular matrix. .

The results of our study suggest that, despite the need of contrast agent and relatively long delay between contrast administration and MR acquisition, dGEMRIC can still be regarded a good method to quantify cartilage sGAG content in human knee OA. T1rho-mapping appears less suitable for this purpose. However, because of its ability to discriminate between healthy subjects, mild and moderate OA patients (36), relatively short acquisition time and the fact that T1rho-mapping does not

require a contrast agent, it may still be a valuable imaging biomarker in large clinical or population based OA research studies.

A potential limitation of our study is the use of OA patients undergoing TKR, whereas dGEMRIC and T1rho-mapping are advocated as imaging biomarkers in early stage OA (2, 3). However, in our opinion this is the only human study population that allows comparison of *in vivo* acquired imaging biomarkers against *ex vivo* references standards performed on cartilage specimens. To minimize the potential of bias we included patients with asymmetrical radiographic OA distribution who nevertheless were indicated for TKR. This way we were able to analyze cartilage with a relatively wide range in quality and sGAG content. Another limitation of the study is the relatively small sample size, which may cause the relatively wide 95% credible intervals we observed. Therefore, our results may not be completely generalizable and future studies with a larger sample size must be performed to reproduce our results. In our study, correlations between dGEMRIC or T1rho-mapping and cartilage composition were not influenced by BMI, but other patient characteristics might be assessed in future research.

Furthermore, it is important to note that the dGEMRIC sequence we used consisted of an IR SPGR protocol, while dGEMRIC can also be acquired using IR FSE or gradient echo sequences with variable flip angles or a Look-Locker method (4). Likewise, our T1rho-mapping protocol consisted of a 3D FSE pulse sequence while others may have used different approaches. Therefore, our results may not be directly generalizable to other research institutes applying other acquisition protocols for dGEMRIC and T1rho-mapping. However, the $T1_{GD}$ and T1rho relaxation times obtained in our study are within the same range as those reported by others using different dGEMRIC and T1rho-mapping protocols at 3T (4). Future research may

compare the outcomes of different protocols for dGEMRIC and T1rho-mapping in patients with knee OA. Such studies may also compare dGEMRIC and T1rho-mapping with other recently introduced biomarkers to measure cartilage sGAG content, e.g. gagCEST or sodium MRI (4).

In conclusion, our results show that dGEMRIC can accurately measure cartilage sGAG content *in vivo* in human knee OA subjects whereas T1rho-mapping appears not suitable for this purpose. Although the technique is not completely sGAG specific and requires a contrast agent, dGEMRIC is a validated and robust method for quantifying cartilage sGAG content in human OA subjects in clinical research.

Acknowledgements

We acknowledge Gavin Houston and Mika Vogel for assistance in implementing the MRI scan protocol. We also acknowledge Nicole Kops and Jessica Snabel for performing the ex vivo reference standards.

Role of funding source

This research project was supported in part by the Anna | NOREF foundation, Leiden, The Netherlands. The funding source had no role in the study design, collection, analysis or interpretation of data; in the writing of the manuscript or in the decision to submit the manuscript for publication.

Competing interests

G.P. Krestin is a consultant to Bracco SA. G.P. Krestin and E.H.G. Oei receive research support from General Electric Healthcare. The other authors have declared that no competing interests exist.

References

1. Litwic A, Edwards MH, Dennison EM, Cooper C. Epidemiology and burden of osteoarthritis. British medical bulletin. 2013;105:185-99.

2. Hunter DJ. Pharmacologic therapy for osteoarthritis--the era of disease modification. Nat Rev Rheumatol. 2011;7(1):13-22.

3. Neu CP. Functional imaging in OA: role of imaging in the evaluation of tissue biomechanics. Osteoarthritis Cartilage. 2014;22(10):1349-59.

4. Oei EH, van Tiel J, Robinson WH, Gold GE. Quantitative radiologic imaging techniques for articular cartilage composition: toward early diagnosis and development of disease-modifying therapeutics for osteoarthritis. Arthritis Care Res (Hoboken). 2014;66(8):1129-41.

