

Cross-sectional Association of 10 Molecular Markers of Bone, Cartilage, and Synovium with Disease Activity and Radiological Joint Damage in Patients with Hip Osteoarthritis: The ECHODIAH Cohort*

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ABSTRACT. Objective. To investigate the associations of molecular markers of joint tissue turnover with clinical and radiological variables in patients with hip osteoarthritis (OA).

Methods. Patients of the ECHODIAH trial cohort (60% female; mean age 63 yrs, disease duration 5 yrs) fulfilling the American College of Rheumatology criteria for hip OA were studied. Pain was assessed using a 100 mm visual analog scale, and the presence of night pain and morning stiffness was observed as the index of joint inflammation. Joint space width (JSW) and subchondral bone sclerosis were assessed on hip radiographs. Ten markers were measured, 8 in serum: N-propeptides of collagen type I (PINP) and type III (PIIINP), cartilage oligomeric matrix protein (COMP), YKL-40, hyaluronan (HA), matrix metalloproteases (MMP1 and MMP3), and ultrasensitive C-reactive protein (CRP); and 2 in urine: C-terminal crosslinking telopeptides of collagen type I (CTX-I) and type II (CTX-II). Analyses of 376 patients with measurements of all the markers included principal component analyses to identify independent clusters of markers; followed by stepwise multivariate regressions to determine associations between markers, clinical variables, and radiographic signs of joint damage.

Results. Markers could be segregated into independent clusters: CTX-II, PINP, and CTX-I for cartilage degradation and bone turnover; COMP, PIIINP, and HA as potential markers of synovitis; and CRP and YKL-40, which are likely to indicate systemic inflammation; plus MMP1 and MMP3. After adjustment for age, sex, and body mass index, pain was significantly associated with CTX-II ($p = 0.0095$) and CRP ($p = 0.046$) and joint inflammation with COMP ($p = 0.013$). Radiographic signs of joint damage were associated with CTX-II ($p = 0.001$ for JSW; $p = 0.007$ for bone sclerosis).

Conclusion. This cross-sectional study of OA molecular markers in a large cohort may provide biological evidence of different pathophysiological processes involved in hip OA. Among the markers measured, CTX-II showed the most consistent association with the symptoms and joint damage of OA. (J Rheumatol 2005;32:697-703)

Key Indexing Terms:

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The most established and validated method for assessing joint damage in osteoarthritis (OA) is joint space width (JSW) measurement using plain radiographs^{1,2}. However, radiographs provide only an indirect measure of cartilage

loss, and when radiological diagnosis of OA is established there is often already significant joint damage. In addition, because changes of JSW are small compared to the precision error of radiographs, at least one year and preferably 2 years

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*ECHODIAH: Evaluation of the Chondromodulating Effect of Diacerein in Osteoarthritis of the Hip

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are necessary to accurately assess the progression of joint damage or its reduction by treatment³. Clearly, for identifying patients with high risk for destructive OA and for monitoring drug efficacy there is an urgent need for techniques more sensitive than plain radiographs. Magnetic resonance imaging is currently being optimized for this purpose; alternatively, specific and sensitive biochemical markers revealing abnormalities of the turnover of bone, cartilage, and synovial tissue may be useful for investigation and monitoring of OA.

Molecular markers are molecules or fragments thereof of connective tissue matrices that are released into biological fluids during the process of tissue biosynthesis and turnover and which can be measured by immunoassays. Several molecular markers of bone, cartilage, and synovium have been described and their changes have been investigated in patients with OA, mainly in cross-sectional studies (as reviewed⁴). In most of these studies, involving mainly small populations of patients with knee OA, molecular markers have been examined one or 2 at a time to evaluate their relationships with clinical and/or radiological disease variables. Recently, Otterness, *et al*^{5,6}, in a small population including patients with knee (n = 34) and hip (n = 8) OA, evaluated 14 different molecular markers and found that they could provide additional information on the clinical symptoms of the disease. However, they did not evaluate relationships with joint damage and did not include the measurements of markers of type II collagen degradation, the hallmark of OA.

We analyzed the relationships between systemic concentrations of 10 different molecular markers of bone, cartilage — including measurement of type II collagen degradation — and synovium metabolism with both clinical variables and radiological signs of the disease in a large homogeneous population of patients with hip OA participating in the ECHODIAH cohort.

