## **Original Article**

# Evaluation of Glycosaminoglycan in the Lumbar Disc Using Chemical Exchange Saturation Transfer MR at 3.0 Tesla: Reproducibility and Correlation with Disc Degeneration<sup>\*</sup>



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## Abstract

**Objective** This study aims to explore the clinical applicability and relevance of glycosaminoglycan Chemical Exchange Saturation Transfer (gagCEST) for intervertebral disc.

**Methods** 25 subjects ranging in age from 24 yrs to 74 yrs were enrolled. gagCEST was acquired using a single-slice TSE sequence on a 3T. Saturation used a continuous rectangular RF pulse with  $B_1$ =0.8 µT and a fixed duration time =1100 ms. Sagittal image was obtained firstly without saturation pulse, and then saturated images were acquired at 52 offsets ranging from ±0.125 to ±7 parts per million (ppm). MR T2 relaxivity map was acquired at the identical location. Six subjects were scanned twice to assess scan-rescan reproducibility.

**Results** GagCEST intraclass correlation coefficient (ICC) of six subjects was 0.759 for nucleus pulposus (NP) and 0.508 for annulus fibrosus (AF). Bland-Altman plots showed NP had a mean difference of 0.10% (95% limits of agreement: -3.02% to 3.22%); while that of AF was 0.34% (95% limits of agreement: -2.28% to 2.95%). For the 25 subjects, gag CEST in NP decreased as disc degeneration increased, with a similar trend to T2 relaxivity. Gag CEST of AF showed a better correlation with disc degeneration than T2 relaxivity.

**Conclusion** GagCEST in NP and AF decreased as disc degeneration increased, while gagCEST in AF showed a better correlation than T2 relaxivity.

**Key words:** Glycosaminoglycan; Chemical Exchange Saturation Transfer (CEST); Reproducibility; Disc degeneration

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## INTRODUCTION

ntervertebral disc degeneration is a process that begins early in life and is the consequence of a variety of genetic, mechanical, traumatic and nutritional factors, as well as normal ageing<sup>[1]</sup>. Early signs of disc degeneration are manifested by biochemical changes, including a loss of proteoglycans, a loss of osmotic pressure and hydration. In the later stages of disc degeneration,

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morphological changes occur, including a loss of disc height, disc herniation, annular tears and radial bulging. On T2-weighted MR images, disc degeneration is seen as a reduction in signal of the nucleus pulposus (NP) and inner fibres of the annulus fibrosus (AF). With more severe disc degeneration, disc height decreases. Pfirrmann et al.<sup>[2]</sup> devised a 5-level grading system for disc degeneration based on MR signal intensity, disc structure, distinction between nucleus and annulus, and disc height. Recently, an 8-level grading system has been proposed and successfully applied in a number of clinical studies<sup>[3-4]</sup>.

Quantitative MR techniques that reflect the intrinsic material properties of disc tissues are being explored to facilitate early disc degeneration detection and assessment, such as in vivo sodium (Na) MRI, quantitative high-resolution magic angle spinning NMR spectroscopy, proton T2 imaging, T1rho imaging, and diffusion weighted imaging<sup>[5-13]</sup>. Ideally, these measurements may have the potential to detect subtle differences in the composition and organization of the degenerative disc from the normal one that may not be apparent with morphologic MRI assessment. However, till now the underlying relationship between these MRI parameters, i.e. T2 and T1rho relaxation time, apparent diffusion coefficient, and disc composition have not yet been well understood yet.

