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Clinically applied CT arthrography to measure the sulphated glycosaminoglycan content of cartilage

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SUMMARY

Objective: Similar to delayed gadolinium enhanced MRI of cartilage, it might be possible to image cartilage quality using CT arthrography (CTa). This study assessed the potential of CTa as a clinically applicable tool to evaluate cartilage quality in terms of sulphated glycosaminoglycan content (sGAG) and structural composition of the extra-cellular matrix (ECM).

Methods: Eleven human cadaveric knee joints were scanned on a clinical CT scanner. Of each knee joint, a regular non-contrast CT (ncCT) and an ioxaglate injected CTa scan were performed. Mean X-ray attenuation of both scans was compared to identify contrast influx in seven anatomical regions of interest (ROIs). All ROIs were rescanned with contrast-enhanced μCT, which served as the reference standard for sGAG content. Mean X-ray attenuation from both ncCT and CTa were correlated with μCT results and analyzed with linear regression. Additionally, residual values from the linear fit between ncCT and μCT were used as a covariate measure to identify the influence of structural composition of cartilage ECM on contrast diffusion into cartilage in CTa scans.

Results: CTa resulted in higher X-ray attenuation in cartilage compared to ncCT scans for all anatomical regions. Furthermore, CTa correlated excellent with reference μCT values (sGAG) ($R = 0.86$; $R^2 = 0.73$; $P < 0.0001$). When corrected for structural composition of cartilage ECM, this correlation improved substantially ($R = 0.95$; $R^2 = 0.90$; $P < 0.0001$).

Conclusions: Contrast diffusion into articular cartilage detected with CTa correlates with sGAG content and to a lesser extent with structural composition of cartilage ECM. CTa may be clinically applicable to quantitatively measure the quality of articular cartilage.

Introduction

The current reference standard for osteoarthritis (OA) staging is the Kellgren and Lawrence score based on knee radiography[1]. However, this technique is not sensitive enough to detect OA at an early stage. Sulphated glycosaminoglycan (sGAG) is a key molecule in articular cartilage and its content is an indicator of cartilage health[2]. Loss of sGAG from the articular cartilage is a hallmark of early OA and occurs well before OA is detected radiographically[3,4].

Micro computed tomography (μCT) used together with a negatively charged contrast agent (ioxaglate) is a well established technique to image sGAG-distribution in cartilage[5–7]. The technique is comparable to delayed gadolinium enhanced magnetic resonance imaging of cartilage (dGEMRIC)[8–12]. Previous *in vitro* work has shown that there is a clear inversed relationship between the amount of ioxaglate in the cartilage measured with μCT and the negatively charged sGAG content of the cartilage measured with biochemical essays ($R^2 = 91–94\%$)[5,6], and histology ($R^2 = 77\%$)[13]. *In vivo* research in small animals has also demonstrated that μCT arthrography is able to accurately measure changes in cartilage quality[14,15].

In humans, CT arthrography (CTa) using intra-articularly injected contrast agent is an established clinical technique for imaging of knee abnormalities[16,17]. However, it is solely used for detection of morphologic derangements rather than assessment of cartilage

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sGAG content. In this cadaver study we determined whether it is possible to quantitatively measure the sGAG content of human articular cartilage with a clinical CT system, after intra-articular injection of a contrast agent. We also investigated to what extent the contrast influx into cartilage is influenced by the structural composition of the extra-cellular matrix (ECM).

Methods

Cadaver specimens

Thirteen cadaveric lower extremities from eleven individuals who had donated their bodies to science (seven female, four male; mean age at death 74 years, age range at death 30–96 years) were available. All extremities were freshly frozen at −20°C until start of the experiment. Prior to first imaging, all specimens were slowly defrosted in a cooled environment (7°C) for 5 days. All extremities were at room temperature during imaging.

Acquisition and post-processing of non-contrast CT (ncCT) and CTa data

ncCT was performed of all knee joints using a second generation dual source multidetector spiral CT scanner (SOMATOM Definition Flash, Siemens Healthcare AG, Erlangen, Germany) with a tube voltage of 80 kV and an effective mAs-value of 3,140. Scan time per ncCT was approximately 30 s per scan. All specimens were scanned in the standard anatomic axial plane. All scans were reconstructed with an effective slice thickness of 0.75 mm and a sharp reconstruction kernel (B75s). Multiplanar reconstruction was performed (image pixel size 0.265 mm) [Fig. 1(A–C)].

Immediately after ncCT, 20 ml of 30% ioxaglate solution (diluted in saline) (Hexabrix 320, Mallinckrodt, Hazelwood, MO, USA)[16] was injected intra-articularly using an 18 gauge needle. All knees were flexed (~120°) and extended (~0°) for 5 min in order to achieve optimal distribution of the contrast agent throughout the joint. Ten minutes after contrast injection, all knees were rescanned using the same CT scanner, scanning parameters (30 s/scan), and reconstruction methods [Fig. 1(D–F)].

