Biochemical Markers of Bone and Cartilage Remodeling in Prediction of Longterm Progression of Knee Osteoarthritis

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ABSTRACT. ***Objective.*** To investigate the relationship between biochemical markers of bone and cartilage remodeling and severity or progression (symptoms and structure) of knee osteoarthritis (OA).

Methods. Mean and minimal joint space width (JSW) of the femorotibial joint were measured from standardized radiographs taken at baseline and at the end of a 3-year longitudinal study of patients with knee OA. Pain, stiffness, and physical function subscales of the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) index were assessed at the same time points. Biochemical markers [serum keratan sulfate (KS), serum hyaluronic acid (HA), urine pyridinoline (PYD) and deoxypyridinoline (DPD), serum osteocalcin (OC), cartilage oligomeric matrix protein (COMP)] were assessed at baseline and after 1 year.

Results. At baseline, no significant correlations were observed between values of biochemical markers and JSW or any of the WOMAC scores. Baseline markers were not correlated with 3-year percentage changes observed in mean or minimal JSW and WOMAC scores. Changes observed after 1 year in OC and HA were significantly correlated with 3-year progression in mean JSW ($r = –0.24$, $p = 0.04$ and $r = 0.27$, $p = 0.02$, respectively) and in minimal JSW ($r = –0.31$, $p = 0.01$ and $r = 0.24$, $p = 0.04$, respectively). In patients from the lowest quartile of 1-year changes in HA (< –21.22 ng/ml), mean JSW decreased after 3 years by 0.76 (1.23) mm compared to an increase of 0.11 (0.83) mm in patients in the highest quartile (> +14.34 ng/ml) ($p = 0.03$).

Conclusion. The 3-year radiological progression of knee OA could be predicted by a 1-year increase in OC or a 1-year decrease in HA levels. (J Rheumatol 2003;30:1043–50)

Key Indexing Terms:
OSTEOARTHRITIS PROGRESSION MARKERS OSTEOCALCIN

Osteoarthritis (OA) is a common cause of pain and disability in the adult population[1], causing a substantial health burden in most developed countries[2]. OA is associated with a loss of balance between synthesis and degradation of the macromolecules needed to provide articular cartilage with its biomechanical and functional properties. During the different stages of the development of OA, significant changes occur in both synthesis and structure of the matrix molecules of cartilage, bone. and synovial membrane[3,4]. These processes result in the destruction of joint cartilage with concomitant structural and functional changes in other joint tissues.

Assessment of standard radiographs is currently the most validated method to evaluate joint damage in OA[5]. This technique allows the measurement of changes in joint space width (JSW), which currently remains the gold standard for evaluation of structure-modifying drugs in OA[6,7]. However, JSW measurement does not allow detection of early structural damage nor does it constitute an efficient way of monitoring the progression of OA in daily practice[5]. The metabolic alterations in joint tissues associated with OA 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. Other potential markers of the OA disease process are associated with the increased production and release of enzymes or cytokines. Markers that reflect the ongoing repair and degradative processes occuring within a joint might be regarded as predictive tools of the rate of OA

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progression. Concentrations in articular fluid of molecular markers for both synthesis and degradation are consistent with the changes in metabolic rate observed for these molecules in human osteoarthritic cartilage *in vitro* and in animal models *in vivo*[3]. 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. Biochemical markers are thus investigated as tools for monitoring OA progression, but many uncertainties regarding their real value remain[5,8]. Biochemical markers are not often assessed in longterm, prospective studies dealing with large patient samples. We investigated the value of single (at baseline) or repeated (at 12-month interval) measurements of bone and cartilage turnover markers to assess the severity of and predict longterm (36-month) progression of knee OA.

Patients and methods. The study population as described[9] comprised 212 subjects over age 50 years, of both sexes, with primary knee OA. They were part of a double blind, placebo controlled study evaluating over a period of 3 years the symptomatic and structural effects of glucosamine sulfate. Knee OA was diagnosed according to the clinical and radiological criteria of the American College of Rheumatology[10]. Major exclusion criteria included: history or active presence of other rheumatic diseases that could be responsible for secondary OA; severe articular inflammation confirmed by physical examination (excluded also by erythrocyte sedimentation rate < 40 mm/h and serum rheumatoid factor < 1:40); traumatic knee lesions; overweight (body mass index > 30); substantial abnormalities in hematological, hepatic, renal, or metabolic functions; and use of intraarticular or systemic corticosteroids in the 3 months preceding enrollment.