Chemical exchange saturation transfer (CEST) has been proposed as a novel MRI contrast mechanism in recent years and been actively explored for a variety of clinical applications<sup>[14-26]</sup>. CEST MRI shares similar theoretical principle as T1rho MRI, while shows the advantages of specificity to certain biochemistry components such as amide, glycosaminoglycan (GAG), glycogen glutamate and glucose. In disc degeneration studies, glycosaminoglycan CEST (gagCEST) been has specifically assess the GAG proposed to concentration loss associated with degeneration procedure. With phantom study, Kim et al.<sup>[18]</sup> reported high correlation between gagCEST and GAG concentrations. In addition, they also demonstrated proof-of-principle the technical feasibility of gagCEST in vivo imaging at 3 Tesla on a cohort of healthy volunteers in axial plane of lumbar discs. Haneder et al.<sup>[19]</sup> applied the gagCEST in sagittal plane at 3T in a small number of patients with low-back pain and investigated the correlation of gagCEST and Pfirrmann grading as well as T2 relaxation time. However, their gagCEST map demonstrated low

signal-to-noise ratio<sup>[19]</sup>.

To facilitate the use of gagCEST MRI for routine clinical use, the aim of the current study was to evaluate the *in vivo* reproducibility of measuring glycosaminoglycan of the lumbar disc using CEST imaging at a 3.0-T system and to determine the feasibility of correlating the MR measurement with the degree of disc degeneration with reference to the 5-level and 8-level semi-quantitative disc degeneration grading systems<sup>[3-4]</sup>, and compare the relative performance of gagCEST *vs.* MR T2 relaxivity.

## MATERIALS AND METHODS

## Subjects

A total of 25 subjects were enrolled in this prospective study, including 12 healthy volunteers (10 males and 2 females; mean age: 30.3 years, age range: 24-47 years), and 13 consecutively patients with unspecific low-back pain (5 males and 8 females; mean age: 59.1 years; age range: 29-74 years). Except for intervertebral disc degeneration, there was no other spine disease in 13 patients, as confirmed by medical history and diagnostic MRI. Six of the healthy volunteers underwent gagCEST MRI scan twice time with two days' time interval to assess scan-rescan reproducibility. Patient scans were performed during Saturday morning, while volunteers were performed during working day evenings. The study was approved by the human research ethics committee of the local university, and all subjects provided written informed consent.

## **MR Data Acquisition**

All subjects were scanned using a Philips Achieva 3T scanner (Philips Healthcare, Best, the Netherlands) with a body coil for transmission and a 12-channel spine coil array for reception. Standard diagnostic MRI sequences were completed including sagittal T1 weighted and T2 weighted images covering whole lumbar spine with the following parameters: T1 weighted sagittal imaging Turbo Spin Echo (TSE) sequence, TSE factor=5, TR=407 ms, TE=8 ms, FOV=(160  $\times$  270) mm<sup>2</sup>, voxel size=0.9 mm  $\times$  2.1 mm, slice thickness= 4 mm, slice gap=0.4 mm, NSA=4, Flip angle=80°; T2 weighted sagittal imaging TSE sequence, TSE factor=30, TR=3700 ms, TE=110 ms, FOV=(160×273) mm<sup>2</sup>, voxel size=0.8 mm × 1.72 mm, slice thickness=4 mm, slice gap=0.4 mm, NSA=2, Flip angle=90°.