All scans were converted into binary datasets using one fixed attenuation threshold (430 Hounsfield units) that was selected visually to render the best possible segmentation of cartilage in all datasets [Fig. 1(G–I), Supplementary Fig. 1 online][14]. Using analysis software (Skyscan, Kontich, Belgium), per knee seven regions of interest (ROIs) were manually defined. Each cartilage ROI extended over 40 contiguous sagittal slices. These cartilage ROIs consisted of the central weight-bearing area of both medial and lateral femoral condyles (wbMC and wbLC), the posterior non-weight bearing area of both femoral condyles (pMC and pLC), both weight-bearing medial and lateral tibial plateaus (wbMP and wbLP) and the mid-portion of patellar cartilage (mpP) [Fig. 1(G–M)]. Anterior margins of the weight-bearing femoral condyles and tibial plateaus were defined at the level of the posterior aspect of the anterior meniscal horn. The posterior margins were defined at the level of

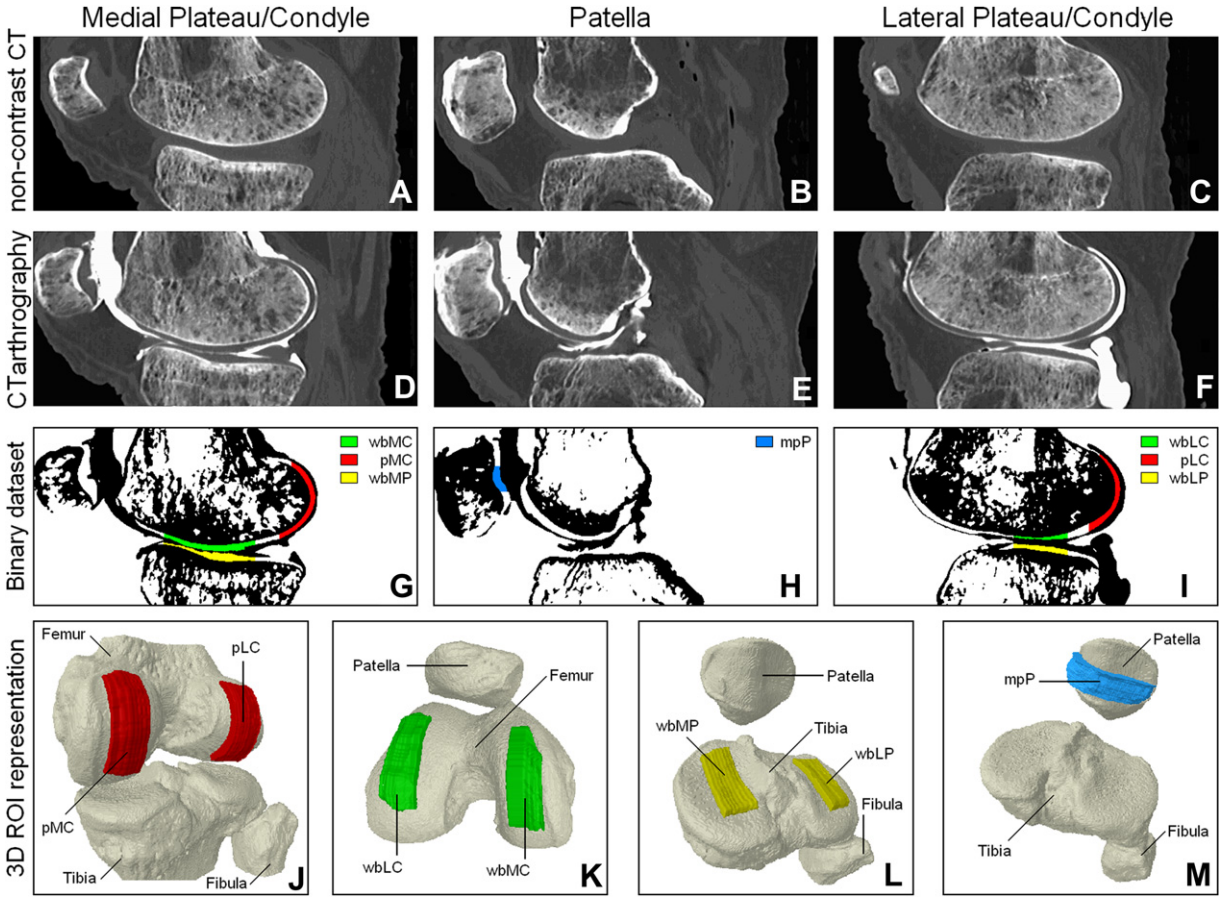

Fig. 1. Representative sagittally reconstructed images of a knee joint from ncCT (A–C) and after intra-articular contrast injection for CTa (D–F), after segmentation into a binary dataset showing the definition of the ROIs. (G–I), and a 3D representation of all seven analyzed ROIs (J–M): weight-bearing medial and lateral condyle (wbMC/wbLC; posterior medial and lateral condyle of the femur (pMC/pLC); weight-bearing medial and lateral plateau of the tibia (wbMP/wbLP); mid portion of patellar cartilage (mpP).

the anterior aspect of the posterior meniscal horn. The posterior non-weight bearing femoral condyle ROI extended backward from the level of the dorsal margin of the posterior meniscal horn. We calculated the mean X-ray attenuation of cartilage in these ROIs on non-contrast and contrast-enhanced clinical CT scans.

