Introduction

Osteoarthritis (OA) of the knee has high socioeconomic impact1,2, is a main source of pain and might eventually end in endoprosthesis3-5. Since untreated injuries of the articular cartilage in young patients may result in premature OA6, early detection of cartilage damage is paramount to prevent further progression. Two main processes within the hyaline cartilage are considered an early event in degeneration leading to OA: the loss of glycosaminoglycans (GAG) and the disturbance of the 3D collagen network7.

Several biochemical or "compositional"8,9 magnetic resonance imaging (MRI) techniques have been proposed to assess these processes, such as delayed gadolinium-enhanced MRI of cartilage (dGEMRIC), 23Na MRI, and T1 rho mapping to assess the GAG processes, such as delayed gadolinium-enhanced MRI of cartilage (dGEMRIC), 23Na MRI, and T1 rho mapping to assess the GAG content and T2 mapping to evaluate the water content and damages to the collagen network. A new promising technique is CEST (chemical exchange saturation transfer) without the need of gadolinium-enhanced MRI of cartilage (dGEMRIC) and T2 mapping to assess the biochemical cartilage properties of the knee.

Method: Sixty-nine subjects were prospectively included (median age, 42 years; male/female = 32/37) in three cohorts: 10 healthy volunteers, 40 patients with clinically suspected cartilage lesions, and 19 patients about 1 year after microfracture therapy. T2 mapping, dGEMRIC, and CEST were performed at a 3 T MRI unit using a 15-channel knee coil. Parameter maps were evaluated using region-of-interest analysis of healthy cartilage, areas of chondromalacia and repair tissue. Differentiation of damaged from healthy cartilage was assessed using receiver-operating characteristic (ROC) analysis.

Results: Chondromalacia grade 2–3 had significantly higher CEST values (P = 0.001), lower dGEMRIC (T1- values (< 0.001) and higher T2 values (P < 0.001) when compared to the normal appearing cartilage. dGEMRIC and T2 mapping correlated moderately negative (Spearman coefficient r = -0.56, P = 0.0018) and T2 mapping and CEST moderately positive (r = 0.5, P = 0.007), while dGEMRIC and CEST did not significantly correlate (r = -0.311, P = 0.07). The repair tissue revealed lower dGEMRIC values (P < 0.001) and higher CEST values (P < 0.001) with a significant negative correlation (r = -0.589, P = 0.01), whereas T2 values were not different (P = 0.54). In healthy volunteers’ cartilage, CEST and dGEMRIC showed moderate positive correlation (r = 0.56), however not reaching significance (P = 0.09). ROC-analysis demonstrated non-significant differences of T2 mapping vs CEST (P = 0.14), CEST vs dGEMRIC (P = 0.89), and T2 mapping vs dGEMRIC (P = 0.12).

Conclusion: CEST is able to detect normal and damaged cartilage and is non-inferior in distinguishing both when compared to dGEMRIC and T2 mapping.
contrast agent administration. CEST has been reported to measure GAG (thus also named gagCEST)\(^1\) and to correlate with \(^{23}\)Na MRI, which is thought to directly measure GAG at 7 T\(^2\). CEST has shown the potential to measure GAG also at 3 T\(^3\). Drawbacks of these methods are: not GAG-specific (T\(_{1,\text{rho}}\)) or non-feasible at clinical MRI systems (\(^{23}\)Na needs ultra-highfield MRI and dedicated hardware\(^2\)). In areas of GAG loss, CEST and dGEMRIC are reported to produce reduced values\(^3\),\(^6\), while T2 values are expected to increase in damaged cartilage\(^7\)\(^--\)\(^10\). Hypothetically, CEST and dGEMRIC should be positively correlated while both should be negatively correlated with T2 mapping in damaged cartilage.

Until now, CEST has not been used in a clinical setting at 3 T to investigate the knee's cartilage properties and a systematic prospective comparison with the more established techniques dGEMRIC and T2 mapping is lacking. Thus, we compared CEST with dGEMRIC and T2 mapping regarding the ability to detect normal and damaged cartilage both in young healthy volunteers and in patients with chondromalacia or repair cartilage after microfracture therapy (MFX).