GagCEST imaging data were acquired using a

single-slice turbo-spin-echo (TSE) sequence with chemical shift-selective fat suppression to reduce possible fat artifacts, and the time interval between two acquired k-space lines was -6 ms<sup>[18]</sup>. Saturation was performed using a continuous rectangular RF pulse with a  $B_1$  field strength of 0.8  $\mu$ T and a fixed duration time of 1100 ms, within the allowable specific absorption rate restriction and RF amplifier capability. To reduce the static magnetic field inhomogeneity  $\Delta B_0$ , localized high-order shimming was also performed for our gagCEST imaging. In sagittal view, the major imaging parameters were as follows: TSE factor (number of refocusing pulses) =27, TR=2500 ms, TE=6 ms, FOV=(160×272) mm<sup>2</sup>, matrix size=80x136, voxel size=(2×2) mm<sup>2</sup>, slice thickness=4 mm, NSA=2, sensitivity encoding (SENSE) factor=1. In axial view, the major imaging parameters were: FOV=(180×352) mm<sup>2</sup>, matrix size=90x176, voxel size=(2×2) mm<sup>2</sup>, slice thickness=8 mm, other parameters are the same as sagittal view. The phase encoding direction was anterior-posterior (AP) for both sagittal and axial view. Sagittal plane was acquired for all subjects, while axial plane was acquired on 6 discs comprising of 3 discs at L4/L5 and 3 discs at L5/S1 as a way of demonstration of technical feasibility. Baseline images were obtained first without application of saturation pulse, and then the saturated images were acquired at 53 offsets ΔΩ of (0, ±0.125, ±0.25, ±0.375, ±0.5, ±0.625, ±0.75, ±0.875, ±1, ±1.125, ±1.25, ±1.375, ±1.5, ±1.625, ±1.75, ±1.875, ±2, ±2.5, ±3, ±3.25, ±3.5,  $\pm 3.75, \pm 4, \pm 4.5, \pm 5, \pm 6, \pm 7$ ) parts per million (ppm). The total data acquisition time was 11 min and 20 s for sagittal plane, and 4 min and 35 s for axial plane.

A multi-echo TSE pulse sequence was applied for T2 map imaging, acquired at identical sagittal locations as gagCEST images with following parameters: FOV=(160×272) mm<sup>2</sup>, Pixel=1.0 mm × 1.0 mm, slice thickness=4 mm, seven echoes of TEs=16, 32, 48, 64, 80, 96, and 112 ms, TR=2300 ms, NSA=2 and SENSE factor=2. Bowel movement artifacts and artifacts due to abdominal wall motion were reduced using a saturation band anterior to the spine.

## **Image Analysis**

Data processing was performed by home-developed Matlab (MathWorks, Natick, MA, USA) programs. For each voxel, the acquired gagCEST signal intensity with regard to offsets, i.e. Z-spectrum, was first least-square fitted by a 12th-order polynomial model and interpolated to a

finer resolution of 0.001 ppm<sup>[16-17]</sup>. To correct the B<sub>0</sub> field inhomogeneity which may strongly affect the CEST data, the actual water resonance (true 0 ppm) was assumed to be at the lowest intensity frequency of the interpolated Z-spectrum. The interpolated Z-spectrum was shifted correspondingly along the offset axis to correct the possible field inhomogeneity  $\Delta B_0^{[16]}$ . Then three regions of the Z-spectrum ( $|\Delta\Omega| \le 1$  ppm,  $\Delta\Omega > 6$  ppm,  $\Delta\Omega < -6$  ppm) were fit to a Lorentzian function simultaneously to estimate the CEST reference signal<sup>[23,25-26]</sup>. CEST effect was quantified by calculating the transfer saturation as the difference between the Lorentzian fitted CEST reference signal curve and the 12th-order polynomial fitted Z-spectrum<sup>[27]</sup>: **CEST effect:** 

 $MTR(\Delta\Omega) = \frac{S_{ref}(\Delta\Omega)}{S_0} - \frac{S(\Delta\Omega)}{S_0}$ (1)

Where  $S_{DS}(\Delta\Omega)$  and  $S(\Delta\Omega)$  donate the signal intensities of the reference signal curve and Z spectrum at the offset frequency of  $\Delta\Omega$  respectively, while  $S_0$  represents the baseline signal intensities obtained without saturation pulse. The CEST effect computed by Equation (1) was integrated over the 0.5-1.5 ppm range on a voxel-by-voxel basis to measure gagCEST values. To exclude the voxels associated with possible unreliable fitting results, a voxel would be excluded if a large  $\Delta B_0$  was shown (>2 ppm or <-2 ppm), or was not fully saturated ( $\Delta B_0$ -corrected Z-spectrum bottom in was larger than 0.15  $S_0$ )<sup>[17]</sup>.