Acquisition of radiographs. Standard radiographs were taken for each knee at baseline and after 3 years. Patients stood with knees fully extended and the posterior aspect of the knees in contact with a vertical cassette in a cassette holder. The lower limbs were internally rotated until the patella was centralized over the lower end of the femur. The feet were parallel and positioned a small distance apart. Foot maps were used for repositioning the patient at the time of subsequent radiographs. The x-ray beam was centered on the joint space and parallel to the tibial plateau. The focus film distance was 110 cm.

Joint space width measurement. Radiographs were digitized and image analysis was performed according to a validated technique[11], which located the proximal and distal joint margins excluding outlier points and calculated the mean JSW of the medial compartment of the femorotibial joint. A further analysis was performed assessing the minimum JSW, i.e., at the narrowest point of the medial compartment of the femorotibial joint, by visual determination using a 0.1 mm graduated magnifying lens[12].

Symptomatic measurement. At baseline and after 3 years, symptoms of OA were evaluated by a validated, disease-specific questionnaire, the Western Ontario and McMaster Universities (WOMAC) Osteoarthritis index, addressing severity of joint pain (5 questions), stiffness (2 questions), and limitation of physical function (17 questions), and referring to the 48 hours before assessment[13]. The visual analog scale version (VAS) of the index was used, i.e., the patient assessing each question with a 100 mm VAS, and the total index score was represented by the sum of the 24 component item scores.

Biochemical marker measurement. Urine and blood collections were taken at baseline and after 12 and 36 months of followup. Venous blood was sampled after an overnight fast and urine was collected on the second morning void.

Serum keratan sulfate (KS) was measured by an in-house competitive indirect ELISA according to the method described by Thonar, *et al*[14] with the same antibodies. The monoclonal anti-keratan sulfate was raised against a proteoglycan core antigen prepared by chondroitinase ABC digestion of human articular proteoglycan monomer. The limit of detection of the method was 5 to 10 ng KS-2 equivalent/ml. The within- and between-assay coefficients of variation were < 10% and < 15%, respectively.

Serum hyaluronic acid (HA) was quantified using the Pharmacia HA Test radiometric assay (Pharmacia & Upjohn, Uppsala, Sweden). The measure is based on the use of specific HA binding protein isolated from bovine cartilage. The limit of detection of the method was 5 ng/ml. The within- and between-assay coefficients of variation were < 10%.

Urine pyridinoline (PYD) and deoxypyridinoline (DPD) were measured using the Pyrilinks® and Pyrilinks-D™ competitive enzyme immunoassays (EIA) (Metra Biosystems, Mountain View, CA, USA). The limits of detection were 7.5 nM for PYD and 1.1 nM for DPD. For the 2 methods the within- and between-assay coefficients of variation were < 10% and < 12%, respectively. PYD and DPD concentrations were corrected for variations in urine concentration by expressing the results as nM/mM creatinine. The creatinine was measured by the method of Jaffé[15] on a MEGA autoanalyzer (Merck, Darmstadt, Germany).

Serum osteocalcin (OC) was quantified using the ELSA-OSTEO radioimmunoassay (IRMA) (CIS-Bio International, Gif-sur-Yvette, France). The antibody was raised against intact osteocalcin. The limit of detection of the method was 0.4 ng/ml. The within- and between-assay coefficients of variation were < 10%.

Serum intact or fragmented cartilage oligomeric matrix protein (COMP) was measured using the Wielisa® COMP inhibition ELISA (Wieslab, Lund, Sweden). The assay utilizes native human articular cartilage COMP coated to 96 well microtiter plates and a rabbit polyclonal antiserum directed to human COMP. The within- and between-assay coefficients of variation were < 11%.

