

# Is T1 $\rho$ Mapping an Alternative to Delayed Gadolinium-enhanced MR Imaging of Cartilage in the Assessment of Sulphated Glycosaminoglycan Content in Human Osteoarthritic Knees?

## An in Vivo Validation Study<sup>1</sup>

Jasper van Tiel, MD  
 Gyula Kotek, PhD  
 Max Reijman, PhD  
 Pieter K. Bos, MD, PhD  
 Esther E. Bron, MSc  
 Stefan Klein, PhD  
 Kazem Nasserinejad, MSc  
 Gerjo J. V. M. van Osch, PhD  
 Jan A. N. Verhaar, MD, PhD  
 Gabriel P. Krestin, MD, PhD  
 Harrie Weinans, PhD  
 Edwin H. G. Oei, MD, PhD

<sup>1</sup>From the Departments of Radiology (J.v.T., G.K., E.E.B., S.K., G.P.K., E.H.G.O.), Orthopedic Surgery (J.v.T., M.R., P.K.B., G.J.V.M.v.O., J.A.N.V.), Medical Informatics (E.E.B., S.K.), Biostatistics (K.N.), and Otorhinolaryngology (G.J.V.M.v.O.), Erasmus University Medical Center, PO Box 2040, 3000 CA Rotterdam, the Netherlands; Department of Biomechanical Engineering, Delft University of Technology, Delft, the Netherlands (H.W.); and Department of Orthopedics and Rheumatology, University Medical Center Utrecht, Utrecht, the Netherlands (H.W.). Received March 22, 2015; revision requested April 27; revision received July 19; accepted August 12; final version accepted September 3. Supported in part by the Anna-NOREF Foundation, Leiden, the Netherlands. **Address correspondence to** E.H.G.O. (e-mail: [e.oei@erasmusmc.nl](mailto:e.oei@erasmusmc.nl)).

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### Purpose:

To determine if T1 $\rho$  mapping can be used as an alternative to delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC) in the quantification of cartilage biochemical composition in vivo in human knees with osteoarthritis.

### Materials and Methods:

This study was approved by the institutional review board. Written informed consent was obtained from all participants. Twelve patients with knee osteoarthritis underwent dGEMRIC and T1 $\rho$  mapping at 3.0 T before undergoing total knee replacement. Outcomes of dGEMRIC and T1 $\rho$  mapping were calculated in six cartilage regions of interest. Femoral and tibial cartilages were harvested during total knee replacement. Cartilage sulphated glycosaminoglycan (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 and T1 $\rho$  mapping and the sGAG and collagen content of the articular cartilage.

### Results:

T1 relaxation times at dGEMRIC showed strong correlation with cartilage sGAG content ( $r = 0.73$ ; 95% credibility interval [CI] = 0.60, 0.83) and weak correlation with cartilage collagen content ( $r = 0.40$ ; 95% CI: 0.18, 0.58). T1 $\rho$  relaxation times did not correlate with cartilage sGAG content ( $r = 0.04$ ; 95% CI: -0.21, 0.28) or collagen content ( $r = -0.05$ ; 95% CI = -0.31, 0.20).

### Conclusion:

dGEMRIC can help accurately measure cartilage sGAG content in vivo in patients with knee osteoarthritis, whereas T1 $\rho$  mapping does not appear 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 osteoarthritis subjects in clinical research.

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**K**nee osteoarthritis is the most common joint disease in middle-aged and elderly subjects, causing serious morbidity and having a large socioeconomic impact (1). Because no definitive treatment options other than joint replacement surgery in end-stage osteoarthritis are available, research mainly focuses on novel interventions such as disease-modifying osteoarthritis drugs. These should be effective in the early stages of osteoarthritis 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 osteoarthritis, accurate in vivo imaging biomarkers are essential. Therefore, quantitative biomarkers that measure cartilage biochemical composites, for

example, sulphated glycosaminoglycan (sGAG) content, have become of interest during the past decade (4).

An example of such a quantitative imaging biomarker for measuring articular cartilage sGAG content is delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC) (5). This technique uses the inverse relationship 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, it does have disadvantages. These disadvantages are mainly related to the administration of contrast material, which increases costs and is potentially harmful to patients with impaired renal function, and the long delay between contrast material administration and MR imaging. Because of these drawbacks, T1 $\rho$  mapping was suggested as a non-contrast-enhanced alternative to dGEMRIC for the measurement of sGAG content (3,4,6). In T1 $\rho$  mapping, the spin relaxation is quantified 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 T1 $\rho$  mapping are increasingly used as outcome measures for cartilage biochemical composition in clinical osteoarthritis research, they have been validated mainly in vitro (9,10) or ex vivo (11,12) by using bovine and cadaveric human cartilage. In vivo validation was performed in only one study of dGEMRIC (13) and only two studies of T1 $\rho$  mapping (14,15). Furthermore, to our knowledge, no investigators have applied both imaging biomarkers in humans in vivo and compared the outcomes with a standard of reference for cartilage sGAG content to validate and

compare their performance. Finally, the influence of the cartilage extracellular matrix integrity, which is mainly provided by the collagen network, has not yet been studied in detail for dGEMRIC and T1 $\rho$  mapping.