Method

Patient enrollment

The study was approved by the institutional review board and conducted according to the declaration of Helsinki. Informed consent was obtained from all patients after the nature of the examination had been fully explained. Sixty-nine patients were prospectively included (median age, 42 years; range, 12–76 years; 37 females and 32 males). All patients underwent a standardized clinical examination by an orthopedic surgeon according to the ICRS recommendations\(^2\)\(^--\)\(^4\). Three subcohorts were included to allow for the evaluation of normal cartilage, areas of chondromalacia and damaged repair cartilage after therapy.

First cohort: healthy volunteers

10 young healthy volunteers (median age 24.5 years, range 20–50 years) without any prior surgery on their lower extremities or any clinical complaints regarding their locomotors system were included. Physical examination was normal. Healthy cartilage was further confirmed using morphological MRI which showed entirely normal cartilage in all sequences including proton density weighted fat-saturated sequences\(^5\).

Second cohort: chondromalacia group

40 consecutive patients (mean age, 47 years; range 12–76 years) that presented with knee pain and clinically suspected cartilage lesions were included to allow analyzing areas of chondromalacia in comparison to adjacent morphologically normal-appearing cartilage. We excluded patients with lack of normal cartilage (no intraindividual comparison), grade 4 (no measurable cartilage layer) and grade 1 lesions (great variety of internal signal changes, underlying pathology not clearly defined). Thus, the inclusion criteria were grade 2 and 3 cartilage lesions according to the modified Noyes score\(^5\)\(^--\)\(^8\). From 41 patients in this group, one had to be excluded who underwent the full MRI study protocol but did not show any normal-appearing cartilage.

Third cohort: repair cartilage group

19 consecutive patients (median age, 43 years; range, 15–62) who underwent MRI about 1 year (mean, 12.8 months; range, 8–26 months) after surgical cartilage repair. The time-interval was chosen because in the first months after therapy, there are ongoing repair processes that would limit the interindividual comparability\(^9\). The treatment consisted of marrow stimulation by arthroscopical microfracturing. Microfractures were generated with specially bent awls (ChondroPick\(^1\), Arthrex, Naples, FL, USA) by creating V-shaped perforation holes (3–4 holes/cm\(^2\)). Bone marrow bleeding from the perforation holes was checked after shutting off the water influx.

Demographic data and distribution of age

The median age of the 69 included individuals was 42 years (range, 12–76 years). The distribution of age in the three groups was comparable between group 2 and group 3 (median age 47 vs 43 years), i.e., not statistically significant different. However, group 1 with a median age of 24.5 years was statistically significant younger when compared to group 2 (\(P = 0.003\)) and group 3 (\(P = 0.013\)). As a consequence, the influence of age regarding the evaluation of the cartilage is minimized when comparing the similar-aged groups 2 and 3, while an additional effect of age may potentially be present when comparing group 1 with the other two groups. However, the younger age is attributed to the study design, as we wanted to have a group with entirely normal cartilage. The highest likelihood of normal cartilage has a young-aged group, when confirmation by arthroscopy and biopsy is not available.

MRI protocol and data post-processing

MRI was performed on a 70 cm open-bore 3-T whole-body scanner (MAGNETOM Verio, Siemens Healthcare, Erlangen, Germany), equipped with an 18-channel total-imaging matrix (Tim [102 × 18] configuration) in combination with a dedicated 15-channel knee coil. Standard and functional MRI was performed during the same session. The MRI protocol consisted of the following sequences: Morphological sequences: Localizer, proton-density (PD)-weighted turbo spin-echo (TSE) with and without fat saturation and T1-weighted spin-echo sequences. Functional sequences: (detailed description below), prescan MAPit T1 volumetric interpolated breath hold examination (VIBE), gagCEST, T2 map, contrast agent administration, 90 min delay, post-contrast MAPit-T1-VIBE (dGEMRIC).