For gagCEST map, 20 discs with the possible unreliable fitting results as described in the image analysis were excluded; 2 discs with motion artifacts were excluded; L1/2 disc was missed in FOV in one patient; and one disc with completely collapsed disc space was excluded. Finally 101 discs were included in further measurements. For T2 relaxivity map, one collapsed disc in a patient was excluded; T2 map scan was not acquired in one patient; L1/2 disc was missed in FOV in one patient; and another 8 discs was not measured due to various artifacts including fold over artifact and aorta pulsing artifact. Finally 2 maps of 110 discs were included for analysis (Figure 1).

Three anatomical regions of interest (ROIs) of disc were manually drawn on sagittal gagCEST and T2 maps by referring to T2-weighted images: nucleous pulposus (NP), anterior part of the annulus fibrosus (AFant) and posterior part (AFpost) (Figure 2). Entire annulus fibrosus (AFtotal) value was equal to (AFant+AFpost)/2. The ROIs area for NP varied from



Figure 1. Study schedule diagram.



**Figure 2.** An example of placement of ROIs over NP (#), anterior AF (\*) and posterior AF (^) in one disc. T2WI: T2-weighted image.



**Figure 3.** Spatial distribution mapping of the gagCEST signal in the axial dimension of L5/S1 disc in one 33-year-old woman. Nucleus pulposus had greater gagCEST value than annulus fibrosus.

22 mm<sup>2</sup> to 90 mm<sup>2</sup>, from 8 mm<sup>2</sup> to 58 mm<sup>2</sup> for AFant, and from 8 mm<sup>2</sup> to 50 mm<sup>2</sup> for AFpost. According to Pfirrmann 5-level grading system as well as modified 8-level Pfirrmann grading system<sup>[3-4]</sup>, ROIs placement and disc degeneration grading were performed using the T2W anatomical images by a radiologist with 3 years' experience in reading spine MRI images who was trained by a senior radiologist (>10 year experience) to score disc degeneration. An example of gagCEST imaging in axial plane is displayed in Figure 3, NP showed higher gagCEST values compared with AF. Randomly 40 discs were selected assessment of intrainter-reader for and reproducibility as part of the pilot study.

## Statistic Analysis

Data were expressed as mean±standard deviation.

The difference in gagCEST values of various disc degeneration grades was evaluated by independent two sample *t*-test or Mann-Whitney U test as appropriate. For intra-reader reproducibility, the same data set was evaluated twice by the same reader. For scan-rescan reproducibility, the repeated scans were evaluated by the same reader. Reproducibility was tested using intraclass correlation coefficient (ICC) and Bland-Altman analysis. ICC values >0.75 represent a good agreement, and values between 0.4 and 0.75 represent fair to moderate agreement. Bland-Altman analysis was performed with a home-developed Matlab (MathWorks, Natick, MA, USA) program. Spearman rank correlation was used to correlate gagCEST values with disc degeneration grading. All other statistical analyses were carried out using SPSS version 17.0 (SPSS, Chicago, IL).

## RESULTS

## GagCEST MR Scan-Rescan Measurement Reproducibility

Pilot study of randomly selected 40 discs showed ICC for intra-reader reproducibility was 0.928 and 0.835 for T2 NP and T2 AFtotal, and 0.826 and 0.786 for gagCEST NP and gagCEST AFtotal. The ICC for inter-reader reproducibility was 0.914 and 0.821 for T2 NP and T2 AFtotal, and 0.828 and 0.757 for gagCEST NP and gagCEST AFtotal.

In the volunteers, six discs (10%) were excluded from analysis with the criteria set to exclude the voxels associated with possible unreliable fitting results<sup>[17]</sup>. For scan-rescan reproducibility with the remaining 24 paired discs gagCEST measurement, ICC for NP was 0.759 indicating good reproducibility, while ICC for AFtotal was 0.508 indicating moderate reproducibility (more details see Supplementary Table 1, www.besjournal.com for details). The scan-rescan mean differences of gagCEST values are shown with Bland-Altman Plots (Figure 4). The scan-rescan mean measurement variability of NP had mean difference of 0.10% (95% limits of agreement: -3.02% to 3.22%); while the scan-rescan mean difference for AFtotal was 0.34% (95% limits of agreement: -2.28% to 2.95%).