The aim of our study was to determine if T1 $\rho$  mapping can be used as an alternative to dGEMRIC for quantifying cartilage biochemical composition in vivo in human knees with osteoarthritis.

### Advances in Knowledge

- The T1 relaxation times after delayed gadolinium-enhanced MR imaging of cartilage (dGEMRIC) at 3.0 T show strong correlation with cartilage sulphated glycosaminoglycan (sGAG) content measured with dimethylmethylene blue assay ( $r = 0.73$ ; 95% credibility interval [CI]: 0.60, 0.83) and weak correlation with cartilage collagen content measured with hydroxyproline assay ( $r = 0.40$ ; 95% CI: 0.18, 0.58).
- Although sGAG is the most important component of cartilage that influences contrast material 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.
- T1 $\rho$  relaxation times at 3.0 T do not show correlation with either cartilage sGAG content or cartilage collagen content.
- T1 $\rho$  mapping cannot be regarded as an alternative for dGEMRIC in the measurement of cartilage sGAG content in clinical osteoarthritis research.

### Implication for Patient Care

- dGEMRIC is a validated and robust method for quantifying sGAG content in human osteoarthritis subjects.

### Materials and Methods

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### Study Design and Participants

For our prospective observational study, which was conducted between October 2012 and December 2013, all consecutive patients scheduled for total knee replacement at our institution were approached. Our study was approved by the Medical Ethical Committee of

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### Abbreviations:

BMI = body mass index  
CI = credibility interval  
dGEMRIC = delayed gadolinium-enhanced MR imaging of cartilage  
ROI = region of interest  
sGAG = sulphated glycosaminoglycan  
SPGR = spoiled gradient echo  
3D = three-dimensional

### Author contributions:

Guarantor of integrity of entire study, J.v.T.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; manuscript final version approval, all authors; agrees to ensure any questions related to the work are appropriately resolved, all authors; literature research, J.v.T., E.H.G.O.; clinical studies, J.v.T., G.K., P.K.B., E.H.G.O.; experimental studies, J.v.T., G.K., E.E.B., S.K., G.J.V.M.v.O.; statistical analysis, J.v.T., M.R., K.N.; and manuscript editing, all authors

Conflicts of interest are listed at the end of this article.

Erasmus MC (MEC-2012-218), and written informed consent was obtained from all participants.

The inclusion criteria were as follows: age of at least 18 years and radiographic knee osteoarthritis with asymmetric distribution and a maximum Kellgren-Lawrence grade of 1–2 (doubtful or definite osteophyte formation without definite joint space narrowing) (16) in the least affected tibiofemoral compartment. Exclusion criteria were as follows: renal insufficiency (glomerular filtration rate, <60 mL/min), history of previous reactions to contrast material, or significant comorbidities in the ipsilateral lower extremity (eg, severe hip osteoarthritis, neurologic or muscular diseases causing hip or knee disability), which prohibit exercising after administration of contrast material for dGEMRIC.

### MR Image Acquisition

MR imaging was performed 1 day before patients underwent total knee replacement by using a 3.0-T MR unit (Discovery MR750; GE Healthcare, Milwaukee, Wis) and a dedicated eight-channel knee coil (Invivo, Gainesville, Fla).

The MR imaging protocol included the following three pulse sequences, all of which were performed in the sagittal plane: (a) a three-dimensional (3D) high-spatial-resolution fat-saturated spoiled gradient-echo (SPGR) sequence, (b) a 3D fast spin-echo T1 $\rho$  mapping sequence with five different spin-lock times (17), and (c) a 3D inversion-recovery non-fat-saturated SPGR sequence with five different inversion times for dGEMRIC (18). Specific imaging parameters are shown in the Table.

Before dGEMRIC, a double dose (0.2 mmol per kilogram of body weight) of gadopentetate dimeglumine (Magnevist; Bayer Schering, Berlin, Germany) was injected intravenously as advocated previously (19). Subsequently, participants cycled for 10 minutes on a stationary bike at a constant speed to promote contrast material distribution into and throughout the knee. After a delay of 90 minutes, the inversion-recovery SPGR sequence was performed (20).