Equilibrium partitioning of an ionic contrast agent using (EPIC-)μCT

Because EPIC-μCT has shown strong correlation with cartilage sGAG content, we selected this as our reference test for sGAG content of cartilage[5,6,13]. In EPIC-μCT an equilibrium-state exists between sGAG and contrast agent after a long incubation period. Due to the equilibrium, structural composition of the cartilage ECM[18] does not influence the interaction between contrast and sGAG content of cartilage[13].

After CTa, the knee joints were dissected into five parts: both medial and lateral femoral condyles, both medial and lateral tibial plateaus and the patella. Soft tissue was removed to a maximal extent, without harming cartilage integrity. In order to achieve equilibrium between the contrast agent and sGAG in cartilage, all dissected specimens were incubated in an ioxaglate contrast solution for 24 h at room temperature[19–21]. It is advocated to use the highest possible concentration of contrast, allowing best cartilage segmentation to achieve highest sensitivity for changes in sGAG content[7,13]. We used a 20% solution of ioxaglate, which resulted in the best cartilage segmentation at the air/cartilage and bone/cartilage interfaces.

EPIC-μCT was performed on a μCT scanner (Skyscan1076, Skyscan, Kontich, Belgium). The following scan settings were used: isotropic voxel size of 35 μm; a voltage of 55 kV; a current of 181 mA; field of view 68 mm; a 0.5 mm aluminium filter; 198° with a 0.4° rotation step. Scanning time per specimen was 6–10 h, depending on the size of the specimen (condyle, plateau or patella) which was scanned. A plastic foil was wrapped around the specimen to avoid dehydration. All scans were performed using the same settings and all data were reconstructed identically.

Using Skyscan analysis software, these datasets were segmented using a fixed attenuation threshold between air (25 gray value) and subchondral bone (120 gray value) that was selected visually for the best segmentation result in all datasets. In all segmented μCT datasets, similar ROIs of the cartilage regions corresponding with ROIs of the clinical CTa were drawn and the mean X-ray attenuation was calculated again. These μCT based mean attenuation values were used as the reference for sGAG content against which the attenuation values on ncCT and CTa were compared.

Contrast diffusion influenced by structural composition of cartilage ECM

An important difference between the μCT and CTa scans is that with μCT scanning, the contrast agent and sGAG are partitioned at equilibrium. However, the principle of CTa is dependent on a diffusion process before equilibrium, which is influenced by the electrostatic interaction between sGAG and ioxaglate[15]. Therefore, measurements from *non-equilibrium* CTa are also influenced by other factors than sGAG content alone[19–21]. In particular, so-called tissue dragging influences the interaction between contrast and sGAG[22,23]. A high tissue drag results from an intact collagen network and is predominantly present in the top layers of healthy cartilage where collagen is densely packed parallel to the cartilage surface and acts as a barrier membrane[24,25]. Consequently, contrast diffusion goes slowly in regions with high tissue drag. When collagen is structurally impaired, e.g., in OA, tissue dragging diminishes and more contrast penetrates in comparison to healthy cartilage due to a higher diffusion rate.

In ncCT, X-ray attenuation of cartilage results only from initial dissimilarities in cartilage composition (e.g., collagen, sGAG and water content). Together with the information on sGAG content from μCT, the influence of this structural composition of the cartilage on CTa outcome was further investigated using statistical models.

Statistical analysis

To assess if the influx of contrast agent into the cartilage could be detected, we compared the attenuation values per anatomical region between ncCT and CTa scans with paired student's *t*-tests. To evaluate to what extent the attenuation values represented sGAG content, we fitted linear regression models of the mean X-ray attenuation values of both the ncCT and CTa to the results of μCT scans for each knee compartment, of which we report the Pearson's correlation coefficients. To test if the correlation with μCT was different between ncCT and CTa, we compared the slopes of both models. These analyses were performed using GraphPad (Graphpad Software Inc., San Diego, USA).

In this study we used 13 knees from 11 individuals. The use of two knees from one individual could potentially lead to an overestimation of the correlation between μCT and CTa measurements[26,27]. Exclusion of either one of the knees in the two patients that were scanned bilaterally did, however, not influence the results of our study. Therefore, we did not apply a statistical correction.

Next, we investigated to what extent the influx of contrast was influenced by structural composition of cartilage ECM itself. The spatial variation in X-ray attenuation inside cartilage from ncCT scans is related to both structural composition of cartilage ECM and its sGAG content. Thus, when ncCT attenuation values are fitted to μCT values (representing sGAG content) using linear regression, the residuals, which is that part of the ncCT values which is not explained by μCT, contain information on structural composition independent of sGAG content. When these residuals are subsequently added as a covariable to the linear regression model that relates CTa to μCT values, the contribution of these residuals to the model represent the extent to which the influx of the contrast is influenced by structural composition of the cartilage ECM, independent of sGAG content. These analyses were performed using SPSS (SPSS Inc., Chicago, USA).

All P-values < 0.05 were considered to be statistically significant.

