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EXTENDED REPORT

Osteoarthritis, magnetic resonance imaging, and biochemical markers: a one year prospective study

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1ST LINE

Objective: To investigate the relation between biochemical markers of bone, cartilage, and synovial remodelling and the structural progression of knee osteoarthritis.

Methods: 62 patients of both sexes with knee osteoarthritis were followed prospectively for one year. From magnetic resonance imaging (MRI), done at baseline and after one year, the volume and thickness of cartilage of the femur, the medial tibia, and the lateral tibia were assessed. A whole organ magnetic resonance imaging score (WORMS) of the knee was calculated for each patient at baseline and at the one year visits. This score consists in a validated, semiquantitative scoring system for whole organ assessment of the knee in osteoarthritis using MRI. Biochemical markers (serum hyaluronic acid, osteocalcin, cartilage glycoprotein 39 (YKL-40), cartilage oligomeric matrix protein (COMP), and C-telopeptide of type I collagen (CTX-I), and urine C-telopeptide of type II collagen (CTX-II)) were measured at baseline and after three months.

Results: Baseline markers were not correlated with one year changes observed in cartilage volume and thickness. However, an increase in CTX-II after three months was significantly correlated with a one year decrease in mean thickness of medial tibial and lateral tibial cartilage. Patients in the highest quartile of three month changes in CTX-II experienced a mean loss of 0.07 (0.08) mm of their medial thickness, compared with a mean increase of 0.05 (0.19) mm for patients in the lowest quartile (p = 0.04) Multiple regression analysis showed that high baseline levels of hyaluronic acid are predictive of a worsening in WORMS (p = 0.004).

Conclusions: These results suggest that a single measurement of serum hyaluronic acid or short term changes in urine CTX-II could identify patients at greatest risk of progression of osteoarthritis.

See end of article for authors' affiliations

Correspondence to:
Dr Olivier Bruyere, University of Liège, Department of Public Health, Epidemiology and Health Economics, CHU Sart-Tilman, 4000 Liège, Belgium; olivier.bruyere@ulg.ac.be

Osteoarthritis of the knee joint is a progressive disorder often leading to permanent disability.[1] Traditionally, physical examination and plain radiographs have been relied upon in diagnosis, treatment selection, and research studies. However, these assessments do not provide a reliable tool for identifying patients who will experience a rapid progression of the disease (that is, in whom earlier intervention may be indicated).[2] Prospective clinical studies of new treatment options in osteoarthritis are lengthy and cumbersome owing to lack of sensitive methods for investigating disease progression and difficulties in the patient selection process. There is an urgent need for reliable, sensitive, and specific tools capable of promptly assessing the progression of this disease.[3]

Radiography is acknowledged to be the best validated method for assessing joint damage in osteoarthritis.[4] It allows the measurement of changes in joint space width (JSW), which currently remains the gold standard for evaluating the use of structure modifying drugs in osteoarthritis.[5] However, JSW measurement does not allow the detection of early structural damage nor is it an efficient way of monitoring the progression of osteoarthritis in daily practice.[4] Magnetic resonance imaging (MRI), with its superior soft tissue contrast, is the ideal technique for assessing normal articular cartilage and cartilage lesions. Joint imaging has the potential of providing morphological information, such as the presence of fissuring, partial or full thickness cartilage defects, and signal changes within residual cartilage.[5] Moreover, MRI, with its ability to discriminate articular tissues, holds the greatest potential as a tool for whole organ imaging of the joint. An MRI global knee joint score has recently been validated.[6]

The metabolic alterations in joint tissues associated with osteoarthritis involve changes in both the synthesis and degradation of matrix molecules, which are then often released as fragments into joint fluid, blood, and urine where they may be detected.[7] Markers that reflect the ongoing repair and degenerative processes occurring within a joint might be regarded as tools capable of predicting the rate of osteoarthritis progression. Biochemical markers have been shown to complement imaging techniques as surrogate markers of disease progression in a variety of diseases such as osteoporosis and rheumatoid arthritis.[4] In osteoarthritis, several biochemical markers of bone, cartilage, and synovium turnover have been reported to be potentially useful in identifying patients at high risk of rapid joint degradation.[3] However, the ability of different biochemical markers to detect early changes occurring in osteoarthritis and thereby to serve as prognostic tools for future cartilage alterations has not been exhaustively tested.[3–5]

This paper describes our investigation into the value of undertaking a single (at baseline) or repeated measurements (at three month intervals) of bone, cartilage, and synovial turnover markers for determining the severity of osteoarthritis and predicting long term (12 month) progression of knee osteoarthritis, as assessed by MRI.