Statistical analysis. Quantitative variables at baseline were expressed as mean ± SD and qualitative variables as frequencies. Relationships between biochemical markers and clinical or radiological severity of knee OA were assessed by calculation of Pearson correlation coefficients, by a multiple regression analysis, and by stepwise regression analysis. The whole study population (n = 212) was included in the evaluation of the relationships observed at baseline between markers and clinical or structural features of OA. The prospective part of the analysis, assessing the links between baseline or short term changes in markers and longterm changes in symptoms or structure, was performed in the placebo and the glucosamine sulfate groups separately, to avoid interaction of the treatment effect. The results were considered significant at the 5% level ($p < 0.05$). Statistical calculations were carried out with SAS software (SAS Institute, Cary, NC, USA).

RESULTS

Baseline demographics of the population are summarized in Table 1. At baseline, mean JSW and minimal JSW were not significantly correlated with any of the bone and cartilage biochemical markers. These markers were not correlated with the total WOMAC score or any of its subscales (pain, function, and stiffness).

Similarly, baseline values of the biochemical markers were not significantly correlated with the percentage changes observed after 3 years in JSW (mean and minimum) and WOMAC (total score, pain, function, and stiffness dimensions).

No significant correlations were observed between changes measured after 1 year in any biochemical marker and the longterm variations in the total WOMAC score or its subscales. One-year changes (value at 12 mo – value at baseline) (Table 2) in OC and HA levels were significantly correlated with 3-year changes in mean JSW (r = –0.24, p = 0.04 and r = 0.27, p = 0.02, respectively) (Figures 1 and 2) and minimal JSW (r = –0.31, p = 0.01 and r = 0.24, p = 0.04, respectively). However, from analysis of variance and a stepwise analysis, 1-year changes in HA were the only measure to be significantly correlated with 3-year changes in mean JSW (p = 0.02), while 1-year changes in OC remained significantly correlated with 3-year changes in minimal JSW (p = 0.004).

Table 1. Demographic and baseline characteristics of all randomized patients (n = 212).

Variables	Mean (SD)
Women, %	76
Age, yrs	66.0 (7.3)
Body mass index, kg/m^2	27.4 (2.9)
Serum osteocalcin, ng/ml	9.24 (3.6)
Urine pyridinoline, nM/mM creatinine	31.29 (11.5)
Urine deoxypyridinoline, nM/mM creatinine	6.41 (2.3)
Serum hyaluronic acid, ng/ml	67.8 (58.8)
Serum keratan sulfate, ngE/ml	456.4 (131.5)
Serum cartilage oligomeric matrix protein, µg/ml	1.5 (0.3)
Mean JSW, mm	5.3 (1.31)
Minimal JSW, mm	3.89 (1.31)
Total WOMAC index, VAS, mm (sum)	985 (481)
WOMAC pain, VAS, mm (sum)	183 (102)
WOMAC function, VAS, mm (sum)	705 (699)
WOMAC stiffness, VAS, mm (sum)	96 (58)

Table 2. One-year changes in biochemical markers (in %) in the placebo group.

Variables	Mean (SD)
Serum osteocalcin	0.07 (54.2)
Urine pyridinoline	5.9 (25.5)
Urine deoxypyridinoline	–14.1 (22.6)
Serum hyaluronic acid	–8.4 (56.4)
Serum keratan sulfate	–8.7 (22.3)
Serum cartilage oligomeric matrix protein	0.7 (18.5)

In patients with 1-year changes in HA below the median (less than –1.96 ng/ml), mean joint space decreased by 0.7 (1.18) mm, while patients with changes above this value had a gain in mean JSW of 0.08 (0.93) mm (p = 0.004). Dividing our population into quartiles of 1-year changes in HA, patients in the first quartile (< –21.22 ng/ml) experienced a mean JSW decrease of 0.76 (1.23) mm after 3 years compared to an increase by 0.11 (0.83) mm in patients in the fourth quartile (> +13.34 ng/ml) (p = 0.03; Figure 3). In the first quartile, 12/22 patients (54.55%) experienced a joint space narrowing > 0.5 mm over 3 years compared to 2/14 (14.29%) in the fourth quartile. The relative risk of experiencing a decrease in mean JSW over 3 years for patients in the first quartile (highest decrease in HA over 1 year) compared to the fourth was 3.818 (95% CI 1.001 to 14.561).