Results

Cadaver subjects

After CT scanning, three extremities were excluded from the study due to clearly visible calcifications inside the cartilage. Thus, a total of 10 cadaveric knee joints from nine individuals were included in the analysis (six female, three male; mean age at death 69 years; age range at death 30–94 years). Furthermore, 12 cartilage ROIs were not included in our data analysis because of (motion) artefacts during μCT scanning and segmentation errors because of severe cartilage loss[15].

sGAG correlation in ncCT and CTa

Mean X-ray attenuation results showed clear differences between the anatomical cartilage locations and between ncCT and CTa outcomes. In all locations, cartilage attenuation increased significantly after injection of contrast agent [Fig. 2(A)].

Cartilage X-ray attenuation in ncCT correlated moderately with μCT ($n = 57$, $R = 0.45$; $R^2 = 0.20$; $P = 0.0003$). The correlation between cartilage X-ray attenuation from CTa scans and μCT was

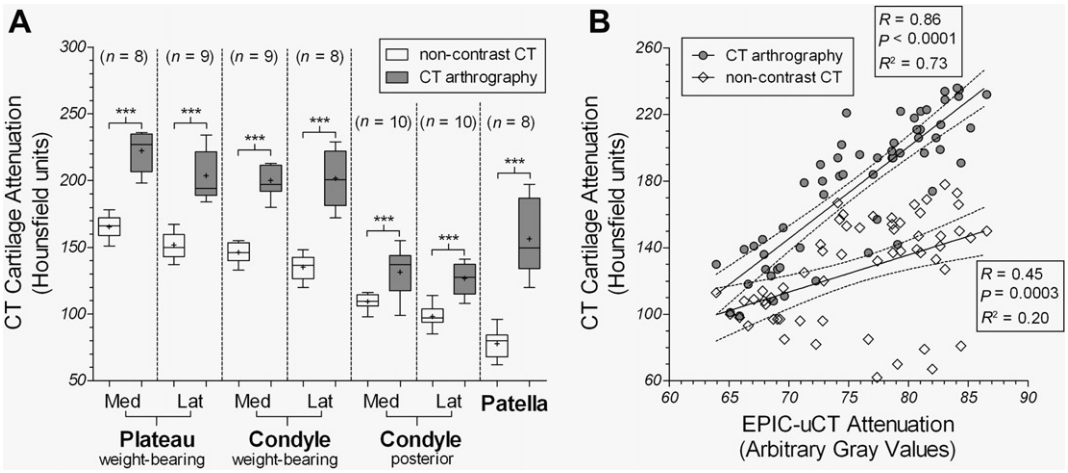

Fig. 2. Contrast diffusion into cartilage. Comparison of cartilage attenuation between ncCT and CTa scans. A: Box plot of mean attenuation in cartilage from CT and CTa scans per anatomical region. Boxes range from twenty-fifth to seventy-fifth percentile, whiskers run from min to max, the horizontal line in the box represents the median and the plus sign shows the mean. B: Correlated results of mean attenuation from EPIC-μCT and clinical CT scans with and without injected contrast for all anatomical regions combined ($n = 57$). ***: $P < 0.0001$.

strong ($n = 57$, $R = 0.86$; $R^2 = 0.73$; $P < 0.0001$) [Fig. 2(B)]. The slopes of both regression lines were significantly different ($P < 0.0001$).

sGAG content per anatomical location

The cartilage attenuation derived from CTa for all separate anatomical compartments correlated strongly with attenuation from μCT [wbMP, wbLP: $n = 17$, $R = 0.89$, $R^2 = 0.79$, $P < 0.0001$; wbMC, wbLC, pMC, pLC: $n = 33$, $R = 0.87$, $R^2 = 0.75$, $P < 0.0001$; mpP: $n = 8$, $R = 0.89$, $R^2 = 0.7$P, $P = 0.003$; Fig. 3(A–C)]. There was a clear trend for all posterior condyle regions to have lower mean attenuation values, indicating that less contrast penetrated this less weight-bearing cartilage. The patellar values were clustered in a different location than the values for the other anatomical regions. When the data was analyzed for the tibio-femoral cartilage, the correlation coefficient was 0.92 [$n = 49$, $R^2 = 0.85$, $P < 0.0001$, Fig. 3(D)]. When all regions (including mpP cartilage) were pooled, the correlation diminished slightly [$n = 57$, $R = 0.86$, $R^2 = 0.73$, $P < 0.0001$, Fig. 3(E)].

To display the spatial agreement of both techniques, Fig. 4 shows representative images for cartilage attenuation for both CTa and μCT.

CTa corrected for structural composition of cartilage ECM

Figure 5 shows the results of the additional analysis into the role of structural composition of cartilage ECM for *non-equilibrium* CTa scans. When residual values from the model that fits μCT to ncCT, which represents structural composition of the ECM independent of sGAG, were added as a covariate to the model that fits μCT to CTa, the correlation coefficient was 0.95 ($n = 57$, $R^2 = 0.90$; $P < 0.0001$).

Discussion

Quantitative imaging techniques are of the utmost necessity for development and monitoring of treatment strategies targeted at early OA. Therefore, imaging techniques (e.g., like dGEMRIC) are extensively studied for their capability to measure sGAG content. This cadaver study demonstrates that cartilage attenuation from CTa is influenced by ioxaglate diffusion. And intra-articular injection of ionic ioxaglate significantly improved the correlation with the outcome of μCT. These results are similar to previous non-clinical reports[5,20,28], supporting our hypothesis that CTa can be used as a quantitative surrogate measure of the cartilage sGAG content.