### MR Imaging Parameters

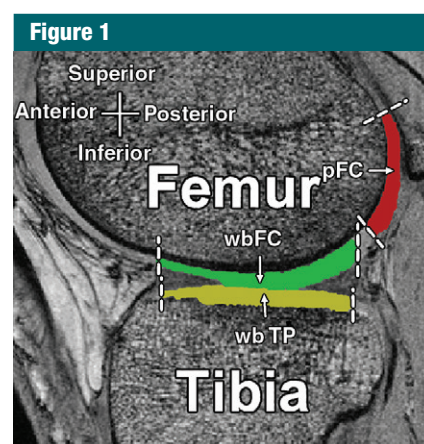
Parameter	High-Spatial-Resolution SPGR Imaging	T1 $\rho$ Mapping	dGEMRIC
Plane	Sagittal	Sagittal	Sagittal
Imaging mode	3D	3D	3D
Sequence	SPGR	FSE	IR SPGR
Matrix			
Frequency	512	288	288
Phase	512	192	192
No. of sections	108	36	36
Field of view (mm)	150	150	150
Section thickness (mm)	1.0	3.0	3.0
Spin-lock time (msec)	NA	1/16/32/64/125	NA
Spin-lock frequency (Hz)	NA	500	NA
Inversion time (msec)	NA	NA	2100/800/400/200/100
Flip angle (degrees)	12	90	15
Bandwidth (Hz/pixel)	122	244	244
No. of signals averaged	0.75	0.5	1
Fat saturation	Yes	Yes	No
Acquisition time	5 min 37 sec	5 min 43 sec	14 min 18 sec

Note.—IR = inversion recovery, FSE = fast spin echo, NA = not applicable.

### MR Image Analysis

With use of Matlab (R2011a; The MathWorks, Natick, Mass), we drew three cartilage regions of interest (ROIs) in both tibiofemoral compartments: the weight-bearing cartilage of the femoral condyles, posterior non-weight-bearing cartilage of the femoral condyles, and weight-bearing cartilage of the tibial plateaus (Fig 1). All ROIs consisted of 15 consecutive sections—the most central section through the medial or lateral tibiofemoral compartment (defined as the sagittal section depicting the most caudal point of the femoral condyle identified with multiplanar reconstructions of the 3D high-spatial-resolution fat-saturated SPGR sequence) along with the neighboring seven sections medially and laterally. All ROIs were drawn on the high-spatial-resolution SPGR images by a researcher with a medical degree and 4 years of experience in musculoskeletal research (J.v.T).

Image analysis was performed with a registration and T1-fitting algorithm developed in-house (Software for Post-processing and Registration of Cartilage of the Knee) (21,22). The image analysis pipeline included registration to correct



**Figure 1:** ROIs used with dGEMRIC and T1 $\rho$  mapping. High-spatial-resolution SPGR image shows three anatomic cartilage ROIs in which outcomes of dGEMRIC and T1 $\rho$  mapping were calculated in 15 consecutive sections in each compartment of tibiofemoral joint (lateral side shown in this example). Posterior non-weight-bearing cartilage of femoral condyle (pFC) is shown in red, weight-bearing cartilage of femoral condyle (wbFC) is shown in green, and weight-bearing cartilage of tibial plateaus (wbTP) is shown in yellow.

for patient motion and fitting of T1 relaxation times with dGEMRIC and T1 $\rho$  relaxation times. First, all images from

T1 $\rho$  mapping and dGEMRIC with different spin-lock times and inversion times were registered to images with a spin-lock time of 1 msec and inversion time of 2100 msec. The femoral condyle and tibial plateau were registered separately. The images were registered by using a 3D rigid transformation model by means of maximization of localized mutual information (23). Cubic interpolation was used to minimize the blurring of the registered images. All registrations were performed by using open-source registration software (Elastix, <http://elastix.isi.uu.nl/>) (24). Second, datasets from both registered T1 $\rho$  mapping and dGEMRIC were registered to the high-spatial-resolution SPGR images. This registration was based on images with a spin-lock time of 1 msec and inversion time of 2100 msec; images with other spin-lock times and inversion times were transformed accordingly. This second registration step allows analysis of matching cartilage ROIs on matching sections from both sequences.