For gagCEST, a modified and segmented three-dimensional (3D) radiofrequency (RF)-spoiled sagittal gradient-echo sequence (TR/TE, 778/3.59 ms, voxel size 0.625 × 0.625 × 3.3 mm\(^3\)), acquisition time 11:12 min, generalized autocalibrating partially parallel acquisitions (GRAPPA) acceleration by a factor of 2) was used. Prior to image acquisition, a series of three Gaussian-shaped RF pulses followed by gradients in x, y, and z directions to spoil residual magnetization were applied for pre-saturation of proton resonances at different offset frequencies on each spectral side of the bulk water resonance. The pulse train was repeated every 90 lines in phase-encoding direction and had a time average B1 amplitude (continuous-wave equivalent) of 1.5 μT. Each saturation pulse had a duration of 99 ms and an interpulse delay of 100 ms. All saturation parameters were specifically optimised to induce maximal gagCEST effects at 3.0 T by simulation of Bloch equations. Thirteen measurements with pre-saturation at different frequency offsets from the bulk water resonance and one reference without pre-saturation were recorded during one experiment. Also, corrections of B0 inhomogeneity and motion artifacts were performed. Residual motion in the 14 CEST image series from one experiment was compensated by using a non-rigid approach. Subsequently, Z-spectra, i.e., residual magnetization after selective pre-saturation (Msat) normalized to the signal of the reference image (M0) and plotted against the saturation offset frequency (\(\Delta f\)), were corrected for B0 heterogeneities on a pixel-by-pixel basis. For this purpose, the minimum of Z-spectra as determined by smoothing
spline interpolation was shifted to $\Delta v = 0$, and assumed to corre-
spond to the resonance frequency of bulk water protons. As a measure for
gagCEST effects, the magnetization transfer asymmetry
$$\text{MTRasym} = \text{MSat}(\Delta v)/\text{M0} - \text{MSat}(\Delta v)/\text{M0}$$
calculated for each pixel and frequency offset. Finally, the average MTRasym in the
offset range 0.5–2 ppm, which corresponds to the resonance fre-
frequency of GAG hydroxyl protons, was calculated. The post-processing
was performed using in-house-modified software (Matlab 7, the
Mathworks, Naticks, MA, USA).

T2 mapping was performed using the standard product sequence of
the manufacturer (Siemens Healthcare, Erlangen, Germany). It is a
multi-echo-spin-echo T2-weighted sequence with a TR of 1,940 ms
and 13 different echo times (11.8, 23.6, 35.4, 47.2, 59, 70.8, 82.6, 94.4,
106.2, 118, 129.8, 141.8 and 153.4 ms), a voxel size of 0.4 × 0.4 × 3 mm³,
and an acquisition time of 5:38 min. T2 relaxation times were
derived from T2 parameter maps by using a pixel-wise, mono-
exponential least-squares-fit analysis (syngo MapIt; Siemens
Healthcare, Erlangen, Germany). The calculation of T2 values in this
sequence included a noise offset correction, which determined sig-
nals that were already hidden in the noise, to discard “noisy” signal
points for fitting of the T2 curve. As long as a sufficient noise
 correction is performed, inclusion of multiple echoes and long echo
times increases the dynamic range. Due to time restriction the ex-
amination comprised only one half of the knee, including either the
lateral or medial femoral condyle. The decision about the particular
side was made by a senior musculoskeletal radiologist during the MRI
examination with regard to the individual clinical question.

For obtaining the dGEMRIC images, a 3D T1-weighted VIBE
sequence (TR/TE, 15/2.5, voxel size 0.4 × 0.4 × 3 mm³, acquisition
time 3.18 min, field-of-view 159 × 159 mm, imaging matrix
384 × 384, echo train lengths: 1.) with two excitation flip angles (5°
and 26°) was performed before and after intravenous administra-
tion of a double dose of gadopentetate dimeglumine (0.2 mmol/kg Gd-
DTPA, Magnevist®, Bayer Vital, Leverkusen, Germany). After Gd-
DTPA administration the subjects had to walk for 15 min, and after
90 min the post-contrast T1-mapping sequences were performed.
The resulting T1 values are referred to as the dGEMRIC values.