## Correlation between GagCEST and Disc Degeneration Grading

The gagCEST measurements for NP in 101 disc ranged from -8.8% to 12.4%, and from -9.2% to 9.6%

for AFtotal (AFant: -9.6% to 11.6%; AFpost: -11.3% to 9.6%). The relationship between 101 discs gagCEST measurements and corresponding 5-level degeneration grading for each disc are shown in Figure 5 (A&C). The T2 relaxation times for NP in 110 discs ranged from 32.96 ms to 133.68 ms, and from 22.81 ms to 51.84 ms for AFtotal (AFant: 23.99 ms to 61.91 ms; AFpost: 20.94 ms to 48.70 ms). The T2 value and corresponding disc degeneration 5-level grading for each disc are shown in Figure 5 (B, D).

GagCEST measurement in NP decreased as disc degeneration grade increased, with a similar trend as T2 relaxation time (Figure 5A, 5B). T2 value in AF showed slightly decreased as disc degeneration grade increased; however, there was no significant difference between grade 2/5 vs. grade 3/5, and grade 3/5 vs. grade 4/5 (Figure 5D). On the other hand, gagCEST measurement showed a steeper decrease following disc degeneration compared with T2. Statistically significant difference was demonstrated between grade 2/5 vs. grade 3/5, and grade 3/5 vs. grade 4/5 (Figure 5C). Similar results were also seen with the correlation of gagCEST measurement and 8-level disc degeneration grading (Supplementary Figure 1, www.besjournal.com for details). The Spearman correlation coefficients exploring relationships of gagCEST and T2 relaxation time with disc degeneration grading are shown in Table 1. GagCEST AF measurement shows a significant correlation with Pfirrmann 5-level disc degeneration grading (P<0.001) while T2 relaxation time does not. Similar results were shown with modified Pfirrmann 8-level disc degeneration grading (Supplementary Table 2, www.besjournal. com for details).

**Table 1.** The Correlation of GagCEST/T2 Value with

 Pfirrmann 5-level Disc Degeneration Grading

| Variables       | r*     | Р      |
|-----------------|--------|--------|
| gagCEST NP      | -0.603 | <0.001 |
| T2 NP           | -0.847 | <0.001 |
| gagCEST AFtotal | -0.577 | <0.001 |
| T2 AFtotal      | -0.191 | 0.046  |
| gagCEST AFant   | -0.556 | <0.001 |
| T2 AFant        | -0.057 | 0.555  |
| gagCEST AFpost  | -0.517 | <0.001 |
| T2 AFpost       | -0.235 | 0.013  |

**Note.** GagCEST AF measurement shows a significant correlation with Pfirrmann 5-level disc degeneration grading while T2 relaxation time does not. r represents Spearman correlation coefficient (significance level set at P<0.01).



**Figure 4.** Bland-Altman plots for the NP, AFtotal, AFant, AFpost of 24 IVDs in 6 subjects. The inter-scan differences of LD values are plotted against the average LD values of two scans for each IVD. (A) For NP, scan-rescan 95% limits of agreement ranges from -3.02% to 3.22% (mean difference: 0.10%). (B) For AFant, scan-rescan 95% limits of agreement ranges from -2.76% to 3.82% (mean difference: 0.51%). (C) For AFpost, scan-rescan 95% limits of agreement ranges from -3.00% to 3.78% (mean difference: 0.41%). (D) For AFtotal, scan-rescan 95% limits of agreement ranges from -2.28% to 2.95% (mean difference: 0.34%). IVD, Intervertebral disc. LD, Lumbar disc.