Patients with 1-year changes in OC below the median (less than 1.3 ng/ml) experienced a minimum joint space narrowing of 0.25 (1.11) mm over 3 years, while patients with changes above that value had a joint space narrowing of 0.67 (0.87) mm (p = 0.09). In the first quartile of 1-year changes in OC (< –2.15 ng/ml), minimum JSW decreased after 3 years by 0.24 (1.04) mm compared to 0.84 (0.90) mm in the fourth quartile (> +3.76 ng/ml) (p = 0.09; Figure 4). In the first quartile, 6/19 patients (31.58%) experienced a joint space narrowing > 0.5 mm over 3 years compared to 7/14 (50%) in the fourth quartile. The relative risk of having a decrease in minimal joint space over 3 years in patients in the first quartile (greatest decrease in OC over 1 year) compared to the fourth was 0.632 (95% CI 0.272 to 1.469).

Eventually, no significant correlation was observed between 3-year changes in biochemical marker values (Table 3) and 3-year changes in JSW (mean or minimal).

In the glucosamine sulfate group, no marker at baseline

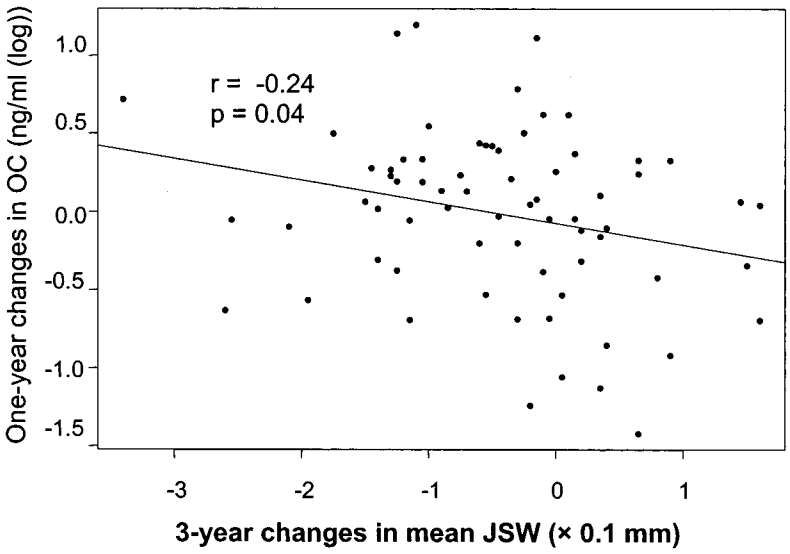

Figure 1. Correlation between 1-year changes in OC and 3-year changes in mean JSW.

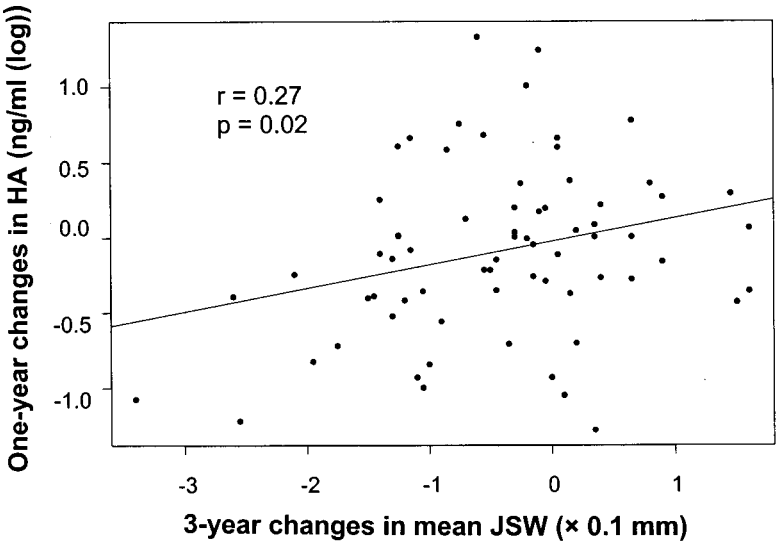

Figure 2. Correlation between 1-year changes in HA and 3-year changes in mean JSW.

was correlated with the 3-year changes in mean or minimal JSW. However, 1-year changes in KS were significantly correlated with 3-year changes in minimal JSW (r = 0.28, p = 0.02). In patients with 1-year changes in KS above the median (> –34.7 ngE/ml), joint space increased over 3 years by 0.35 (0.93) mm, while patients with changes in KS below the value (< –34.7 ngE/ml) had a mean joint space narrowing of 0.16 (0.82) mm. The difference between the 2 groups was statistically significant (p = 0.02).