Image analysis

The morphological image analysis was performed on our picture
archiving and communication system (Centricity PACS, version
3.0.4, GE Healthcare Integrated IT Solutions, Barrington, IL) that
allowed for direct comparison and co-registration of anatomic and
functional data sets. The CEST, T1 and T2 parameter maps were
analyzed using an ROI analysis [Fig. 1] according to previous rec-
ommendations11,23,28 on a separate multimodality workstation
(Leonardo, Siemens Healthcare, Erlangen, Germany) by two readers
in consensus.

In healthy cartilage, three ROIs were placed in three consecutive
slices within the normal-appearing cartilage in fat-saturated PD-
weighted images and average values were calculated for further
analysis. Planar ROIs comprised the full thickness of cartilage in one
direction and followed the cartilage curvature about 1 cm along the
other direction. Using the morphological sequences, areas of grade
2 and grade 3 cartilage defects24 and areas after microfracture
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slices within the normal-appearing cartilage in fat-saturated PD-

comparison, three ROIs in three slices were placed into the adjacent
normal-appearing cartilage with a minimum distance of 1 cm to
the defect. Regarding T2 mapping, also the healthy cartilage was
subdivided into a superficial and a deep layer, and equally sized
ROIs were placed separately within these layers as recommended
previously25. Also, for comparison of the T2 mapping results with
those of CEST and dGEMRIC, the mean value of the superficial and
deep ROIs was used.

Statistical analysis

Statistical analysis was performed using the software SAS for
Windows version 9.3 (SAS Institute Inc, North Carolina, USA). Continuous
variables were summarized using descriptive statistics
(e.g., mean, standard deviation, median). Demographic data are
presented as median age and range. The Wilcoxon rank sum test
was performed to check homogeneity in demographic character-
istics within the different populations and included the distribution
of age within all three groups. For this purpose statistical tests were
applied but their outcome will be interpreted solely in a descriptive
manner and no formal statistical conclusions will be drawn from.
Analysis of the examination techniques comprised descriptive
statistics for each group and separately for healthy and damaged
cartilage. Intraindividual differences between healthy and damaged
cartilage were compared using Wilcoxon signed-rank test. We
 refrained from using analysis of covariance (ANCOVA) models
to correct for age differences in normal cartilage, because T2 mapping
and dGEMRIC data of the normal cartilage were not normally
distributed and normality could also not induced by log trans-
formation and because the residuals of CEST data were not nor-
mally distributed in the Shapiro–Wilk test. Thus, to assess
differences within normal cartilage between the three techniques
the Kruskal–Wallis test was used. In case of a significant result of
the Kruskal-Wallis-test, further post-hoc two-method comparisons
were performed using Wilcoxon rank sum test. Correlation was
assessed using Spearman’s correlation coefficient and 95% confi-
dence intervals were calculated. Coefficient values were rated ac-

cording to Zou et al.29 as no association (0) as well as weak (<0.2,
<0.5), moderate (0.5–0.8), strong (>0.8 < 1.0) and perfect (1.0)
correlations. In all tests, an effect was considered to be significant if
the P-value is less than 0.05. This study was not planned as
confirmatory study with the aim of final decision making but rather as
exploratory study for a descriptive purpose generating hypothe-
sis which have to be confirmed in further confirmatory studies.
Due to the characteristics of exploratory studies, no adjustment for
multiplicity is required30.