Abbreviations: COMP, cartilage oligomeric matrix protein; FOV, field of view; JSW, joint space width; WORMS, whole organ magnetic resonance imaging score

METHODS

Patients

The cohort consisted in 62 patients (49 women) with medial compartment primary knee osteoarthritis. They were part of a non-intervention, prospective, study of 12 months' duration. They were enrolled if knee osteoarthritis was diagnosed at screening by the radiographic Kellgren-Lawrence grading scale for knee osteoarthritis.[8] All patients were classified as grade 2 or grade 3 at baseline. Osteoarthritis at other sites besides the knee was permissible. The main exclusion criteria were history or active presence of other rheumatic diseases that could be responsible for secondary osteoarthritis, medical or surgical treatment on the knee within the previous six months, weight exceeding 114 kg (250 lb), and any MRI contraindications. Patients had no known cancer or liver disease. We have also excluded from this study patients who had had intra-articular injections of steroids, in any joints, within the previous 30 days or more than three injections in the previous six months, as well as those having intra-articular injections of hyaluronic acid within the previous 90 days. During the study, no patient received intra-articular steroid injections or glucosamine treatment. However, during follow up, patients could have treatment with non-steroidal anti-inflammatory drugs for symptom relief.

MRI acquisition

MRI of the study knee of each patient was acquired with a 1.5 Tesla whole body scanner, using a commercial circumferential knee coil. Imaging sequences were as follows:

- axial T1 weighted spin echo: time of repetition (TR) in ms/ time of echo (TE) in ms, 700/11; 20 cm field of view (FOV); slice thickness/interslice gap, 5 mm/1 mm; 256×192 matrix, frequency encoding (FE) anterior-posterior, one excitation;

- coronal T1 weighted spin echo: 600/11, 16 cm FOV, 4 mm/ 0.5 mm, 256×192, FE superior-inferior, two excitations averaged;

- sagittal T1 weighted spin echo: 600/11, 16 cm FOV, 4 mm/ 0.5 mm, 256×92, FE anterior-posterior, two excitations averaged;

- sagittal T2 weighted fast spin echo: 2500/90; echo train length = 8; 14 cm FOV, 4 mm/0 mm, 256×192, FE superior-inferior, two excitations averaged, with fat suppression (frequently selective presaturation);

- sagittal fat suppressed T1 weighted, three dimensional, spoiled gradient echo (FS-3DSPGR): 58/6, 40° flip angle, 14 cm FOV, 256×128 matrix, 60 contiguous 2 mm slices covering all articular cartilage plates in the knee, FE, superior-inferior, one excitation, frequency selective fat saturation, and superior-inferior saturation bands to minimise pulsation artefacts.

The total time required for MRI, including patient set up, was 60 minutes. All images were visually inspected for quality. In case of poor quality, repeat images were acquired within a window of 10 days.