Patellar cartilage is known to have a different structural ECM composition[29,30]. In the μCT and CTa scatter plot the patellar values were located differently than the other anatomical locations. Exclusion of patellar cartilage from our analysis improved the predictive value of CTa for sGAG content (R^2 from 73% to 85%), indicating that structural composition of cartilage ECM influences the outcome of *non-equilibrium* CTa. When residual ncCT values representing structural composition of cartilage ECM were combined with μCT (sGAG content) as a predictive value for CTa, the R^2 values from the model fit to CTa increased from 73% to 90%. This improvement indicates to what extent contrast diffusion into cartilage is influenced by structural composition of cartilage ECM. In clinical practice, a correction for different contrast diffusion rates cannot be calculated, since a reference standard for sGAG like EPIC-μCT is not available in clinical practice. Therefore, cartilage X-ray attenuation from CTa does not solely resemble sGAG content, but reflects a quality measure of cartilage which also concerns the structural integrity of the ECM.

Despite these encouraging results, there are limitations of CTa that need to be addressed. For example, the intra-articular injection introduces the risk of infection and also increases the risk of patient complaint of knee pain after injection. Furthermore, the high concentrations of ioxaglate used in this study, could influence cartilage electro-mechanical properties[31].

CTa is best applied in early stages of OA, because with severe sGAG loss in advanced stages of OA segmentation errors will occur[15]. Usually, early OA progression develops in relatively young patients and obviously the main concern with (repetitive) CT scans at a younger age is radiation exposure. The total dose of the scanning protocol in this study (~2 mSv) was 10 times higher in comparison to previously defined radiation doses of routine knee CT scans (~0.2 mSv)[32]. More research is needed to determine whether the same correlation with sGAG content can be measured if radiation dose is reduced.

Magnetic resonance imaging (MRI) uses no ionizing radiation and during the last years, has seen a rapid improvement with several newly developed MR-based imaging techniques to measure articular cartilage quality (e.g., dGEMRIC, Na^{23} mapping, T2 mapping, and T1rho[33,34]). Thanks to the more widespread availability of 3.0 Tesla MR systems and the development of novel MRI sequences (e.g., Ultrashort TE[35], SSFP[36], UTE T2*[37], and DENSE-FSE[38]), relatively fast MR scans can be acquired with high in plane resolution for (semi)quantitative cartilage imaging in OA