Performance of the three techniques regarding the differentia-
tion of cartilage lesions from healthy cartilage was assessed using
receiver-operating characteristic (ROC) analysis and included 64
individuals (only those patients were included in whom all imaging
methods were performed). Nonparametric estimates and 95%-con-
fidence intervals for the area under the ROC curves (AUC) were

calculated with AUC = 1 indicating perfect discrimination and
AUC = 0.5 indicating no or random discrimination. Because in pa-

tients of groups 2 and 3 the three biochemical cartilage imaging
techniques were performed both in normal and in damaged car-
tilage, the populations were not independent but had to be consid-
ered as clustered data. Therefore, the statistical comparison of the
AUCs was performed using a non-parametric test for a paired

design with clustered data, described by Lange31.

Results

All results of the ROI analysis of dGEMRIC, T2 mapping and CEST
are presented in Fig. 2 and Table I and their correlations in Table II.
Imaging examples are illustrated in Fig. 3 and Fig. 4. Analysis of dGEMRIC, T2 mapping and CEST was possible in all volunteers and in all patients of repair cartilage group. In chondromalacia group, analysis was possible for dGEMRIC in 34/40 patients, for T2 mapping in 28/40 and for CEST in 34/40 patients. Reasons were contraindications against the used contrast agent ($n = 1$), MR sequence problems ($n = 7$), and lack of grade 2 or 3 defect within the examination area when regarding T2 mapping (eight patients).

Normal cartilage

CEST showed a statistical significant difference ($P = 0.02$) between groups 1 (normal-appearing cartilage of healthy volunteers) and 3 (normal-appearing cartilage of patients who presented with knee pain and patients after microfracturing), but no statistical significant difference between groups 1 and 2 as well as between groups 2 and 3 could be detected. dGEMRIC and T2 mapping revealed no statistically significant different T1 and T2 values. There was some variation of the values of all three techniques (Table I) both in healthy volunteers (especially dGEMRIC) and in the patient groups (especially CEST), while T2 mapping produced more constant values with lower standard deviations. In healthy cartilage mean dGEMRIC values were 915 (group 1: healthy volunteers), 802 ms (chondromalacia group) and 747 (repair cartilage group). Mean T2 values for group 1, 2, and 3 were 52, 52, and 53 ms, respectively. Mean T2 value of the deep zone was 47 ± 3.5 ms and of the superficial zone 57 ± 5.3 ms. Mean CEST values were 1.6, 1.4% and 0.7% for group 1, 2 and 3. In healthy volunteers’ cartilage, a moderate positive correlation of dGEMRIC and CEST ($r = 0.56$) was observed without reaching a significance level ($P = 0.09$, confidence interval $[-0.13, 0.87]$). The other methods assessed showed no or only weak correlations without significance regarding the healthy cartilage (Table II).

Chondromalacia

Areas of grade 2 and 3 chondromalacia had statistically significant lower T1 values (mean, 707 vs 802 ms, $P < 0.0001$), higher T2 values (mean, 75 ms vs 52 ms, $P < 0.0001$) and higher CEST values (mean, 4.8 vs 1.4%, $P < 0.001$) when compared to the normal-appearing cartilage of group 2. In this group dGEMRIC and T2 mapping correlated moderately negative ($r = -0.56$, $P < 0.01$), and T2 mapping and CEST correlated moderately positive ($r = 0.5$, $P < 0.01$; Fig. 3). Of note is considerable variation of the CEST values even in normal appearing cartilage (Table I and Fig. 3(d)).
Repair cartilage

Areas of repair cartilage [Fig. 4] presented with statistically significant higher CEST values when compared to normal cartilage \((P < 0.0001)\). Typically, there were focal areas of increased values within the microfractured area that lead to the overall increased values [Fig. 4(d)]. Also, some variation of CEST values was also present in normal appearing cartilage. The repair tissue also revealed lower T1 values (mean, 592 vs 747 ms, \(P < 0.0001\)) but higher standard deviation of values in the repair tissue (SD, 28.7 vs 4.8) was observed. There were examples with no difference, higher and also lower T2 values compared with healthy cartilage.