5. Bashir A, Gray ML, Hartke J, Burstein D. Nondestructive imaging of human cartilage glycosaminoglycan concentration by MRI. Magn Reson Med. 1999;41(5):857-65.

6. Wang L, Regatte RR. T1rho MRI of human musculoskeletal system. J Magn Reson Imaging. 2015;41(3):586-600.

7. Regatte RR, Akella SV, Wheaton AJ, et al. 3D-T1rho-relaxation mapping of articular cartilage: in vivo assessment of early degenerative changes in symptomatic osteoarthritic subjects. Acad Radiol. 2004;11(7):741-9.

8. Li X, Han ET, Ma CB, Link TM, Newitt DC, Majumdar S. In vivo 3T spiral imaging based multi-slice T(1rho) mapping of knee cartilage in osteoarthritis. Magn Reson Med. 2005;54(4):929-36.

9. Duvvuri U, Kudchodkar S, Reddy R, Leigh JS. T(1rho) relaxation can assess longitudinal proteoglycan loss from articular cartilage in vitro. Osteoarthritis Cartilage. 2002;10(11):838-44.

10. Regatte RR, Akella SV, Borthakur A, Reddy R. Proton spin-lock ratio imaging for quantitation of glycosaminoglycans in articular cartilage. J Magn Reson Imaging. 2003;17(1):114-21.

11. Keenan KE, Besier TF, Pauly JM, et al. Prediction of glycosaminoglycan content in human cartilage by age, T1rho and T2 MRI. Osteoarthritis Cartilage. 2011;19(2):171-9.

12. Li X, Cheng J, Lin K, et al. Quantitative MRI using T1rho and T2 in human osteoarthritic cartilage specimens: correlation with biochemical measurements and histology. Magn Reson Imaging. 2011;29(3):324-34.

13. Watanabe A, Wada Y, Obata T, et al. Delayed gadolinium-enhanced MR to determine glycosaminoglycan concentration in reparative cartilage after autologous chondrocyte implantation: preliminary results. Radiology. 2006;239(1):201-8.

14. Wong CS, Yan CH, Gong NJ, Li T, Chan Q, Chu YC. Imaging biomarker with T1rho and T2 mappings in osteoarthritis - In vivo human articular cartilage study. Eur J Radiol. 2013;82(4):647-50.

15. Nishioka H, Hirose J, Nakamura E, et al. T1rho and T2 mapping reveal the in vivo extracellular matrix of articular cartilage. J Magn Reson Imaging. 2012;35(1):147-55.

16. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis. 1957;16(4):494-502.

Met opmaak: Engels (V.S.)

17. Chen W, Takahashi A, Han ET. 3D Quantitative Imaging of T1rho and T2. In: 19th, ed. International Society for Magnetic Resonance in Medicine: Annual Meeting & Exhibition. Montréal2011.

18. McKenzie CA, Williams A, Prasad PV, Burstein D. Three-dimensional delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) at 1.5T and 3.0T. J Magn Reson Imaging. 2006;24(4):928-33.

19. Tiderius CJ, Olsson LE, de Verdier H, Leander P, Ekberg O, Dahlberg L. Gd-DTPA2)-enhanced MRI of femoral knee cartilage: a dose-response study in healthy volunteers. Magn Reson Med. 2001;46(6):1067-71.

20. Tiderius CJ, Olsson LE, Leander P, Ekberg O, Dahlberg L. Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) in early knee osteoarthritis. Magn Reson Med. 2003;49(3):488-92.

21. Bron EE, van Tiel J, Smit H, et al. Image registration improves human knee cartilage T1 mapping with delayed gadolinium-enhanced MRI of cartilage (dGEMRIC). Eur Radiol. 2013;23(1):246-52.

22. van Tiel J, Bron EE, Tiderius CJ, et al. Reproducibility of 3D delayed gadolinium enhanced MRI of cartilage (dGEMRIC) of the knee at 3.0 T in patients with early stage osteoarthritis. Eur Radiol. 2013;23(2):496-504.