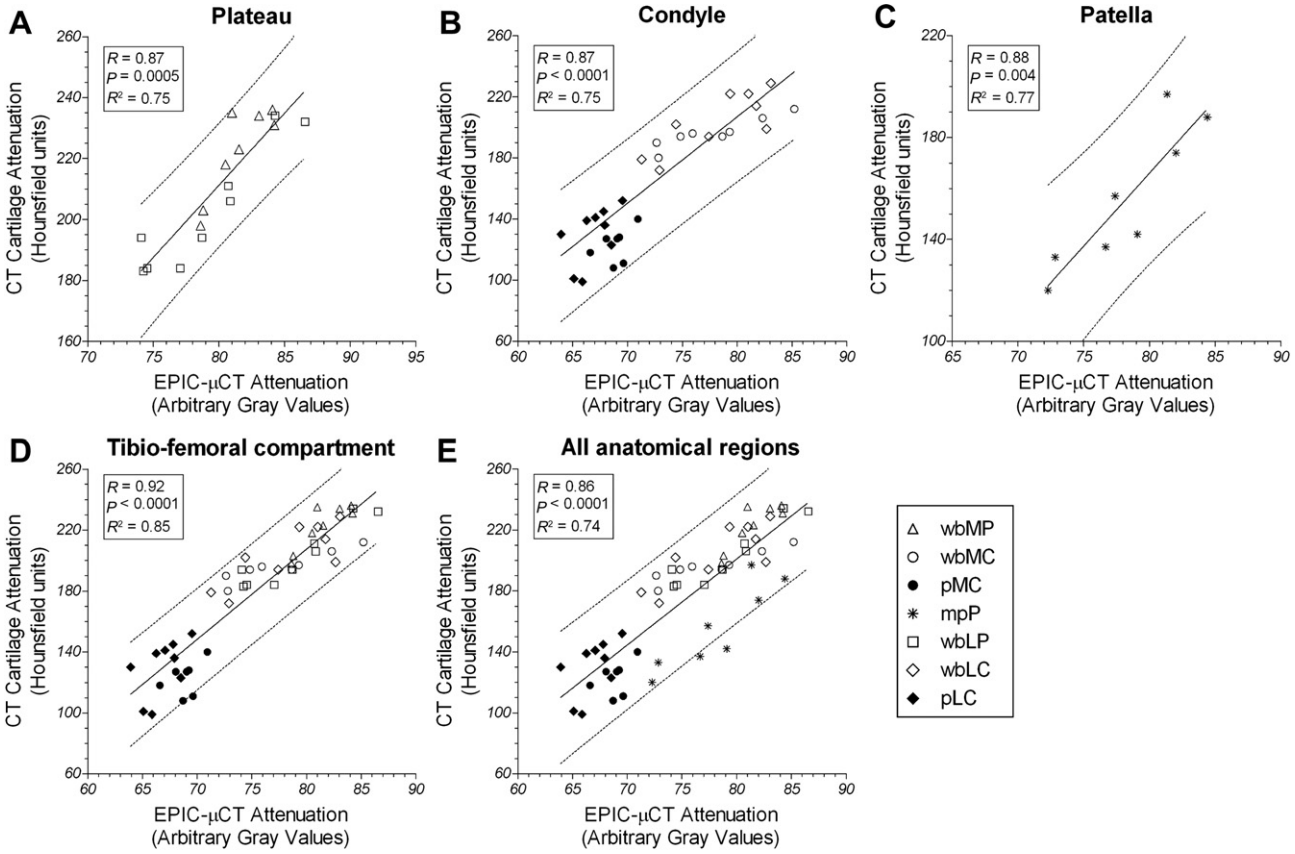

Fig. 3. Correlation plots of mean attenuation from EPIC-µCT and CTa. A: weight-bearing cartilage of medial and lateral plateaus ($n = 17$). B: weight-bearing and posterior cartilage of medial and lateral condyles ($n = 17$). C: mid-portion of patellar cartilage ($n = 8$). D: pooled results for both tibial and femoral compartments ($n = 49$). E: pooled results for all ROIs ($n = 57$). The dashed lines indicate the 95% confidence interval of the best fit regression line. wbMP: weight-bearing medial plateau, wbMC: weight-bearing medial condyle, pMC: posterior medial condyle, mpP: mid-portion patella, wbLP: weight-bearing lateral plateau, wbLC: weight-bearing lateral condyle, pLC: posterior lateral condyle.

research. However, these techniques still have several limitations: relative (e.g., claustrophobia) or absolute (e.g., pacemaker) contraindications for patients to undergo MRI, relatively low spatial resolution, and costs[39].

Given our results in relation to previously reported outcomes of *in-vivo* µCT arthrography studies in small animals with an intact circulation[14,15], we believe that CTa may be able to measure cartilage quality in human patients in a clinical setting. CT has a short

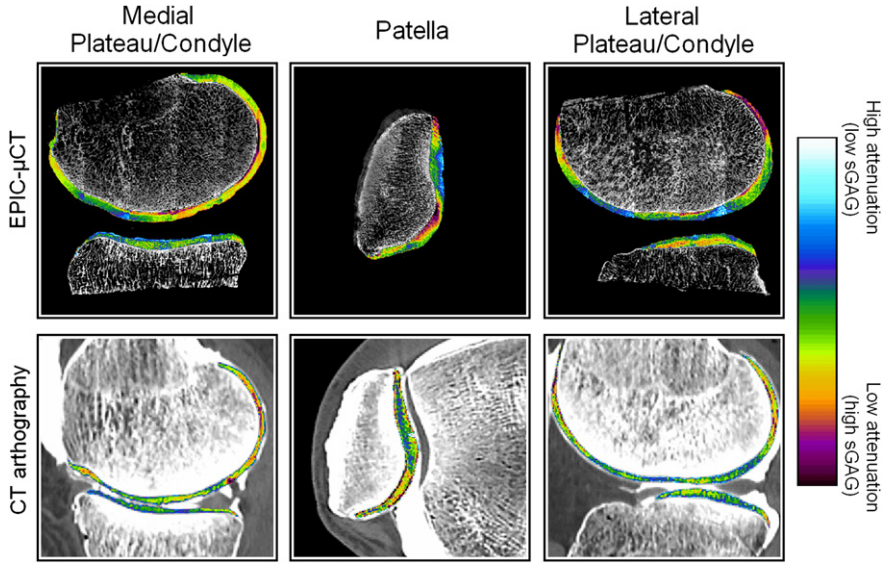

Fig. 4. Images of both EPIC-µCT and CTa. The attenuation of cartilage regions is visualized in colour and representative for sGAG content. High levels of attenuation represent a low sGAG-distribution.

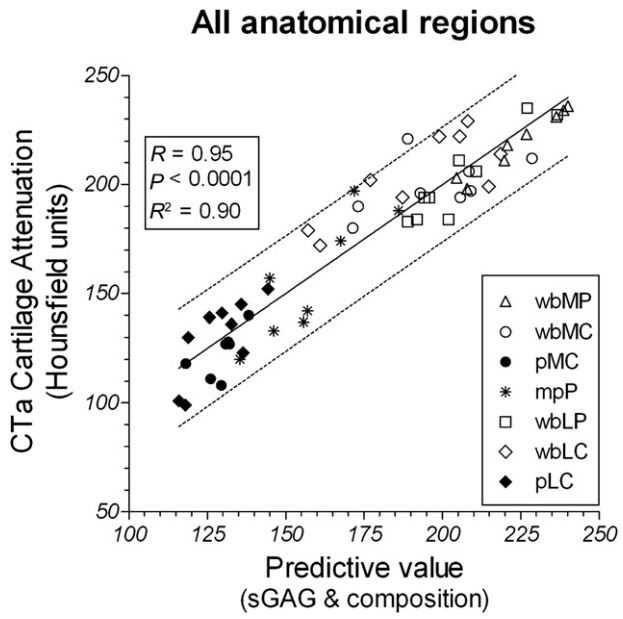

Fig. 5. Predictive CTa value (horizontal axis) based best fitted model from EPIC-μCT (sGAG) and ncCT residuals (cartilage ECM composition) correlated with mean attenuation of CTa (vertical axis) ($n = 57$). The dashed lines indicate the 95% confidence interval of the best fit regression line. wbMP: weight-bearing medial plateau, wbMC: weight-bearing medial condyle, pMC: posterior medial condyle, mpP: mid-portion patella, wbLP: weight-bearing lateral plateau, wbLC: weight-bearing lateral condyle, pLC: posterior lateral condyle.

scanning time (~30 s), generates images with a high isotropic resolution. Therefore, CT techniques may be a valuable alternative to MR techniques, but more research is needed for this technique to find its place in clinics and research.