Diagnostic performance in differentiating healthy and damaged cartilage

Fig. 5 presents the ROC curves for analyzing the differentiation of cartilage lesions from healthy cartilage. Areas under the curve (AUCs) for dGEMRIC, T2 mapping, and CEST were 0.8, 0.68, and 0.8. Comparisons of the AUCs yielded non-significant differences of T2 mapping vs CEST \((P = 0.14)\), CEST vs dGEMRIC \((P = 0.89)\), and T2 mapping vs dGEMRIC \((P = 0.12)\).

Discussion

Biochemical cartilage imaging is a rapidly growing field with several recent advances but there is controversy which functional technique is superior in detecting or excluding cartilage damage and in monitoring repair tissue after cartilage therapy\(^9,32\). New techniques like CEST are promising but not established in clinical routine. We present the first prospective study that compared CEST with dGEMRIC and T2 mapping in healthy cartilage, chondromalacia and repair tissue after MFX of the knee at 3 T.

Healthy cartilage

In healthy cartilage of all 3 groups CEST produced values of <2% which is in the range of what could be expected from other cartilage-investigating studies at 3 T\(^3\). Thus, CEST proved to reliably measure cartilage. In statistically significant older, but morphologically normal cartilage of group 3, CEST values were reduced which can be explained by a reduced GAG-content in older

<table>
<thead>
<tr>
<th>Table I</th>
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<tr>
<td>Results of the ROI-analysis of healthy and pathologic cartilage is presented as mean and standard deviation in the three cohorts for the three functional methods. Differences between areas of healthy and pathologic cartilage and their corresponding significant (P)-values are highlighted.</td>
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<td></td>
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<td></td>
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<tr>
<td>Group 1: healthy volunteers</td>
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<td></td>
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<tr>
<td>Group 2: chondromalacia</td>
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<td></td>
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<td>Group 3: repair tissue</td>
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Note: n.s.: not significant, ms: milli seconds, SD: standard deviation, N: number.
Table II

<table>
<thead>
<tr>
<th>Group</th>
<th>Healthy Pathologic</th>
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<td></td>
<td>dGEMRIC and T2 mapping</td>
<td>dGEMRIC and CEST</td>
<td>T2 mapping and CEST</td>
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<td>Correlation coefficients between region-of-interest (ROI) measurements and the corresponding p-values are highlighted. Correlations &lt; 0.5 and their significance are highlighted.</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>f</td>
<td>r</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>0.3</td>
<td>0.15</td>
</tr>
<tr>
<td>Group 1: volunteers</td>
<td>10</td>
<td>0.3</td>
<td>0.15</td>
</tr>
<tr>
<td>Group 2: chondromalacia</td>
<td>36</td>
<td>0.31</td>
<td>0.24</td>
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<tr>
<td>Group 3: repair cartilage</td>
<td>18</td>
<td>0.28</td>
<td>0.37</td>
</tr>
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</table>

Note: N: number, r: correlation coefficient, CI = confidence interval.

All three techniques could distinguish normal from damaged cartilage, and ROC analysis showed non-superiority of either technique. Chondromalacia grade 2 and 3 had significantly higher CEST values, lower T1 values and higher T2 values when compared to the normal appearing cartilage in the same patients. We could also underline our hypothesis, that dGEMRIC and T2 mapping are negatively correlated in chondromalacia. Also, cartilage areas after microfracturing presented with significantly lower T1 values when compared to normal-appearing cartilage. Lower T1 values are known to correspond to a lower GAG content due to various underlying pathologies, mainly OA. Trattnig et al. reported statistically significant lower T1 values in 10 patients after microfracture therapy when compared to the healthy-appearing cartilage of the same patients. Kijowski et al. reported 228 arthroscopically proven cartilage lesions where 204 (89.5%) showed increased T2 values. Negative correlations between T1 and T2 values have also been reported in repair cartilage, overall T2 values were slightly but not statistically significant different including cases with increased or decreased and non-altered T2 values. Consequently, T2 mapping did not correlate with dGEMRIC as we hypothesized. One factor that contributes to the lack of statistical significance might be the reduced sample size of the repair cartilage group compared with the healthy cartilage group. However, Welsch et al. also reported no differences between healthy cartilage and full-thickness T2 values of repair tissue after MACT. In other studies, areas after microfracture therapy showed decreased values. Apart from the water content and the collagen network, repair cartilage is supposed to be influenced by many other factors, like hemorrhage and calcification processes.
Thus, when evaluating the repair cartilage using T2 mapping, the longitudinal follow-up might be of most importance rather than the exact value.