After registration, T1 $\rho$  maps and T1 maps with dGEMRIC were estimated by 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 T1 relaxation times with T1 $\rho$  mapping and dGEMRIC were calculated by using the reciprocal of the uncertainty of the estimated T1 relaxation time with each technique in each voxel (21). This uncertainty was measured with the square root of the Cramér-Rao lower bound, which gives a lower bound for the standard deviation of the estimated T1 relaxation time with T1 $\rho$  mapping or dGEMRIC (25–27). Images from T1 $\rho$  mapping and dGEMRIC that are not perfectly aligned after registration might result in implausible T1 relaxation times, especially at tissue boundaries. With use of the weighted mean, these implausible relaxation times will not heavily influence the results of the analyses (21). Finally, as proposed by Tiderius et al (28), T1 relaxation times at dGEMRIC were corrected for the participants' body mass index (BMI).

The weighted T1 relaxation times at T1 $\rho$  mapping and dGEMRIC for each anatomic cartilage ROI were averaged over the 15 consecutive MR images. Thus, for each patient, six mean T1 relaxation times from six cartilage ROIs were obtained with T1 $\rho$  and dGEMRIC.

### Harvesting of Cartilage and Biochemical Cartilage Analyses

During total knee replacement, weight-bearing and non-weight-bearing femoral cartilage and weight-bearing tibial cartilage were harvested and stored in saline for 30 minutes to 1 hour before further processing in the laboratory. Four full-thickness cartilage explants measuring 6 mm in diameter were obtained from posterior femoral cartilage and six or eight were obtained from weight-bearing femoral and plateau cartilage (with the number of explants dependent on specimen size) with use of a biopsy punch. The explants corresponded to cartilage of the ROIs analyzed with dGEMRIC and T1 $\rho$  mapping. All explants were cut in half and stored separately in airtight tubes at  $-20^{\circ}\text{C}$ .

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 assay, as described by Farn-dale et al (29). The other half of each explant was not digested and was used to quantify collagen content on the basis of 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 number of explants taken from that specific ROI.

### Statistical Analysis

To assess the correlation between dGEMRIC or T1 $\rho$  mapping and reference tests (sGAG content and collagen content), a four-dimensional multivariate mixed-effects model was applied. In

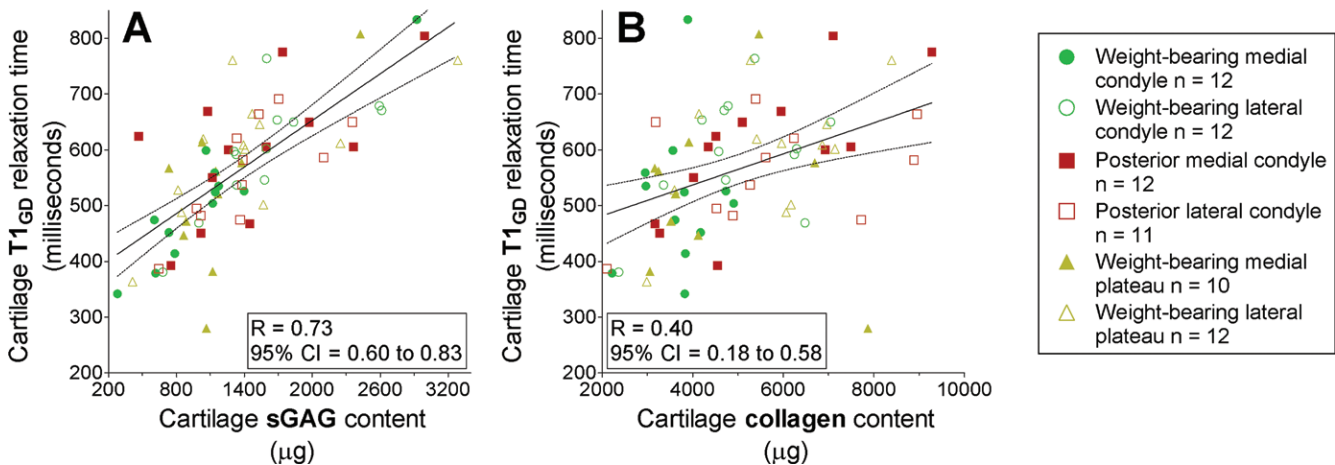
this model, it is assumed that dGEMRIC, T1 $\rho$  mapping, and sGAG and collagen content are multivariately normally distributed (ie,  $Y \sim N_4[\mu, \Sigma]$ , where  $Y = \text{dGEMRIC, T1}\rho \text{ mapping, sGAG content, or collagen content}$  and  $\mu$  and  $\Sigma$  are the mean vector [ie,  $\mu_1 = \text{dGEMRIC, } \mu_2 = \text{T1}\rho \text{ mapping, } \mu_3 = \text{sGAG content, } \mu_4 = \text{collagen content}$ ] 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 (eg,  $\mu_{i,j} = \beta_i + b_{1,i}$ ;  $i = 1, \dots, 12$ ;  $j = 1, \dots, 63$ ). We also included the participant's BMI as a predictor in our multivariate mixed-effects model. Pearson correlation coefficients of dGEMRIC and T1 $\rho$  mapping and each reference test were extracted from the results of this model. These analyses were performed by using a Bayesian approach with Markov chain Monte Carlo sampling with use of software (WinBUGS; MRC Biostatistics Unit, Cambridge Institute of Public Health, Cambridge, England; <http://www.mrc-bsu.cam.ac.uk/software/bugs/>) (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 prior information (your belief) 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 the 95% credibility interval (CI). The 95% CI was calculated for all Pearson correlation coefficients. To assess goodness of fit, we used an omnibus posterior predictive check (32). We computed a Bayesian  $P$  value; extreme  $P$  values (eg,  $<.05$  or  $>.95$ ) were indicative of a poor fit of the model to the data (32). In our study, we used relatively noninformative priors for the model parameters, that is, a normal distribution with a mean of zero and a large variance for the fixed-effects parameters, and an inverse gamma distribution with small shape and rate values for the variance of the random effects. We also