In our opinion, research should first focus on optimizing the CTa protocol for clinical use. The reproducibility of CTa measurements should be evaluated in an *in vivo* environment in which all factors that influence CTa outcomes are present (intact circulation, muscle tension, joint capsule strength, etc). Future studies could also focus on the fact that recent *in-vitro* studies indicate that X-ray attenuation of cartilage can predict certain biomechanical properties such as compressive stiffness[28]. Our finding that CTa outcome is influenced by sGAG and structural composition of cartilage ECM could be used to predict the biomechanical function of articular cartilage with CT.

In conclusion, the results of this cadaver study demonstrate the proof-of-principle that CTa is able to measure cartilage quality in human knee joints. A wide implementation of this quantitative analysis of articular cartilage may detect early changes in OA patients and may contribute to the development of new treatment strategies.

Contributions

All authors have substantially contributed to the conception and design of the study, acquisition of data, or analysis and interpretation of data. All authors have participated in the writing process and approved the final version of the manuscript.

Role of funding source

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Conflict of interest

All authors indicate that they have no conflicts of interest.

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Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.joca.2011.07.006.

References

1. Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957;16:494–502.
2. Grushko G, Schneiderman R, Maroudas A. Some biochemical and biophysical parameters for the study of the pathogenesis of osteoarthritis: a comparison between the processes of ageing and degeneration in human hip cartilage. Connect Tissue Res 1989;19:149–76.
3. Felson DT, Lawrence RC, Dieppe PA, Hirsch R, Helmick CG, Jordan JM, *et al*. Osteoarthritis: new insights. Part 1: the disease and its risk factors. Ann Intern Med 2000;133:635–46.
4. Buckwalter JA, Mankin HJ. Articular cartilage: degeneration and osteoarthritis, repair, regeneration, and transplantation. Instr Course Lect 1998;47:487–504.
5. Palmer AW, Guldberg RE, Levenston ME. Analysis of cartilage matrix fixed charge density and three-dimensional morphology via contrast-enhanced microcomputed tomography. Proc Natl Acad Sci U S A 2006;103:19255–60.
6. Kallioniemi AS, Jurvelin JS, Nieminen MT, Lammi MJ, Toyras J. Contrast agent enhanced pQCT of articular cartilage. Phys Med Biol 2007;52:1209–19.
7. Xie L, Lin AS, Levenston ME, Guldberg RE. Quantitative assessment of articular cartilage morphology via EPIC-microCT. Osteoarthritis Cartilage 2009;17:313–20.
8. Eckstein F, Cicuttini F, Raynauld JP, Waterton JC, Peterfy C. Magnetic resonance imaging (MRI) of articular cartilage in knee osteoarthritis (OA): morphological assessment. Osteoarthritis Cartilage 2006;14(Suppl A):A46–75.
9. Eckstein F, Burstein D, Link TM. Quantitative MRI of cartilage and bone: degenerative changes in osteoarthritis. NMR Biomed 2006;19:822–54.
10. Bashir A, Gray ML, Hartke J, Burstein D. Nondestructive imaging of human cartilage glycosaminoglycan concentration by MRI. Magn Reson Med 1999;41:857–65.
11. Bashir A, Gray ML, Burstein D. Gd-DTPA2 – as a measure of cartilage degradation. Magn Reson Med 1996;36:665–73.
12. Bashir A, Gray ML, Boutin RD, Burstein D. Glycosaminoglycan in articular cartilage: in vivo assessment with delayed Gd(DTPA)(2-)-enhanced MR imaging. Radiology 1997;205:551–8.
13. Xie L, Lin AS, Guldberg RE, Levenston ME. Nondestructive assessment of sGAG content and distribution in normal and degraded rat articular cartilage via EPIC-microCT. Osteoarthritis Cartilage 2010;18:65–72.