23. Klein S, van der Heide UA, Lips IM, van Vulpen M, Staring M, Pluim JP. Automatic segmentation of the prostate in 3D MR images by atlas matching using localized mutual information. Med Phys. 2008;35(4):1407-17.

24. Klein S, Staring M, Murphy K, Viergever MA, Pluim JP. elastix: a toolbox for intensity-based medical image registration. IEEE Trans Med Imaging. 2010;29(1):196-205.

453 25. Cavassila S, Deval S, Huegen C, van Ormondt D, Graveron-Demilly D.
454 Cramer-Rao bounds: an evaluation tool for quantitation. NMR Biomed.
455 2001;14(4):278-83.
456 26. Sijbers J, Den Dekker AJ, Raman E, Van Dyck D. Parameter estimation from
457 magnitude MR images. Int J Imag Syst Tech. 1999;10(2):109-14.
458 27. Rao CR. Minimum variance and the estimation of several parameters.
459 Cambridge Univ Press. 1946;43:280-3.
460 28. Tiderius C, Hori M, Williams A, et al. dGEMRIC as a function of BMI.
461 Osteoarthritis Cartilage. 2006;14(11):1091-7.
462 29. Farndale RW, Buttle DJ, Barrett AJ. Improved quantitation and discrimination
463 of sulphated glycosaminoglycans by use of dimethylmethylene blue. Biochim
464 Biophys Acta. 1986;883(2):173-7.
465 30. Bank RA, Krikken M, Beekman B, et al. A simplified measurement of
466 degraded collagen in tissues: application in healthy, fibrillated and osteoarthritic
467 cartilage. Matrix biology : journal of the International Society for Matrix Biology.
468 1997;16(5):233-43.
469 31. Lunn DJ, Thomas A, Best N, Spiegelhalter D. WinBUGS – A Bayesian
470 modelling framework: Concepts, structure, and extensibility. Statistics and
471 Computing. 2000;10:325-37.
472 32. Gelman A, Meng XL, Stern H. Posterior predictive assessment of model
473 fitness via realized discrepancies. Statistica Sinica. 1996;6:733-807.
474 33. Watanabe A, Boesch C, Anderson SE, Brehm W, Mainil Varlet P. Ability of
475 dGEMRIC and T2 mapping to evaluate cartilage repair after microfracture: a goat
476 study. Osteoarthritis Cartilage. 2009;17(10):1341-9.

34. Salo EN, Nissi MJ, Kulmala KA, Tiitu V, Toyras J, Nieminen MT. Diffusion of Gd-DTPA(2)(-) into articular cartilage. Osteoarthritis Cartilage. 2012;20(2):117-26.

35. van Tiel J, Kotek G, Reijman M, et al. Delayed gadolinium-enhanced MRI of the meniscus (dGEMRIM) in patients with knee osteoarthritis: relation with meniscal degeneration on conventional MRI, reproducibility, and correlation with dGEMRIC. Eur Radiol. 2014;24(9):2261-70.

36. Mosher TJ, Zhang Z, Reddy R, et al. Knee articular cartilage damage in osteoarthritis: analysis of MR image biomarker reproducibility in ACRIN-PA 4001 multicenter trial. Radiology. 2011;258(3):832-42.

Met opmaak: Engels (V.S.)

Figure captions

Figure 1: Regions of interest in dGEMRIC and T1rho-mapping

Representation of the three anatomical cartilage ROIs in which outcomes of dGEMRIC and T1rho-mapping were calculated in 15 consecutive slices in each compartment of the tibiofemoral joint (lateral side shown in this example). The posterior non-weight-bearing cartilage of the femoral condyle (pFC) is shown in red, the weight-bearing cartilage of the femoral condyle (wbFC) is shown in green and the weight-bearing cartilage of the tibial plateaus (wbTP) is shown in yellow.