Figure 2



**Figure 2:** Average regression line of dGEMRIC and ex vivo reference standards for sGAG and collagen content of articular cartilage. Scatterplots show relationship between mean T1 relaxation times at dGEMRIC in all anatomic ROIs and, *A*, sGAG content of cartilage measured with dimethylmethylene blue assay and, *B*, collagen content of cartilage measured with hydroxyproline assay. Dashed lines indicate 95% confidence band of average regression lines.

used an inverse Wishart distribution with an identity scale matrix and 4 *df* for the covariance matrix of the four-dimensional multivariate normal distribution. Finally, the Mann-Whitney *U* test was used to compare age and BMI distributions between male and female patients.

## Results

### Participants

Fourteen patients participated in our study. Two patients were excluded because their total knee replacement was postponed after inclusion. Therefore, 12 patients (six men, six women; six left and six right knee joints) were analyzed. T1 $\rho$  mapping data were not be obtained in one patient because 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 excluded from the analysis.

The mean patient age was 63 years (interquartile range = 61–65 years), and the mean BMI was 32 kg/m<sup>2</sup> (interquartile range = 28–38 kg/m<sup>2</sup>). Women had

a mean age of 64 years (interquartile range = 63–65 years) and a mean BMI of 33 kg/m<sup>2</sup> (interquartile range = 30–37 kg/m<sup>2</sup>). Men had a mean age of 62 years (interquartile range = 58–66 years) and a mean BMI of 32 kg/m<sup>2</sup> (interquartile range = 27–36 kg/m<sup>2</sup>). *P* values for age and BMI were .94 and .75, respectively, between women and men individuals, which indicates that both distributions were similar for both sexes.

The Kellgren-Lawrence grades in the medial tibiofemoral compartments were 3 or 4 in seven patients and 1 or 2 in four. The Kellgren-Lawrence grades in the lateral tibiofemoral compartments were 1 or 2 in nine patients and 3 in two.

### Correlation of dGEMRIC and T1 $\rho$ Mapping with Biochemical Cartilage Analyses

For the applied four-dimensional mixed-effects model, the Bayesian *P* value of the omnibus posterior predictive check was .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.

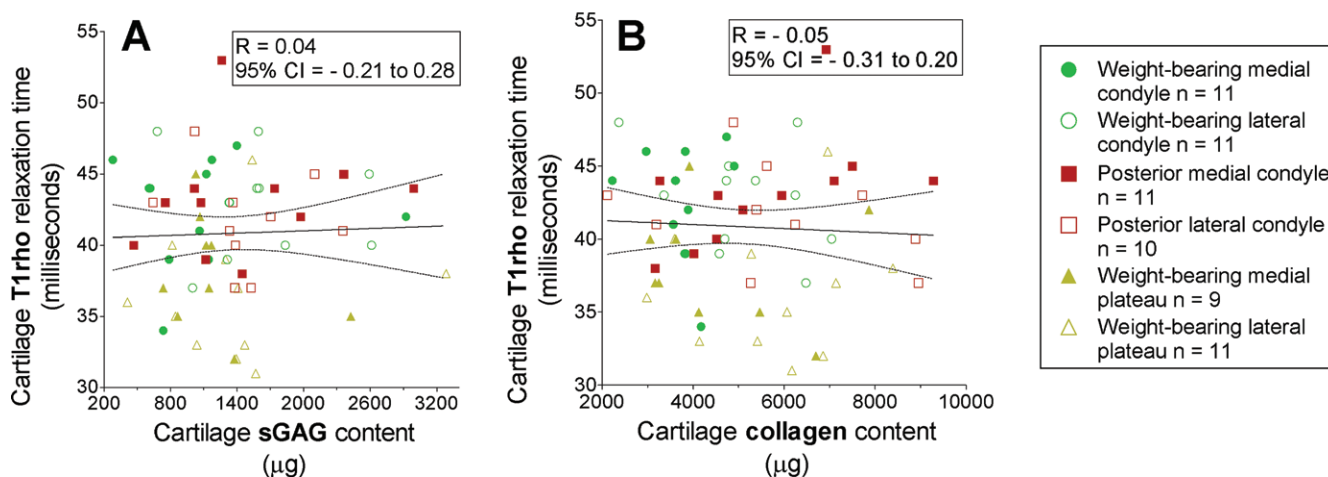
The T1 relaxation times at dGEMRIC for all femoral and tibial cartilage ROIs showed strong correlation with cartilage sGAG content measured with the