Volume and thickness assessment

Cartilage volume and thickness of the femur, the medial tibia, and the lateral tibia were assessed using three dimensional image processing. The cartilage was measured using a semiautomated region growing approach that has been presented and validated previously. In this technique, trained readers mark seed points within the cartilage and draw boundaries between low contrast regions of concurrent cartilage plates; the region growing technique then delineates the entire cartilage. Cartilage volumes were read in a paired fashion, but blinded to visit sequence.[9]

Whole organ MRI scoring (WORMS)

All images were transferred to a Sun Workstation and analysed using MRVision software (MRVision Inc, Menlo Park, California, USA) and were scored by validated readers. Images were scored with respect to 14 independent articular features: cartilage signal and morphology, subarticular bone marrow abnormality, subarticular cysts, subarticular bone attrition, marginal osteophytes, medial and lateral meniscal integrity, anterior and posterior cruciate ligament integrity, medial and lateral collateral ligament integrity, synovitis, loose bodies, and periarticular cysts or bursae. Two musculoskeletal radiologists undertook the readings independently. The final WORMS scores were tabulated as: (a) independent values for each feature in each of the three compartments of the knee (patello-femoral joint; medial femoro-tibial joint; lateral femoro-tibial joint); (b) cumulative surface feature scores (cartilage, marrow abnormality, subarticular cysts, bone attrition, osteophytes) for each compartment; (c) cumulative scores for each feature throughout the knee; and (d) a total combined score for the entire knee.[6] One reader read the entire set of images. The images were read paired, with the visit sequence being blinded. Before reading, the readers were trained by Dr Charles Peterfy. The reader showed excellent reproducibility, as described in a previous paper.[6]

Measurement of biochemical markers

Urine and blood collection were taken at baseline and after three months of follow up. Venous blood was sampled after an overnight fast and urine was collected on the second morning void. Blood was collected from the most visible vein near the elbow of the non-dominant arm (unless this was contraindicated), at least one hour after rising. Patients were not admitted to hospital. The time interval between blood sample collection and centrifugation for serum preparation was within two hours. The samples were stored at $-80°C$. The analyses were done within three months after storage.

Serum hyaluronic acid

Serum hyaluronic acid was measured by an enzyme linked binding protein assay, the ''Hyaluronic Acid (HA) Test'' (Corgenix Inc, Westminster, Colorado, USA), using hyaluronic acid binding protein isolated from bovine cartilage. The limit of detection of the method was 10 ng/ml. The within and between assay coefficients of variation were less than 10%.

Serum osteocalcin

Serum osteocalcin was measured by an immunoradiometric assay (IRMA), ''OSTEO-RIACT'' (CIS bio International, Gif-sur-Yvette, France), using two monoclonal antibodies raised against two sterically remote sites of the intact molecule. The limit of detection of the method was 0.4 ng/ml. The within and between assay coefficients of variation were less than 10%.

Serum intact or fragmented cartilage oligomeric matrix protein

Serum intact or fragmented cartilage oligomeric matrix protein (COMP) was measured by inhibition enzyme linked immunosorbent assay, ''Wielisa® hCOMP'' (Wieslab, Lund, Sweden), using a rabbit polyclonal antiserum directed to human COMP. The limit of detection of the method was estimated at 500 ng/ml. The within and between assay coefficients of variation were less than 11%.

Serum cartilage glycoprotein 39

Serum cartilage glycoprotein 39 (YKL-40) was measured using a sandwich enzyme immunoassay, ''Metra[TM] YKL-40

EIA'' (Quidel Co, San Diego, California, USA), employing a Fab fragment of a mouse monoclonal anti-human YKL-40 antibody as catcher and a rabbit polyclonal anti-human YKL-40 antibody for detection. The limit of detection of the method was estimated at 20 ng/ml. The within and between assay coefficients of variation were less than 10%.

Serum C-telopeptides of type I collagen

Serum C-telopeptides of type I collagen (CTX-I) was measured with an enzyme linked immunosorbent assay, "Serum CrossLaps® ELISA" (Nordic Bioscience Diagnostics A/S, Herlev, Denmark). This assay uses two monoclonal antibodies directed against a β-isomerised form of an eight amino acid sequence (EKAHD-βGGR) of the C-telopeptide of the α1 chain of type I collagen. The limit of detection of the method was estimated at 125 pM. The within and between assay coefficients of variation were less than 16%.