MATERIALS AND METHODS

Patients with hip OA. Outpatients fulfilling the American College of Rheumatology criteria for the diagnosis of hip OA⁷ were recruited from 26 rheumatology departments in France to participate in the randomized prospective study ECHODIAH, as described⁸. Briefly, clinical criteria for inclusion were the presence of symptomatic disease, as defined by the presence of daily hip pain for at least one month during the past 2 months, and a Lequesne algofunctional index of at least 3 points⁹. The radiographic criterion for inclusion was JSW between 1 and 3 mm. If JSW exceeded 3 mm, it had to be at least 0.5 mm thinner than the JSW of the contralateral hip, measured at its narrowest point. The main criteria for exclusion were evidence of secondary hip OA, medial femoral head migration, intraarticular injection or arthroscopy or corrective surgery of the hip joint during the 3 months prior to inclusion in the study, and total replacement of the contralateral hip joint < 6 months prior to inclusion. Analysis was performed on the 376 patients among the total 507 patients of the study who had baseline serum and urine samples and clinical and radiological data available.

The clinical characteristics of this subgroup of patients were similar and did not differ significantly from the 131 who could not be assessed for biochemical markers (Table 1).

Symptomatic outcome variables of OA. At the time of collection of serum

Table 1. Baseline characteristics of 376 patients with hip OA included in the study population and 131 patients who did not have serum and/or urine for biochemical marker assessment.

Characteristic	Study Population, n = 376	Not in the Study Population, n = 131
Age, mean \pm SD yrs	62.4 \pm 7.0	62.9 \pm 6.5
Sex, % male	40.4	38.9
Body mass index, mean \pm SD, kg/m ²	25.7 \pm 3.5	26.0 \pm 3.7
Disease duration, mean \pm SD yrs	4.5 \pm 4.8	4.6 \pm 4.8
Hip OA localization, %		
Superolateral	59.3	57.3
Superomedial	31.9	30.5
Concentric	8.8	12.2
Total OA score mean \pm SD score	280.5 \pm 157.3	268.9 \pm 137.5
Symptomatic severity		
Pain score on VAS, mean \pm SD, mm	44.3 \pm 20.0	46.3 \pm 20.1
Functional impairment by Lequesne index, mean \pm SD score	2.29 \pm 1.17	2.50 \pm 1.25
Global patient assessment, mean \pm SD, mm	42.9 \pm 24.4	45.2 \pm 24.4
Night pain and/or morning stiffness, %	89.9	92.4
Structural severity by hip radiographs		
Joint space width, mean \pm SD, mm	2.30 \pm 0.87	2.27 \pm 0.80
Subchondral bone sclerosis, %	28.5	26.2

and urine, 4 different symptomatic domains were evaluated: (1) “pain,” using a visual analog scale (VAS; 0–100 mm) in response to a question related to the level of pain occurring after physical activities during the last 3 days; (2) “functional impairment,” using the 4 questions of the Lequesne index related to physical activities, and for the response of each question, a 4-point Likert scale was used (0 = easy, 0.5 = some difficulty, 1.0 = with difficulty, 1.5 = with a lot of difficulty, 2.0 = impossible). The functional impairment scale (the sum of the answers from the 4 questions) ranged from 0 to 8. (3) “Patient’s global assessment,” using a VAS (0–100 mm) in response to questions related to the level of handicap estimated by the patients; and (4) “inflammation,” using 2 questions from the Lequesne index related to night pain (a 2-point Likert scale: yes = 1, no = 0) and to morning stiffness (a 3-point Likert scale: none = 0, less than 15 minutes = 1, more than 15 minutes = 2). The inflammation scale for scoring answers from the 2 questions ranged from 0 to 4.

Radiological variables of OA. Pelvic radiographs were obtained with patients placed in a weight-bearing position and standing 1 m from the x-ray source with 20° internal foot rotation as described¹⁰. As predefined in the protocol, the JSW of the signal hip was measured centrally by a single, senior expert radiologist (ML) who was unaware of patient’s identity. The reader determined the location of the narrowest point of the JSW on the radiographs. The anatomic limits for the measurement of JSW were the bone contour of the femoral head and that of the acetabular roof; both were marked with a dedicated pencil. Finally, the distance between these limits was measured using a 0.1 mm graduated magnifying glass. The intraobserver reproducibility of this technique has an intraclass correlation coefficient of 0.96¹¹. The reader also determined the presence (coded 1) or absence (coded 0) of subchondral bone sclerosis.

Molecular markers. Fasting blood samples were collected in all patients between 8 and 10 A.M. Second morning void urine samples were also collected in plastic containers. Several aliquots of serum and urine were performed and stored centrally at –20°C until assay. All molecular markers were measured in the baseline samples, i.e., before diacerein treatment was initiated.