Figure 2: Average regression line of dGEMRIC and *ex vivo* reference standards for sGAG and collagen content of articular cartilage

Average regression line of mean $T1_{GD}$ relaxation times in all anatomical ROIs with sGAG content of the cartilage measured and DMMB assay (A) and outcomes of dGEMRIC and collagen content of the cartilage measured with hydroxyproline assay (B). The dashed lines indicate the 95% confidence band of the average regression lines of the analyzed subjects in our study.

Figure 3: Average regression line of T1rho-mapping and *ex vivo* reference standards for sGAG and collagen content of articular cartilage

Average regression line of mean T1rho relaxation times in all anatomical ROIs with sGAG content of the cartilage measured and DMMB assay (A) and outcomes of T1rho-mapping and collagen content of the cartilage measured with hydroxyproline assay (B). The dashed lines indicate the 95% confidence band of the average regression lines of the analyzed subjects in our study.

Figure 4: Spatial agreement between MRI, EPIC-μCT and histology

Representative images of matching sagittal slides of dGEMRIC and T1rho-mapping, EPIC-μCT and histology. The relaxation time and/or attenuation of cartilage are visualized in color and representative for sGAG content. In dGEMRIC high $T1_{GD}$ represent high sGAG content and low $T1_{GD}$ represent low sGAG content. In T1rho mapping the opposite is true for T1rho relaxation times. In EPIC-μCT high attenuation represents a low sGAG content of cartilage and a low attenuation represents high sGAG content. In the Safranin-O staining used on the histological sections, the location and intensity of the redness is representative for cartilage sGAG distribution and content. A high intensity represents high sGAG content and a low intensity or discoloration represents a low or absent sGAG content. The top row shows visual agreement for dGEMRIC and disagreement for T1rho-mapping in relative high cartilage sGAG content and the bottom row shows the same for a relative low cartilage sGAG content in the superficial and partially mid zone of the cartilage. The example images are obtained form male subjects of which the relative low sGAG content subject was 66 years of age and the relative high sGAG content subject was55 years at the time of inclusion. Visual slice matching was performed for the histological slices and the microscopic magnification was 10 times.

534 **Tables and captions**

535 Table 1: MRI protocol parameters

Sequence	**High resolution SPGR**	**T1rho-mapping**	**dGEMRIC**
Plane	Sagittal	Sagittal	Sagittal
Imaging mode	3D	3D	3D
Sequence	SPGR	FSE	IR SPGR
Matrix (frequency)	512	288	288
Matrix (phase)	512	192	192
Number of slices	108	36	36
FOV (mm)	150	150	150
Slice thickness (mm)	1.0	3.0	3.0
TSL (ms)	NA	1 / 16 / 32 / 64 / 125	NA
FSL (Hz)	NA	500	NA
TI (ms)	NA	NA	2100 / 800 / 400 / 200 / 100
Flip angle (˚)	12	90	15
Bandwidth (Hz/pixel)	122	244	244
Number excitations averaged	0.75	0.5	1
Fat saturated	Yes	Yes	No
Acquisition time (min)	05:37	05:43	14:18

536 FOV: field of view; FSE: fast spin-echo; FSL: spin lock frequency;

IR SPGR: inversion recovery spoiled gradient-echo; NA: not applicable; SPGR: spoiled gradient-echo; TI: inversion time; TSL: spin lock time