Urine C-telopeptides of type II collagen

Urine C-telopeptides of type II collagen (CTX-II) was measured with an enzyme linked immunosorbent assay, "u-CartiLaps® ELISA" (Nordic Bioscience Diagnostics). The assay uses a monoclonal antibody (MAbF46) highly specific for a six amino acid epitope (EKGPDP) derived from C-telopeptide of type II collagen. The limit of detection of the method was estimated at 0.17 µg/l. The within and between assay coefficients of variation were less than 12%. The assay values were corrected for urinary dilution by urine creatinine levels and finally expressed in µg/mmol creatinine. Urine creatinine was measured with the Jaffé method on a "Modular" auto-analyser (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

Quantitative variables at baseline were expressed as mean (SD) and qualitative as frequencies. Because the biomarkers levels were not normally distributed, they were log transformed before analysis. Relations between biochemical markers and clinical or radiological severity of knee osteoarthritis were assessed by the calculation of Spearman's correlation coefficients, by a multiple regression analysis and by a stepwise regression analysis. The results were considered significant at the 5% level ($p < 0.05$). No adjustment for multiple comparisons was made to the type I error rate, as the analysis was exploratory in nature. Statistical calculations were carried out with the Stastistica software.

RESULTS

The cohort included 62 patients (46 women), with a mean (SD) age of 64.9 (10.3) years and a mean body mass index of 28.1 (4.7) kg/m^2. Of these patients, 28 (45%) were classified as grade 2 by the Kellgren-Lawrence grading scale, and 34 (55%) as grade 3. MRI assessment of the knees showed a lateral tibial volume of 1742 (528) ml, a medial tibial volume

Table 1 Baseline biochemical marker levels

Variable	Median (range)
Serum hyaluronic acid (ng/ml)	69 (16 to 293)
Serum osteocalcin (ng/ml)	12.3 (5.4 to 39.3)
Serum COMP (ng/ml)	1627 (912 to 2755)
Serum YKL-40 (ng/ml)	82 (25 to 221)
Serum CTX-I (pM)	3030 (1035 to 8873)
Urine CTX-II (µg/mmol creatinine)	0.26 (0.07 to 0.89)

COMP, cartilage oligomeric matrix protein; CTX-I, C-telopeptides of type I collagen; CTX-II, C-telopeptides of type II collagen; YKL-40, cartilage glycoprotein 39.

Table 2 Three month biochemical marker levels

Variable	Median (range)
Serum hyaluronic acid (ng/ml)	68 (19 to 293)
Serum osteocalcin (ng/ml)	12.6 (5.2 to 31.3)
Serum COMP (ng/ml)	1711 (931 to 2784)
Serum YKL-40 (ng/ml)	77 (24 to 439)
Serum CTX-I (pM)	3112 (997 to 9688)
Urine CTX-II (µg/mmol creatinine)	0.27 (0.09 to 0.85)

COMP, cartilage oligomeric matrix protein; CTX-I, C-telopeptides of type I collagen; CTX-II, C-telopeptides of type II collagen; YKL-40, cartilage glycoprotein 39.

of 1301 (337) ml, a femur volume of 6316 (1231) ml, a lateral tibial thickness of 1.83 (0.35) mm, a medial tibial thickness of 1.52 (0.22) mm, a femur thickness of 1.17 (0.15) mm, and a WORMS of 43.8 (34.9). Biochemical markers rates of the population are summarised in tables 1 and 2.

Baseline markers were not correlated with one year changes observed in cartilage volume and thickness. In particular, we did not find any significant associations between CTX-II rate and cartilage changes (r values between 0.03 and 0.19 and p values between 0.16 and 0.87). However, an increase in CTX-II after three months was significantly correlated with a one year decrease in mean thickness of medial tibial ($r = -0.30$, $p = 0.03$) and lateral tibial cartilage ($r = -0.38$, $p = 0.005$). After adjusting for age, sex, and body mass index with multiple regression analysis, three month increases in CTX-II were significantly predictive of one year loss in thickness of the medial ($p = 0.03$) and lateral ($p = 0.001$) tibial cartilage. Patients within the highest quartile of three month changes in CTX-II (>0.056 µg/mmol) experienced a mean loss of 0.07 (0.08) mm of their medial thickness compared with a mean increase of 0.05 (0.19) mm for patients within the lowest quartile (< -0.055 µg/mmol) ($p = 0.04$ between the two groups) (fig 1). In patients with a decrease in the medial tibial cartilage thickness, CTX-II values did not change after three months ($+0.01$ (0.11)) compared with a small decrease of -0.11 (0.43) in patients with an increase of the medial tibial cartilage thickness ($p = 0.19$ between the two groups).