Serum markers. Eight different serum molecular markers were measured. Intact procollagen type I N-propeptide (S-PINP) was measured by an electrochemiluminescence immunoassay based on monoclonal antibodies raised against purified intact human PINP and detecting both intact mono and trimeric forms, but not fragments, using an automated analyzer (Elecsys; Roche Diagnostics, Penzberg, Germany). Intraassay coefficient of variation (CV) is lower than 2% and interassay CV, lower than 4%^{12,13}. Serum procollagen type III N propeptide (S-PIIINP) was measured by radioimmunoassay based on a polyclonal antibody (PIIINP-RIA kit; Farnos Diagnostica, Oulunsala, Finland). The assay detects the authentic propeptide and other larger related antigens, but is insensitive to the degradation products of the propeptide¹⁴. Intra and interassay CV ranged from 6% to 8%. Serum cartilage oligomeric matrix protein (S-COMP) was measured by a 2-site immunoassay (COMPTM ELISA kit; AnaMar Medical, Lund, Sweden)¹⁵. Intra and interassay CV are below 7% and 8%, respectively. Serum hyaluronic acid (S-HA) was measured by RIA (Pharmacia HA test; Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden), based on the use of specific HA binding protein isolated from bovine cartilage¹⁶. Intra and interassay CV are lower than 10%. Serum human cartilage glycoprotein 39 or YKL-40 was measured by a 2-site ELISA using antibodies raised against YKL-40 purified from supernatants of MG63 human osteosarcoma cell line (Chondrex, Metra Biosystems, Mountain View, CA, USA). Intra and interassay CV are lower than 4% and 6%, respectively¹⁷. Matrix metalloproteinases 1 and 3 (S-MMP1 and S-MMP3) were measured using 2-site ELISA using 2 antibodies raised against the human MMP and recombinant human MMP as a standard (Amersham Pharmacia Biotech, Buckinghamshire, UK). The assays recognize total MMP including pro-MMP, free MMP, and the complexes of MMP and tissue inhibitors of MMP. The assays do not crossreact with MMP bound to the nonspecific protease inhibitor α_2 -macroglobulin. Intra and interassay CV are lower than 8% and 13%, respectively^{18,19}. Serum C-reactive protein (S-CRP) was assayed using an ultrasensitive immunonephelometry method (N Latex CRP mono; Behringwerke AG, Marburg, Germany) on a BNA Behring nephelometer. The intra and interassay variations are lower than 5% and the detection limit is 0.2 mg/l.

Urinary markers. Urinary excretion of β -isomerized C-terminal crosslinking telopeptide of type I collagen (U-CTX-I) was measured by the Crosslaps ELISA (Nordic Bioscience, Herlev, Denmark). This assay uses a polyclonal antiserum raised against β -isomerized EKAH β DGGR sequence of the C-telopeptide of $\alpha 1$ chains of human type I collagen. Intra and interassay CV are lower than 6% and 9%, respectively²⁰. Urinary C-terminal crosslinking telopeptide of type II collagen (U-CTX-II) was measured by ELISA based on a mAb raised against the EKGPD linear amino acid peptide specific of the type II collagen C-telopeptide (Cartilaps; Nordic Biosciences). Intra and interassay CV are lower than 8% and 10%, respectively²¹.

All measurements were performed centrally, blinded for patient's identity and clinical and radiographic data, in the following laboratories: (1) Synarc, Lyon, France (Dr. P. Garnero) for S-PINP, S-PIIINP, S-HA, S-CRP, U-CTX-I, and U-CTX-II; (2) Laboratoire d'immunologie CHU Toulouse — Hôpital Rangueil, Toulouse, France (Dr. M. Abbal) for S-MMP1, S-MMP3; (3) Service de Biochimie 3, Hôpital de la Grave, Toulouse, France (Dr. J-P. Salles) for S-YKL-40; and (4) AnaMar Medical AB, Lund, Sweden (Dr. C. Freiburghaus) for S-COMP.

Statistical analyses. All data are expressed as mean \pm SD unless otherwise specified. Because molecular markers were not normally distributed, data were normalized using logarithmic transformation for all analyses. Molecular markers were grouped into clusters of related measures by principal component analyses using repeated oblique component analysis (SAS procedure VARCLUS). In this analysis, markers are consecutively divided into different clusters when their coefficient was ≤ 0.85 .

Associations between molecular marker concentrations and clinical and radiological OA indicators were assessed by multivariate linear (continuous) or logistic (dichotomous variables) regression analyses. Differences in continuous and categorical variables between tertiles of markers were ana-

lyzed by analysis of variance and chi-square test, respectively. All analyses were performed using SAS (SAS Institute, Cary, NC, USA).