DISCUSSION

Biochemical markers of bone and/or cartilage remodeling have been proposed as potential tools for the diagnosis of OA. An increase in serum or urine concentrations of COMP[16-20], KS[21-23], HA[16,24,25], and PYD and DPD[26] was described in patients with OA compared to controls. Similar observations have been reported for other molecular markers like C-reactive protein (CRP)[27,28], human cartilage glycoprotein-39 (YKL-40)[27], C-telopeptide of type II collagen[16], type II collagen propeptide[29,30], type III collagen N-propeptide[16,31], bone sialoprotein (BSP)[32], and chondroitin sulfate[33,34]. Most of these data were obtained from cross-sectional studies that rarely took into account the clinical and/or radiological ACR criteria[10] for OA diagnosis. On

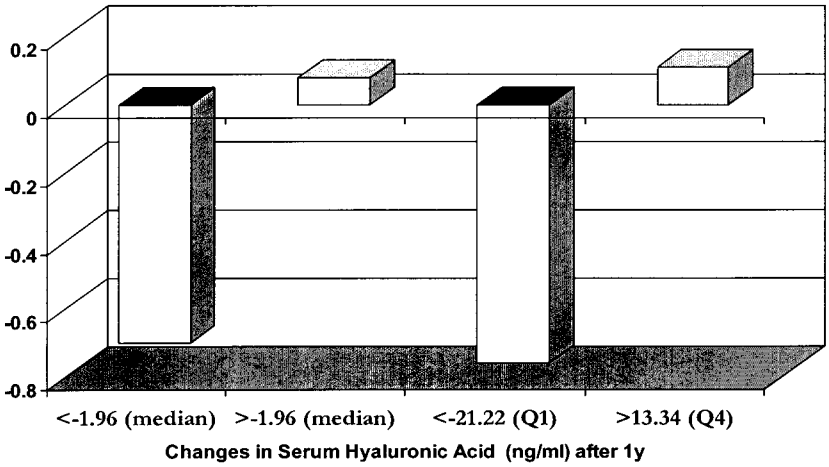

Figure 3. Mean JSW changes (mm) after 3-year followup in 106 patients taking placebo, both sexes.

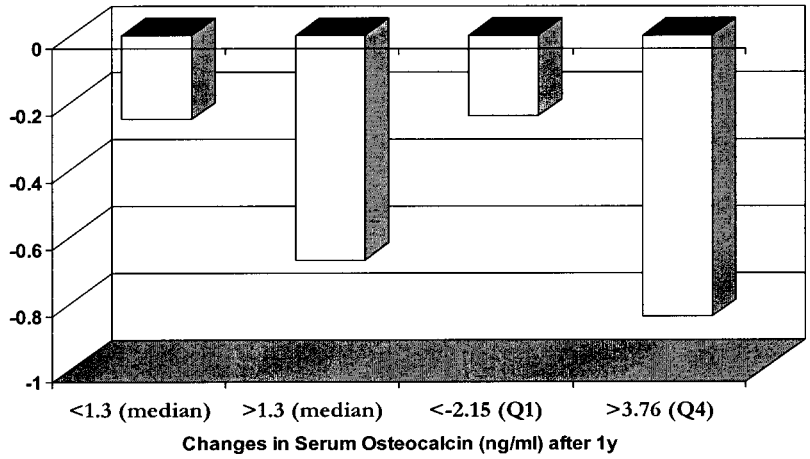

Figure 4. Minimal JSW changes (mm) after 3-year followup in 106 patients taking placebo, both sexes.

Table 3. Three-year changes in biochemical markers (in %) in the placebo group.