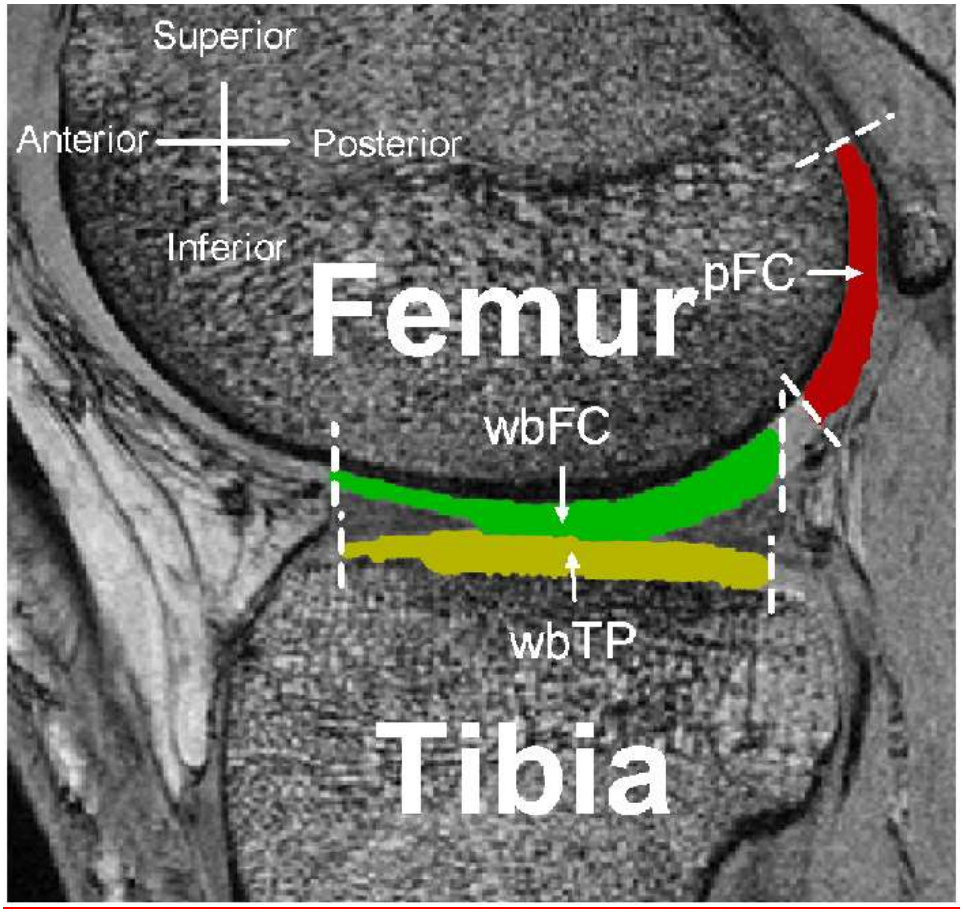

Superior
Anterior
Posterior
Inferior
Femur
pFC
wbFC
wbTP
Tibia

Fig.1

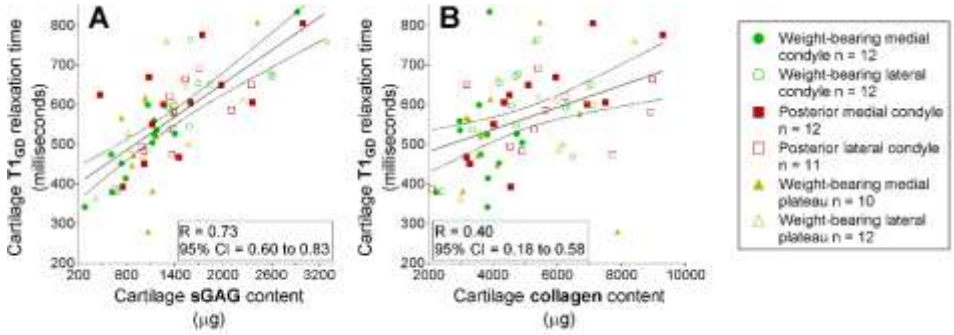
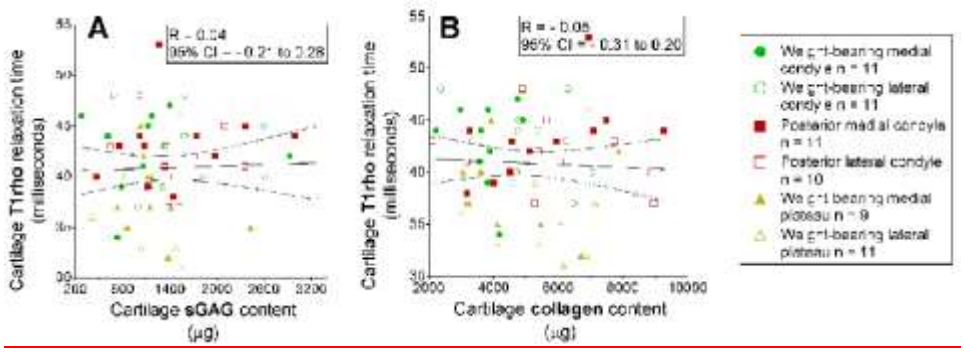