**Figure 5.** (A) NP gagCEST value *versus* 5-level disc degeneration grading. (B) NP T2 value *versus* 5-level IVD degeneration grading. (C) AF T2 value *versus* 5-level IVD degeneration grading. (D) AF gagCEST value *versus* 5-level IVD degeneration grading. *\*\*\*P*<0.01; \*\*\**P*<0.001; n.s. not significant. IVD, Intervertebral disc.

## DISCUSSION

The loss of GAG is known to be an initiating factor in disc degeneration, followed by a reduction in the osmotic pressure and shrinkage of the disc height as a consequence<sup>[28]</sup>. Lyons et al. suggested a decrease in GAG in the NP corresponding to the disc possible earliest changes<sup>[29]</sup>. degeneration as Conceptually it is important to diagnose degenerative changes at the stage of GAG loss in structurally intact discs by quantification of the GAG content using imaging techniques. With CEST approach, selective radiofrequency irradiation of exchangeable solute protons, such as amide (-NH) and hydroxyl (-OH) is detected through progressive saturation of the water signal consequential to chemical exchange. CEST observation of solutes and particles in the millimolar to nanomolar range has been demonstrated both in vitro and in vivo<sup>[14-17]</sup>.

Recently, investigators have quantified CEST in cartilage as well as in disc and have demonstrated a relationship between gagCEST and GAG content<sup>[18]</sup>. The proof of principle for imaging gagCEST in animal disc in vitro and the description of the underlying concepts have been reported<sup>[30]</sup>. Kim et al's proof-of-principle the technical demonstrated feasibility of gagCEST in vivo imaging at 3 Tesla on a cohort of healthy volunteers in axial plane of lumbar discs<sup>[18]</sup>. They used a TSE sequence for acquisition with similar saturation pulse strength (0.75 µT) but much shorter saturation duration (400 ms). As such, direct water saturation might not be as complete as in our study, and hence gagCEST effect and contrast might be smaller. We acquired saturated images at 53 offsets, comparable to 49 offsets in Kim's study, but with a broader frequency range (-7 to 7 ppm vs. -4 to 4 ppm). We did not apply WASSR for Z-spectrum  $\Delta B_0$  correction as we found the acquired Z-spectrum was sufficiently steep for accurate self-correction of  $\Delta B_0$ , and it also saved the scan time of gagCEST. Haneder et al.<sup>[19]</sup> investigated the correlation of gagCEST and Pfirrmann grading as well as T2 relaxation time. They applied gagCEST in sagittal plane at 3T in a small number of patients; however, their gagCEST map demonstrated low signal-to-noise ratio (Figure 4 of ref 19). They used a segmented 3D gradient echo sequence which was more subject to tissue susceptibility compared to TSE sequence used in current study. The saturation pulse consisted of three 99 ms Gaussian pulses with an inter-pulse delay of 100 ms, considerably shorter than ours and Kim's, while the effective saturation

strength was 1.5 µT, i.e. much higher than ours and Kim's. Haneder et al. did not mention the offset frequencies applied for gagCEST acquisition. WASSR was not applied either in Haneder et al.'s study. In the aspect of Z-spectrum analysis, both Kim Haneder used asymmetric magnetization and (MTR<sub>asvm</sub>) for gagCEST transfer ratio effect quantification based on asymmetric analysis of Z-spectrum. Despite its simplicity, **MTR**<sub>asym</sub> calculation could be subject to asymmetric conventional magnetization transfer (MT) and nuclear overhauser effect (NOE)<sup>[14,17]</sup>.