Against our expectations, we observed increased CEST values in areas of damaged cartilage in patients with chondromalacia and even more increased values in repair tissue. Also, the significant negative correlation of CEST and dGEMRIC in repair tissue and the positive correlation of CEST and T2 mapping in chondromalacia was surprising. We would have expected a positive correlation of CEST and dGEMRIC as a decrease in the values indicate a GAG loss\textsuperscript{10,11,16}. One explanation is that evaluation of CEST effects at 3.0 T not only depends on the GAG content but also on the tissue’s T2 value. CEST quantification depends strongly on the quality of the bulk water signal, i.e., narrow line width and high intensity, which is likely to be compromised in cartilage areas with T2 increases. High T2 values might arise both in chondromalacia and especially after MPX. Here, complex remodeling processes which include bleeding, calcification, migration of stem cells together with alterations in the content of molecules like proteins and glucose can be expected and contribute to the CEST signal\textsuperscript{5,26,50–52}. At higher field strengths the influence of T2 on CEST quantification is mitigated by higher chemical-shift dispersion. At 7 T, CEST values in repair cartilage were found to be decreased and a high correlation ($r = 0.701$) of CEST and sodium ($^{23}$Na) values, which are supposed to directly correlate with the GAG content, was observed\textsuperscript{11}. Krusche-Mandl et al.\textsuperscript{10} also found decreased CEST values in nine patients 8 years after autologous osteochondral transplantation (AOT) however these consist of hyaline or hyaline-like cartilage\textsuperscript{53}, whereas cartilage after microfracture therapy consists of a rather fibrous tissue\textsuperscript{54,55}. Singh et al.\textsuperscript{15} indicated in a small number of only four volunteers, that the CEST effect can be expected to be reduced at 3 T compared to 7 T. They concluded that CEST might not be valuable at 3 T. This assumption may be partly valid as more sophisticated measures allowing for high image signal intensities and reliable B0 and motion correction must be used to enable reliable quantification of CEST effects at 3.0 T. However, with these measures in place, CEST is very well expected to be a valuable clinical tool at 3.0 T. Nevertheless, it must be noted that evaluation of cartilage areas with strongly increased T2 values or severely altered composition may be an ultimate obstacle for CEST at 3.0 T. As a future perspective, one potential solution to minimize this issue might be to correct CEST values for T2 effects, which may be possible using modifications of existing CEST correction algorithms when T1 or T2 values are known\textsuperscript{56,57}. Other studies showed that the CEST technique is feasible in measuring GAG of intervertebral discs at 3 T after that the proof of concept was made by Saar et al. at ultra-highfield strengths\textsuperscript{13}. Haneder et al. showed that evaluation of GAG

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![Fig. 3. Appearance of chondromalacia. Sagittal proton-density weighted image shows thinning of the cartilage (arrows in A) and normal adjacent cartilage (black arrow). In the functional sequences (B–D) the area of chondromalacia exhibits decreased dGEMRIC values (B) and increased T2 mapping values (C) when compared to the normal cartilage. Of note is an increase in T2 in the dorsal femoral condyle, partly explained through a magic angle effect. In this case the CEST values in the damaged area were only slightly and focally increased (arrows in D). Overall CEST values were not significantly altered in this specific case. Of note is variation of the CEST values in the normal cartilage as well.](image-url)
content in degenerated intervertebral discs in patients with low back pain is feasible at 3 T.