dimethylmethylene blue assay (*n* = 69; *r* = 0.73; 95% CI: 0.60, 0.83; Fig 2, *A*) and weak correlation with cartilage collagen content measured with the hydroxyproline assay (*n* = 69; *r* = 0.40; 95% CI: 0.18, 0.58; Fig 2, *B*). 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 correlation coefficients ranged from 0.30 to 0.49.

T1 $\rho$  relaxation times for all femoral and tibial cartilage ROIs did not show correlation with either cartilage sGAG content (*n* = 63; *r* = 0.04; 95% CI: −0.21, 0.28; Fig 3, *A*) or cartilage collagen content (*n* = 63; *r* = −0.05; 95% CI: −0.31, 0.20; Fig 3, *B*). A range of −0.07 to 0.06 was observed for the correlation coefficients between T1 $\rho$  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 T1 $\rho$  mapping and collagen content.

Figure 4 shows representative images of cartilage, with relatively high and low sGAG content measured with dGEMRIC, T1 $\rho$  mapping, equilibrium partitioning of an ionic contrast material by using micro-computed

Figure 3



**Figure 3:** Average regression line of T1ρ mapping and ex vivo reference standards for sGAG and collagen content of articular cartilage. Scatterplots show relationship between mean T1ρ relaxation times in all anatomic ROIs and, *A*, sGAG content of cartilage measured with dimethylmethylene blue assay and, *B*, collagen content of cartilage measured with hydroxyproline assay. Dashed lines indicate 95% confidence band of average regression lines.

tomography (CT) (visual representation of sGAG content), and histologic examination (visual representation of sGAG content with use of safranin O staining). These images confirm the strong correlation between dGEMRIC and cartilage sGAG.