In current study, we utilized a Lorentzian fitting approach that could effectively separate CEST effect from DS, MT and NOE effects<sup>[27,31]</sup>. Kim et al. used the mean of the integrated gagCEST effect from 0.5 ppm to 1.5 ppm, the identical frequency range as ours, for disc assessment while Haneder et al. used the frequency range from 0.5 ppm to 2 ppm. In the aspect of ROI placement and result interpretation, Kim et al.'s study only contained axial scan, Kim et al's interpretation of NP might be contaminated by inner AF (Figure 4 of ref 18; 32, 33). In Haneder et al.'s study, semi-automatic approach with 5 equal-sized ROI was used which is prone to partial volume measurement error (Figure 1 of ref 19; 34). In our study, we carefully placed ROIs on location of NP, AFant, and AFpost manually guided by a radiologist. Haneder et al's study showed that gagCEST measurement of the AFtotal was not affected by the degeneration. On the contrary, our study showed AFtotal gagCEST signal decreased as disc degeneration progressed (Figure 6).

For volunteers our study demonstrated good reproducibility for NP with the ICC value of 0.759, while the ICC for AF was moderate (=0.508). The scan-rescan reproducibility ICC value for AF could be compromised by the subjectivity of ROI placement (intra-reader ICC=0.786 and inter-reader reproducibility=0.757 for the same data set in our pilot study). These results support recent studies on the repeatability of the CEST imaging measurement<sup>[35-36]</sup>. Müller-Lutz et al. also demonstrated an age-dependency of lumbar disc measurement<sup>[36]</sup>. Further standardization in ROI placement may improve this measurement<sup>[34]</sup>.

In our study disc gagCEST of volunteers and those of non-specific back pain patients were mixed together for analysis against T2 relaxivity, as it is well-known that disc degeneration is commonly seen in non-symptomatic, even young, subjects, and disc degeneration *per se* is not specifically related to back

pain, instead maybe part of physiological aging process<sup>[1,37-38]</sup>. Despite the moderate reproducibility, our data still showed gagCEST measurement offers advantage for assessment of AF in disc degeneration compared to T2 relaxivity. We tentatively tried to compare the performance of gagCEST vs. T1rho for AF assessment using the slope over 5-level Pfirrmann disc degeneration grading, gagCEST seems to outperform T1rho (Supplementary Figure 2, www.besjournal.com for details). However, these subjects are of different cohorts and conclusion cannot be readily made. Further comparative study of T1rho vs. gagCEST imaging will be of interest.

There are a few limitations in this study. This study remains a proof of concept study and the patient number is still small, and how gagCEST measurement is related to future patient clinical symptom development was not investigated. Clinically, there is no gold standard to assess early disc degeneration. How to best evaluate in vivo disc gagCEST measurement itself remains unknown, therefore the same as many previous studies we could only utilized the semi-quantitative grading systems for comparative study<sup>[11,19]</sup>. However, these gradings are concerned with T2 relaxivity based image and also they are inherently subjective. One major drawback of gagCEST MR measurement is that data acquisition time is very long, i.e. 11 min and 20 s in our setting for single slice in sagittal plane. This resulted in 22 discs (20%) had motion artifacts and unreliable for quantification. It has been suggested that approaches with motion correction can improve CEST imaging<sup>[39]</sup>. Due to the limited access to MR scan time, patient scans were performed during morning, while volunteer reproducibility were performed during day evenings. However, we expect this would not affect the paired comparison of gagCEST vs. T2 relaxivity. Strategies to shorten data acquisition time while maintain sufficient signal-to-noise ratio is warranted<sup>[34-45]</sup>. GagCEST MR will also benefit from a higher magnetic field strength because of the increased CEST effect and better absolute offset frequency separation.

In summary, this study shows that gagCEST MR imaging on a clinical 3.0 T system is feasible with acceptable reproducibility. GagCEST demonstrated a decrease in the NP of degenerated discs, matching the known loss of GAG in the NP with an increasing grade of degeneration. GagCEST MR imaging is also able to demonstrate signal decrease in AF with an increasing grade of degeneration, which is an advantage over T2 relaxation time based technique. To translate gagCEST MR imaging into a practical tool and thereby positively influence clinical management, technological advancement including acceleration of data acquisition and improvement in signal-to-noise ratio remain to be further realized.

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#### DISCLOSURE

Queenie Chan is an employee of Philips Healthcare. The other authors declare no conflict of interest.

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