Thus, both T2 mapping and dGEMRIC could be recommended to detect and characterize damaged cartilage in chondromalacia patients. dGEMRIC is by far more time-consuming and needs contrast agent. T2 mapping is easy to perform and to evaluate and produces concordant values. Therefore, T2 mapping can be recommended as first-line method that could be integrated into a routine protocol to early detect or to follow-up patients with chondromalacia. However, not the T2 value itself is of most importance but the intraindividual difference. dGEMRIC is also valuable in analysis of repair tissue after microfracture therapy. Here, the areas of GAG-depleted repair tissue can be easily detected and quantitatively evaluated. CEST has a high potential to measure GAG non-invasively and appears to reliably detect normal cartilage and to be as good as the other methods to show cartilage damage. CEST might measure GAG at least in entirely healthy cartilage at 3 T and might also indicate early GAG depletion in older but morphologically normal cartilage but analysis especially of repair cartilage is challenging.

Limitations

The three subpopulations were not balanced. The age of the healthy volunteers was significantly lower, and this has probably influenced the comparison of the normal-appearing cartilage. A future goal would therefore be to evaluate whether similar-aged but non-symptomatic cartilage of volunteers is equal to morphologically normal-appearing cartilage in these patient groups. Not all of the patients received all imaging techniques. This is mainly attributed to the clinical setting of this study. Due to time restriction and also patients’ compliance, it was not always possible to repeat sequences in cases of inadequate imaging, for instance due to technical problems, especially if the examination time was too long. Moreover, we had no arthroscopic or histologic confirmation. This would be desirable to characterize the areas that showed increased CEST values and to assess early degeneration in morphological normal-appearing cartilage. The CEST sequence is currently at an early stage of its development and some variation of values even in normal appearing cartilage is present that currently may limit the detection of subtle cartilage changes. However, this variation is also present at higher field strengths11,16 and the presented 3 T CEST image quality is high when compared with recent publications at 3 T13,58.

Conclusion

All three 3 T techniques can readily be used to evaluate cartilage properties with particular advantages for each technique. CEST is feasible in a clinical setting at 3 T and detects normal and damaged

Fig. 4. Appearance of repair tissue. The area of repair tissue after microfracturing is depicted in sagittal proton-density weighted image (arrows in A). In the functional sequences (B–D) the area of repair tissue exhibits decreased dGEMRIC values (arrows in B) when compared to the normal cartilage. T2 mapping values (C) where superficially slightly (arrows) but overall not increased. Of note is again an increase in T2 in the dorsal femoral condyle, partly explained through a magic angle effect. CEST (D) depicts an area of focally increased values (arrows). Some variation of the CEST in normal cartilage is also present.
cartilage with non-inferiority when compared with dGEMRIC and T2 mapping. CEST might indicate early GAG-loss in older cartilage. At this early stage of development, some variation of the CEST signal is present in normal appearing cartilage and confined by complex remodeling processes including T2 effects in severely damaged cartilage, especially after MFX. Thus, T2 correction algorithms may be helpful at 3 T. T2 mapping may be recommended to assess chondromalacia, while dGEMRIC is rather advantageous in the analysis of repair cartilage after microfracture therapy.

**Author contribution**

Each author contributed substantially in drafting or critically revising the manuscript and approved the final version to take public responsibility of the content.

Conception and study design: C.R.; M.-A.W.; B.S.; L.L.

Acquisition of data, analysis and interpretation of data or statistical expertise: C.R.; J.K.; N.S.; I.B.; H.-U.K.; M.-A.W.

The first and corresponding author (C.R. Christoph.Rehnitz@med.uni-heidelberg.de) also declares full responsibility for the integrity of the work as a whole.

**Competing interests**

Two co-authors (B.S. and L.L.) are employees of Siemens Healthcare. Both were involved in establishing sequence protocols but not in data acquisition, processing, and analysis. All other authors have reported no conflicts of interest and guarantee that they had full sovereignty of all data and their interpretation. Thus, no conflict of interest is declared.

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**References**