Baseline levels of COMP and CTX-II were correlated with baseline WORMS ($p = 0.03$ and $p = 0.0002$, respectively). These results remain significant after multiple regression analysis. Baseline hyaluronic acid was not significantly associated with baseline WORMS ($p = 0.06$). However, multiple regression analysis showed a strong positive association between the one year variation in the WORMS and the baseline sample of hyaluronic acid ($p = 0.004$). This indicates that high levels of hyaluronic acid are predictive of worsening osteoarthritis as assessed by MRI.
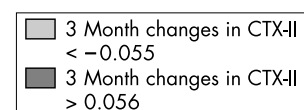

Figure 1 One year changes in medial tibia cartilage thickness stratified for quartiles of three month changes in CTX-II level. CTX-II, C-telopeptides of type II collagen.

DISCUSSION

Using a panel of biochemical indices of bone, cartilage, and synovial tissue turnover, we found that a single measurement of serum hyaluronic acid or short term changes in urine CTX-II could identify the patients at greatest risk of osteoarthritis progression.

Biochemical markers of bone, cartilage, or synovial remodelling have the potential to serve as diagnostic tools for osteoarthritis. Increases in serum or urine levels of COMP,[10–12] hyaluronic acid,[10 13 14] and CTX-II[10 15–17] have been described previously in osteoarthritis patients compared with controls. On the other hand, very few studies have assessed the relation between the structural severity of osteoarthritis and biochemical markers.[13 15 16 18] In this study, we showed no significant correlation at baseline between the level of any of the various biochemical markers and any single MRI feature (volume and thickness of cartilage of the medial tibia, the lateral tibia and the femur). However, when using a validated global MRI score, a significant association was found between damage severity score and two biochemical markers (COMP and CTX-II, $p = 0.03$ and $p = 0.0002$, respectively). To the best of our knowledge, the only other study using MRI assessment of joint damage showed that YKL-40, measured in synovial fluid, correlated with synovial membrane and the joint effusion volumes determined by MRI.[19] Using standard x ray, we previously reported the absence of significant associations, at baseline, between biochemical markers (serum keratan sulphate, serum hyaluronic acid, urine pyridinoline and deoxypyridinoline, serum osteocalcin, and COMP) and femoro-tibial joint space width in more than 200 patients with knee osteoarthritis.[18] Garnero et al reported that an increased CTX-II and glucosyl-galactosyl pyridinoline levels were significantly correlated with cartilage loss.[10] However, no significant associations were reported between joint space width and other biochemical markers (COMP, CTX-I, YKL-40, hyaluronic acid, and C reactive protein).[10] Obviously, joint space narrowing on standard x rays only produces an indirect assessment of cartilage damage. However, pathologically, osteoarthritis is an episodical inflammatory disorder of synovial joints characterised by the focal deterioration and abrasion of articular cartilage, with sclerosis and cyst formation in the underlying bone, as well as the formation of osteophytes at the joint surface.[20] Thickening of the joint capsule and chronic synovitis are also commonly reported features. In this study, we showed a significant association between global joint damage severity score and CTX-II and COMP levels. Our results corroborate the hypothesis that, because hyaluronic acid and COMP are not cartilage specific and because these biochemical markers levels were not correlated with knee cartilage volume or thickness, the pathogenesis of osteoarthritis not only involves cartilage but also bone and synovium.[21–23] Our results obtained with COMP and hyaluronic acid may suggest that synovial inflammation plays a central role in the pathogenesis of osteoarthritis. The key role of inflammation in osteoarthritis is supported by histological evidence of severe inflammation, raised levels of biomarkers related to synovitis, and heightened levels of pro-inflammatory cytokine expression in osteoarthritic chondrocytes. Moreover, MRI and ultrasonography have demonstrated synovitis in early osteoarthritis.