14. Piscaer TM, Waarsing JH, Kops N, Pavljasevic P, Verhaar JA, van Osch GJ, *et al*. In vivo imaging of cartilage degeneration using microCT-arthrography. Osteoarthritis Cartilage 2008;16: 1011–7.
15. Siebelt M, Waarsing JH, Kops N, Piscaer TM, Verhaar JA, Oei EH, *et al*. Quantifying osteoarthritic cartilage changes accurately using in vivo microCT arthrography in three etiologically distinct rat models. J Orthop Res 2011.
16. Subhas N, Freire M, Primak AN, Polster JM, Recht MP, Davros WJ, *et al*. CT arthrography: in vitro evaluation of single and dual energy for optimization of technique. Skeletal Radiol 2010;39:1025–31.
17. De Filippo M, Bertellini A, Pogliacomi F, Sverzellati N, Corradi D, Garlaschi G, *et al*. Multidetector computed tomography arthrography of the knee: diagnostic accuracy and indications. Eur J Radiol 2009;70:342–51.
18. Wiener E, Settles M, Weirich G, Schmidt C, Diederichs G. The Influence of Collagen Network Integrity on the Accumulation of Gadolinium-Based MR Contrast Agents in Articular Cartilage. Fortschr Röntgenstr Online-Publikation; 2010.
19. Silvast TS, Jurvelin JS, Aula AS, Lammi MJ, Toyras J. Contrast agent-enhanced computed tomography of articular cartilage: association with tissue composition and properties. Acta Radiol 2009;50:78–85.
20. Silvast TS, Jurvelin JS, Lammi MJ, Toyras J. pQCT study on diffusion and equilibrium distribution of iodinated anionic contrast agent in human articular cartilage – associations to matrix composition and integrity. Osteoarthritis Cartilage 2009;17:26–32.
21. Silvast TS, Kokkonen HT, Jurvelin JS, Quinn TM, Nieminen MT, Toyras J. Diffusion and near-equilibrium distribution of MRI and CT contrast agents in articular cartilage. Phys Med Biol 2009;54:6823–36.
22. Maroudas A. Distribution and diffusion of solutes in articular cartilage. Biophys J 1970;10:365–79.
23. Perlewitz TJ, Haughton VM, Riley 3rd LH, Nguyen-Minh C, George V. Effect of molecular weight on the diffusion of contrast media into cartilage. Spine (Phila Pa 1976) 1997;22: 2707–10.
24. Bullough PG, Yawitz PS, Tafra L, Boskey AL. Topographical variations in the morphology and biochemistry of adult canine tibial plateau articular cartilage. J Orthop Res 1985;3:1–16.
25. Weiss C, Mirow S. An ultrastructural study of osteoarthritis changes in the articular cartilage of human knees. J Bone Joint Surg Am 1972;54:954–72.
26. Bryant D, Havey TC, Roberts R, Guyatt G. How many patients? How many limbs? Analysis of patients or limbs in the orthopaedic literature: a systematic review. J Bone Joint Surg Am 2006;88:41–5.
27. Park MS, Kim SJ, Chung CY, Choi IH, Lee SH, Lee KM. Statistical consideration for bilateral cases in orthopaedic research. J Bone Joint Surg Am 2010;92:1732–7.
28. Bansal PN, Joshi NS, Entezari V, Grinstaff MW, Snyder BD. Contrast enhanced computed tomography can predict the glycosaminoglycan content and biomechanical properties of articular cartilage. Osteoarthritis Cartilage 2010;18:184–91.
29. Kiviranta P, Rieppo J, Korhonen RK, Julkunen P, Toyras J, Jurvelin JS. Collagen network primarily controls Poisson's ratio of bovine articular cartilage in compression. J Orthop Res 2006;24:690–9.
30. Julkunen P, Korhonen RK, Nissi MJ, Jurvelin JS. Mechanical characterization of articular cartilage by combining magnetic resonance imaging and finite-element analysis: a potential functional imaging technique. Phys Med Biol 2008;53: 2425–38.
31. Urban JP, Hall AC, Gehl KA. Regulation of matrix synthesis rates by the ionic and osmotic environment of articular chondrocytes. J Cell Physiol 1993;154:262–70.
32. Biswas D, Bible JE, Bohan M, Simpson AK, Whang PG, Grauer JN. Radiation exposure from musculoskeletal computerized tomographic scans. J Bone Joint Surg Am 2009;91:1882–9.
33. Crema MD, Roemer FW, Marra MD, Burstein D, Gold GE, Eckstein F, *et al*. Articular cartilage in the knee: current MR imaging techniques and applications in clinical practice and research. Radiographics 2011;31:37–61.
34. Trattnig S, Domayer S, Welsch GW, Mosher T, Eckstein F. MR imaging of cartilage and its repair in the knee – a review. Eur Radiol 2009;19:1582–94.
35. Gatehouse PD, Thomas RW, Robson MD, Hamilton G, Herlihy AH, Bydder GM. Magnetic resonance imaging of the knee with ultrashort TE pulse sequences. Magn Reson Imaging 2004;22:1061–7.
36. Bieri O, Scheffler K, Welsch GH, Trattnig S, Mamisch TC, Ganter C. Quantitative mapping of T(2) using partial spoiling. Magn Reson Med 2011.
37. Williams A, Qian Y, Bear D, Chu CR. Assessing degeneration of human articular cartilage with ultra-short echo time (UTE) T2* mapping. Osteoarthritis Cartilage 2010;18:539–46.
38. Neu CP, Walton JH. Displacement encoding for the measurement of cartilage deformation. Magn Reson Med 2008;59: 149–55.
39. Guermazi A, Burstein D, Conaghan P, Eckstein F, Hellio Le Graverand-Gastineau MP, Keen H, *et al*. Imaging in osteoarthritis. Rheum Dis Clin North Am 2008;34:645–87.