Variables	Mean (SD)
Serum osteocalcin	–23.6 (42.7)
Urine pyrydinoline	–18.7 (36.3)
Urine deoxypyridinoline	13.5 (40.0)
Serum hyaluronic acid	31.7 (0.52)
Serum keratan sulfate	–39.3 (20.4)
Serum cartilage oligomeric matrix protein	–9.3 (18.7)

the other hand, few studies have assessed the relationship between structural severity of OA and biochemical markers. Moreover, structural severity is generally based on structural change scores only. For example, joint space narrowing, assessed by the Kellgren-Lawrence grading scale[35], was found to be correlated with CRP[28]. With the same grading method, urine pyridinium crosslinks of collagen were increased in severe OA (with subchondral bone changes)[26]. The Kellgren-Lawrence score is widely used for OA studies and is simple but may sometimes lack accuracy[36]. Another method, more accurate and more reliable than scores, to evaluate OA severity is measuring JSW[36]. It can be measured manually (with a caliper, graduated plastic ruler, or graduated magnifying lens) or with a computer after digitization of the radiographs. In our study, JSW measured by either the computer assisted method or with the graduated magnifying lens failed to correlate significantly with any of the biochemical markers. To our knowledge, only one previous study correlated molecular markers and JSW, assessed with a computer assisted method at the medial knee joint. Garnero and colleagues reported[16] (67 OA patients, mean age 64 yrs) that within the biochem-

ical markers measured at baseline (serum OC, serum and urine C-telopeptide of type I collagen, serum COMP, urinary type II collagen C-telopeptide, serum HA, serum type III collagen N-propeptide, and urinary glucosyl-galactosyl PYD), increased urinary type II collagen C-telopeptide and glucosyl-galactosyl PYD were the only ones that correlated with cartilage loss assessed by joint space area ($r = –0.30$, $p < 0.03$ and $r = –0.38$, $p < 0.007$, respectively). This absence of correlation between JSW and OC, COMP, or HA is consistent with those observed in our study. Some elements may explain why our molecular markers did not correlate with JSW, measured with the computer or magnifying lens. JSW is only an indirect measure of cartilage volume and does not take into account other components of OA severity like the subchondral bone. It is now clear that subchondral bone is intimately involved in the pathology of OA[37]. Clinical and laboratory evidence indicates an altered subchondral bone metabolism in OA, possibly due to abnormal osteoblast behavior[4]. Further, none of our molecular markers are unequivocally cartilage-specific. OC, PYD and DPD are markers more specific for bone turnover[38], HA is considered a marker of synovial inflammation[5], and COMP lacks clear specificity[8]. Only KS epitopes could be considered as markers of aggrecan metabolism[5]. Indeed, it is not clear whether this marker reflects degradation of mature resident proteoglycan or of newly synthesized molecules[5].

When considering studies on hip OA[27,39], only serum COMP (but not YKL-40, BSP, or CRP) was correlated with baseline JSW measurement obtained by a digitized image analyzer. Serum COMP concentrations were also correlated with the yearly mean narrowing of the JSW[39]. These interesting results with COMP are not fully consistent with results obtained by us or others[16,40] on knee OA. Pathogenesis of OA is likely to be different depending upon the joint being considered, and results obtained at the hip should only be extrapolated to the knee with the greatest caution.

Garnero, *et al* also reported[16] that a higher WOMAC index at baseline was associated with increased levels of CRP and type III collagen N-propeptide. We found no statistical correlation between clinical severity of knee OA, assessed by the WOMAC, and any of the biochemical markers. This absence of association of the WOMAC score with the molecular markers we studied but not with type III collagen N-propeptide (a potential marker of synovium turnover[5,31]) or with CRP (a marker of disease activity) could be explained by the fact that while some pain may arise from accumulating structural damage shown by radiographs, pain may also be caused by intermittent phases of activity in the synovium or in the joint capsule[37].