## Discussion

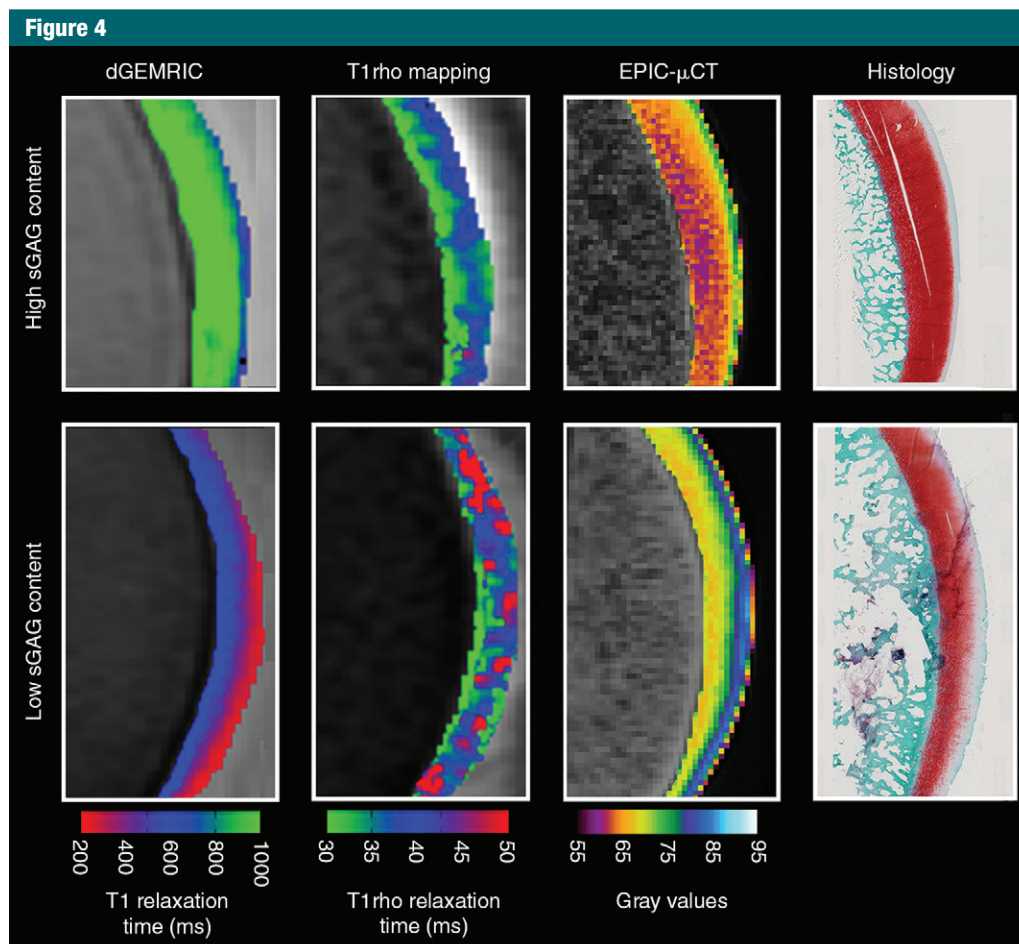
The results of our study showed that outcomes of in vivo dGEMRIC in patients with osteoarthritis showed a strong correlation with cartilage sGAG content measured with dimethylmethylene blue assay. This finding indicates that dGEMRIC performed in vivo enables accurate measurement of sGAG content in patients with osteoarthritis. These results are consistent with those from previous research showing a strong correlation between T1 relaxation times at dGEMRIC acquired in vitro and ex vivo in cadaveric animal cartilage after microfracture treatment and human osteoarthritis cartilage (5,33). Our results are also in agreement with those of the only in vivo validation study of dGEMRIC, which was performed by Watanabe et al (13) in 2006 and found a strong correlation ( $r = 0.82$ ) between outcomes of dGEMRIC after treatment of focal cartilage defects and cartilage

sGAG content measured with high-performance liquid chromatography in nine cartilage explants.

We found a weak correlation between outcomes of dGEMRIC and the amount of collagen in the articular cartilage (correlation with intact collagen content measured with the hydroxyproline assay was comparable; there was no correlation between dGEMRIC and degraded collagen content [data not shown]). Despite the weak correlation, this finding suggests that, in addition to sGAG content, the integrity of cartilage extracellular matrix also has an influence on contrast material influx into cartilage. Therefore, dGEMRIC outcomes appear not to be dependent on sGAG content alone, which was recently also suggested by others (34,35). The difference between the strength in correlation between the outcomes of dGEMRIC and cartilage sGAG and collagen content, however, suggests that sGAG is the composite that has the most influence on contrast material distribution throughout the articular cartilage.

We did not observe any correlation between T1ρ relaxation times and cartilage sGAG content. These results are surprising when compared with those from previous in vitro and ex vivo research in which T1ρ relaxation

times showed a modest to strong correlation with sGAG amount in bovine and human cartilage (9,15). Our results, however, are more consistent with those from one of the two previous in vivo validation studies of T1ρ mapping, which showed only a weak correlation ( $r = 0.44$ ) between T1ρ relaxation times and sGAG content in the lateral tibial plateau cartilage of 20 patients with osteoarthritis (14). A possible explanation for the difference in the strength of reported correlation values between in vivo and in vitro or ex vivo acquired T1ρ maps and cartilage sGAG content may be the differences in specific acquisition parameters. For example, the number and duration of spin-lock times, field of view, and in-plane image matrix are usually different for in vitro or ex vivo experiments (9–11) compared with in vivo experiments (7,14,15). Optimization of these parameters might improve the ability of T1ρ mapping to help assess cartilage sGAG content but will likely increase the acquisition time. Moreover, the spin-lock frequency was usually higher in vitro and ex vivo (8,10) compared with in vivo (14,15). A higher spin-lock frequency causes less B0 inhomogeneity, which may improve the accuracy of T1ρ mapping; however, increased spin-lock frequency is a limiting factor