Another field of interest for biochemical markers is the identification of patients at increased risk of rapid disease progression. Some studies found a predictive value of hyaluronic acid,[13 24] C reactive protein,[25] COMP,[26 27] bone sialoprotein,[26] osteocalcin,[18] procollagen of type II collagen (PIINP),[28] CTX II,[15–17] Coll 2-1,[29] and Coll 2-1 NO_2[29] for radiographic progression. To the best of our knowledge, our study is the first to assess the value of biochemical markers for predicting the progression of osteoarthritis using MRI. We found that a single measurement of serum hyaluronic acid could predict a one year progression of the validated whole-organ MRI score of the knee in patients with osteoarthritis. Our results are in accordance with previous reports of the predictive value of hyaluronic acid[13 24] for joint space narrowing, assessed on x rays. Recently, Pavelka et al reported that osteoarthritis patients with high basic serum level of hyaluronic acid had a rapid radiological progression ($r = 0.30$, $p<0.005$).[13] In our study, we used a validated global joint score to assess structural severity. WORMS combines individual scores for articular cartilage, osteophytes, bone marrow abnormality, subchondral cysts, and bone attrition in 14 locations. It also incorporates scores for the medial and lateral menisci, anterior and posterior cruciate ligaments, medial and lateral collateral ligaments, and synovial distension.[6] Our results suggest that hyaluronic acid level predicts global damage in patient with knee osteoarthritis, and not only cartilage loss. This seems reasonable because, as a major product of synovial cells, hyaluronic acid is considered to be a marker of synovial inflammation[4] and so is not cartilage specific. This study suggests that short term changes in CTX-II identify patients at greatest risk of losing tibial cartilage after one year. These results are in accordance with our previous report that, when using x ray to assess joint damage severity, 12 month changes in CTX-II correlated with the changes in joint space width (on x ray assessment) observed after 36 months ($r = 0.43$, $p<0.05$).[16] Garnero et al reported that an increased level of U-CTX-II was correlated with the one year progression of knee joint damage in 67 patients with knee osteoarthritis (though the significance was borderline: $r = -0.27$, $p = 0.056$).[15] A recent study showed that, in an osteoarthritis cohort of 1235 men and women followed for a mean of 6.6 year, subjects with CTX-II levels in the highest quartile had a sixfold increased risk for progression of radiographic osteoarthritis in the knee.[20] It should be noted that the correlation coefficients were low (0.32 and 0.38) and account for little of the variance of cartilage lost. However, the association between biological markers and cartilage volume were stronger when the analysis was adjusted for age, sex, and body mass index, suggesting that the variance accounts for more than is apparent. It should also be noted that the importance of changes in thickness of less than 1 mm is not yet established. Several large ongoing studies such as the Osteoarthritis Initiative will be able to address this question. Our study, however, does clearly show a significant association between CTX-II and thickness loss. We must also acknowledge that the matrix of acquisition for the cartilage volume sequences might not be adequate to pick up small changes in volume. However, other studies have shown important results using a similar matrix size. In our present study, the size was chosen to minimise the time taken for the investigation, and thereby patient discomfort.

Conclusion

These results suggest that a single measurement of serum hyaluronic acid or short term changes in urine CTX-II may identify patients at greatest risk of osteoarthritis progression. This could be helpful for the clinician in identifying patients who should be treated with a structure modifying drug rather than with simple analgesics.

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Authors' affiliations
O Bruyere, J Collette, N Burlet, J-Y Reginster, WHO Collaborating Centre for Public Health Aspects of Osteoarticular Disorders, University of Liège, Liège, Belgium
M Kothari, S Zaim, D White, H Genant, C Peterfy, Synarc Inc, San Francisco, California, USA

D Ethgen, T Montague, C Dabrowski, GlaxoSmithKline, Collegeville, Pennsylvania, USA

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