Another objective of our study was to investigate whether biochemical markers are useful to predict the outcomes of OA in untreated patients. Symptoms and radiological changes of OA may reach a steady state in some patients, while in other individuals OA seems to continue to progress[41]. Identification of patients at risk for such a progression is thus of a clear interest. Moreover, because changes that occur in cartilage can be closely related to those observed in other tissues, it could theoretically be possible to identify OA progression[37] with biochemical markers of cartilage, bone, or synovium turnover. Unfortunately, the markers we studied were poorly correlated with 3-year clinical (WOMAC) or structural (JSW) changes in knee OA. Other studies found predictive values for HA[42], CRP[28] or COMP[32,39,40], and bone sialoprotein[32] for radiographic progression. For example, patients whose knee OA had progressed over 5 years were shown to have significantly higher concentrations of HA at baseline compared to those whose disease had not progressed[42]. In the same study population, an increase in serum COMP during the first year after study entry was associated with radiographic progression of the knee after 5 years of followup[40]. Nevertheless, as in previous cross-sectional studies, these studies only assessed changes in the JSW without computer assisted or graduated magnifying lens methods. In our study, 1-year changes in OC and HA were correlated with 3-year progression in mean and minimal JSW. So a more severe joint space narrowing after 3 years could be predicted by a greater increase in OC or a greater decrease in HA after 1 year. Progression of knee OA is characterized by phases of disease activity that involve the cartilage, bone, and synovium. Our results suggest that a higher level of bone remodeling, evidenced by a short term increase in the level of OC, could be predictive of longterm cartilage loss. Dieppe, *et al*, assessing the activity of subchondral bone by scintigraphy, showed that patients with positive scintiscans were the most likely to experience further cartilage loss over a period of 5 years[43]. A hypothesis to explain the more severe cartilage loss over 3 years in patients with the greatest decrease in HA levels after 1 year (Figure 3) is that a decrease in synovium activity (and the decrease in HA) could be a transient phenomenon announcing a phase of more abundant joint remodeling evidenced by a narrowing of the mean joint space width. Indeed, in our placebo population, HA levels decreased by 8.4% after 1 year, but increased by 31.7% after 3 years, compared to values at baseline. The implication of a dramatic structural alteration in some patients is that these patients are the best candidates for treatment aiming not only at improvement of OA symptoms but also at prevention of cartilage loss. We showed[9] that glucosamine sulfate acts as a disease-modifying drug by preventing cartilage loss over a period of 3 years compared to placebo. Such treatments should probably be preferentially directed to patients with the lowest increase in HA, who are most likely to experience an alteration of their structural integrity.

Eventually, we showed that short term (1-year) changes in KS concentrations predict longterm (3-year) radiological

evolution of knee OA in patients treated with glucosamine sulfate, and then could be useful to monitor structural response to therapy.

We should acknowledge, as a possible limitation of our study, that the weightbearing radiographic view that we used might not be the most accurate to assess JSW. In prospective studies, a frequent criticism concerning the weightbearing radiographic view is the change in patient positioning due to symptom modification. However, we believe it is unlikely that the symptom changes we observed in the 2 groups might have affected the results, given the mild to moderate disease and symptom conditions at baseline and throughout the initial study from which these results are derived[8]. Similarly, the relationship between symptom and structure modification in our study was of poor magnitude and marginal clinical relevance[44].

In conclusion, the markers we studied were not highly correlated with clinical (WOMAC) or structural (mean and minimum JSW) severity of knee OA. Consequently, they cannot be used as diagnostic tools in knee OA. Nevertheless, in view of our results, measurements of HA, and to a lesser extend OC, help to predict those patients who will experience the most dramatic structural progression over a long period, but only when considering patients with the lowest or highest levels. This could help the clinician decide which patients to treat with a structure-modifying drug rather than with simple analgesics. In addition, because HA and OC are not cartilage-specific, our results confirm that the pathogenesis of OA does not only involve cartilage but also bone and synovium. Synovial fluid markers may better reflect the local situation of the joint, but such samples are technically more difficult and ethically more problematic to get. Research in the field of biochemical markers of OA is growing and tests for other markers will soon be developed. Studies on potential OA molecular markers should include a large population over a long period with validated and sensitive techniques to assess severity or progression of OA. Moreover, with the emergence of effective symptomatic and/or structural treatments of OA, biochemical markers should also be assessed in longterm clinical trials with the aim of monitoring response to therapy appropriately.

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