**Figure 4:** Spatial agreement between dGEMRIC, T1 $\rho$  mapping, equilibrium partitioning of an ionic contrast material by using micro-CT (*EPIC- $\mu$ CT*), and histologic examination. Representative images are matching sagittal sections from dGEMRIC, T1 $\rho$  mapping, equilibrium partitioning of an ionic contrast material by using micro-CT, and histologic examination (safranin O stain; original magnification,  $\times 10$ ). Relaxation time and/or attenuation of cartilage are visualized in color and are representative of sGAG content. In dGEMRIC, a high T1 relaxation time represents high sGAG content and a low T1 relaxation time represents low sGAG content. The opposite is true for T1 $\rho$  relaxation times. In equilibrium partitioning of an ionic contrast material by using micro-CT, high attenuation represents low sGAG content and low attenuation represents high sGAG content. On photomicrographs obtained with safranin O staining, the location and intensity of redness is representative of cartilage sGAG distribution and content. High intensity represents high sGAG content, and low intensity or discoloration represents low or absent sGAG content. Top row shows visual agreement for dGEMRIC and disagreement for T1 $\rho$  mapping with regard to relative high cartilage sGAG content, and bottom row shows the same for a relatively low cartilage sGAG content in superficial and partially middle zone of cartilage. Images were obtained from male subjects. The subject with relatively low sGAG content was 66 years of age at study inclusion, and the subject with relatively high sGAG content was 55 years. Visual section matching was performed for the histologic slices.

in vivo because it induces a higher specific absorption rate (4). T1 $\rho$  mapping performed with a spin-lock frequency higher than 500 Hz has been described as safe (8); however, we applied a 500-Hz spin-lock frequency because this is most commonly used in vivo, enabling us to compare our results with those

from the previous literature. Another option to improve T1 $\rho$  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.

T1 $\rho$  relaxation times also did not correlate with cartilage collagen content

(they also did not show correlation with intact and degraded collagen content measured with the hydroxyproline assay [data not shown]). Although this finding is consistent with those from previous research in human cartilage after total knee replacement (12), it suggests that T1 $\rho$  mapping measures other elements

of cartilage, for example, water content or a combination of composites of the cartilage extracellular matrix.

The results of our study suggest that, despite the need for contrast material and the relatively long delay between contrast material administration and MR image acquisition, dGEMRIC can still be regarded as a good method for quantifying cartilage sGAG content in human knee osteoarthritis. T1 $\rho$  mapping appears less suitable for this purpose. However, because of its ability to enable differentiation between healthy subjects and patients with mild to moderate osteoarthritis (36), its relatively short acquisition time, and the fact that T1 $\rho$  mapping does not require contrast material, it may still be a valuable imaging biomarker in large clinical or population-based osteoarthritis research studies.

A potential limitation of our study is the use of patients with osteoarthritis who are undergoing total knee replacement; dGEMRIC and T1 $\rho$  mapping are advocated as imaging biomarkers in early-stage osteoarthritis (2,3). In our opinion, however, this is the only human study population that allows comparison of in vivo acquired imaging biomarkers against ex vivo reference standards obtained from cartilage specimens. To minimize the potential of bias, we included patients with an asymmetric radiographic osteoarthritis distribution who nevertheless were indicated for total knee replacement. 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 have caused the relatively wide 95% CIs 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 T1 $\rho$  mapping and cartilage composition were not influenced by BMI; however, other patient characteristics might be assessed in future research.

Furthermore, it is important to note that the dGEMRIC sequence we used consisted of an inversion-recovery SPGR protocol, whereas dGEMRIC can also be

performed with inversion-recovery fast spin-echo or gradient-echo sequences with variable flip angles or a Look-Locker method (4). Likewise, our T1 $\rho$  mapping protocol consisted of a 3D fast spin-echo pulse sequence; others may have used different approaches. Therefore, our results may not be directly generalizable to other research institutes that apply different acquisition protocols for dGEMRIC and T1 $\rho$  mapping. However, the T1 relaxation times obtained in our study with dGEMRIC and T1 $\rho$  mapping are within the same range as those reported by others using different dGEMRIC and T1 $\rho$  mapping protocols at 3.0 T (4). Future research may compare the outcomes of different protocols for dGEMRIC and T1 $\rho$  mapping in patients with knee osteoarthritis. Such studies may also compare dGEMRIC and T1 $\rho$  mapping with other recently introduced biomarkers to measure cartilage sGAG content (eg, glycosaminoglycan chemical exchange saturation transfer or sodium MR imaging) (4).

In conclusion, our results showed that dGEMRIC can enable accurate measurement of cartilage sGAG content in vivo in human subjects with knee osteoarthritis, whereas T1 $\rho$  mapping does not appear to be suitable for this purpose. Although the technique is not completely sGAG specific and requires contrast material, dGEMRIC is a validated and robust method for quantifying cartilage sGAG content in human osteoarthritis subjects in clinical research.

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