A
B
Cartilage T1Gd relaxation time (milliseconds)
800
700
600
500
400
300
200
R = 0.73
95% CI = 0.60 to 0.83
200
800
1400
2000
2600
3200
Cartilage sGAG content (µg)
R = 0.40
95% CI = 0.18 to 0.58
2000
4000
6000
8000
10000
Cartilage collagen content (µg)
Weight-bearing medial condyle n = 12
Weight-bearing lateral condyle n = 12
Posterior medial condyle n = 12
Posterior lateral condyle n = 11
Weight-bearing medial plateau n = 10
Weight-bearing lateral plateau n = 12

Fig.2



551

552 Fig. 3

553

554

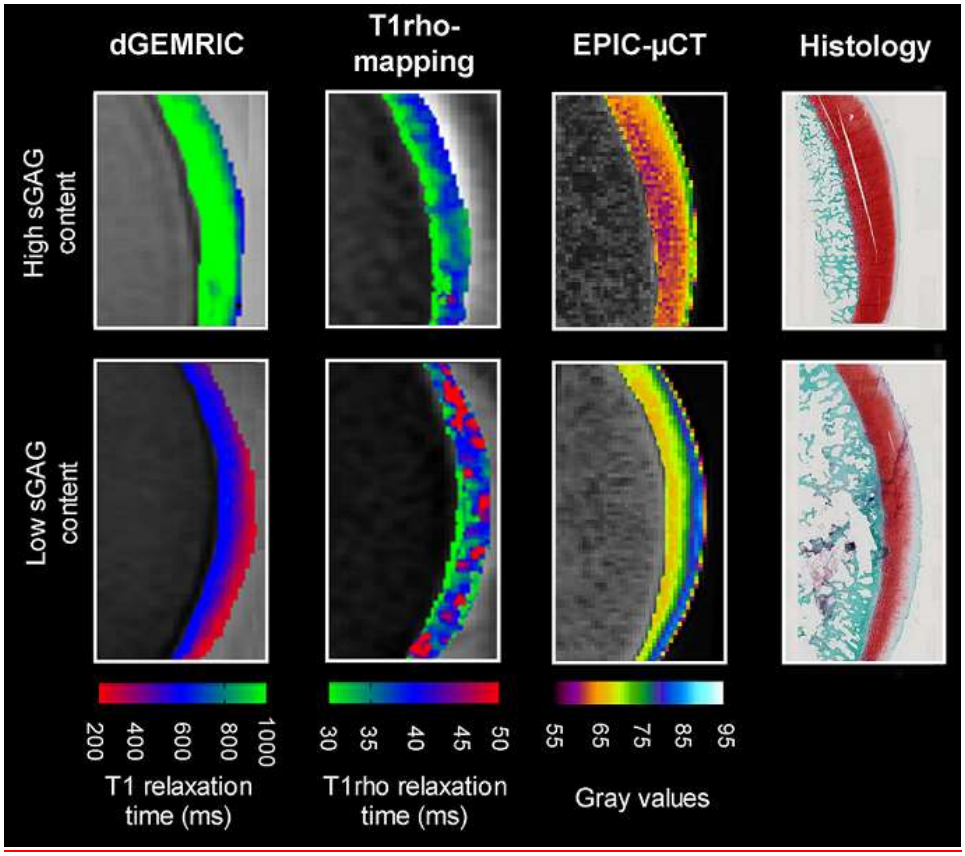

dGEMRIC
T1rho-mapping
EPIC-µCT
Histology
High sGAG content
Low sGAG content
200 400 600 800 1000
T1 relaxation time (ms)
30 35 40 45 50
T1rho relaxation time (ms)
55 65 75 85 95
Gray values

Fig.4