RESULTS

The mean, median, and variability of molecular markers in patients with hip OA are shown in Table 2. Factor analysis by the principal component method was carried out to determine associations between molecular markers. The 5 principal components, which accounted for 65% of the total variance, summarized the relationships between the different molecular markers in this population of patients with hip OA. Table 3 shows the loading coefficients of each of the 10 molecular markers of the 5 principal components, where each of the primary contributors is indicated in bold type. Factor 1 comprised markers of bone (S-PINP, U-CTX-I) and cartilage (U-CTX-II) turnover. Factor 2 comprised S-COMP, S-HA, and S-PIIINP. Factor 3 comprised the markers of systemic inflammation S-CRP and S-YKL-40. MMP-1 and MMP-3 segregated into 2 separate factors 4 and 5, respectively.

In bivariate analyses, urinary CTX-II correlated significantly with joint pain ($r = 0.13$, $p = 0.0095$), joint space width ($r = -0.20$, $p < 0.0001$), and presence of bone sclerosis ($r = 0.15$, $p = 0.007$) after adjustment for sex, age, and body mass index (BMI). Among the 9 other markers, significant associations were found between S-CRP and pain ($r = 0.15$, $p = 0.045$) and between S-COMP and joint inflammation ($r = 0.13$, $p = 0.013$).

To establish the independent associations of the markers with indicators of severity of hip OA, we performed multivariate linear (for continuous variables) or logistic (for inflammation and subchondral bone sclerosis) regression

Table 2. Levels of molecular markers of joint tissue turnover in 376 patients with hip OA. Samples were available for all markers in the 376 patients.

Molecular Markers	Mean \pm SD	Median (interquartile range)
Serum markers		
S-PINP, ng/ml	41.9 \pm 18.3	40.3 (22.6)
S-PIIINP, μ g/l	4.27 \pm 1.94	4.03 (1.51)
S-COMP, U/l	10.77 \pm 2.70	10.51 (3.35)
S-HA, ng/ml	135 \pm 137	99 (96)
S-YKL-40, ng/ml	87.9 \pm 85.7	64.5 (63.0)
S-MMP-1, ng/ml	6.50 \pm 4.68	5.20 (5.68)
S-MMP-3, ng/ml	25.4 \pm 28.6	19.6 (21.1)
S-CRP, mg/l	3.07 \pm 4.58	1.60 (2.40)
Urinary markers		
U-CTX-I, μ g/mmol Cr	184 \pm 100	167 (107)
U-CTX-II, ng/mmol Cr	317 \pm 211	257 (239)

PINP: procollagen type I N-propeptide; PIIINP: procollagen type III N propeptide; COMP: cartilage oligomeric matrix protein; HA: hyaluronic acid; YKL-40: human cartilage glycoprotein 39; MMP-1: matrix metalloproteinase 1; MMP-3: matrix metalloproteinase 3; CRP: C-reactive protein; CTX-I: C-terminal cross-linking telopeptide of type I collagen; CTX-II: C-terminal cross-linking telopeptide of type II collagen.

Table 3. Principal component analysis coefficients of independent molecular marker factors in 376 patients with hip OA. Molecular markers were grouped into factors of related measures by principal component analyses using repeated oblique component analysis (primary components of each factor are shown in bold type). In this analysis, markers are consecutively divided into different factors when their coefficient was ≤ 0.85 . All marker values were logarithmic transformed before analyses.

Molecular Marker	Factor 1 [†]	Factor 2	Factor 3	Factor 4	Factor 5
S-PINP	0.751	0.365	−0.063	−0.051	−0.187
U-CTX-I	0.867	0.058	−0.056	0.028	−0.225
U-CTX-II	0.741	0.204	0.067	0.049	−0.223
S-COMP	0.115	0.641	0.106	−0.005	0.053
S-PINP	0.179	0.798	0.196	−0.113	−0.021
S-HA	0.246	0.674	0.187	−0.064	0.064
S-YKL-40	0.022	0.244	0.760	0.088	0.145
S-CRP	−0.060	0.110	0.760	0.083	0.050
S-MMP1	0.012	−0.091	0.112	1.000	0.047
S-MMP3	−0.268	0.040	0.128	0.047	1.000

Abbreviations for each marker are given in Table 2.

including sex, age, BMI, and logarithmic transformed molecular marker data. As shown in Table 4, U-CTX-II and S-CRP were independently associated with pain; U-CTX-II and S-PINP with patient global assessment; S-COMP with joint inflammation; and U-CTX-II with JSW and subchondral bone sclerosis. The contribution of these molecular markers to the interindividual variation of the clinical variables and radiological signs of hip OA was minor, and accounted for less than 7.1% of the variance together with age, sex, and BMI. The contribution of urinary CTX-II to explain structural damage was, however, more important than age, sex, and BMI together, with r^2 value increasing from 1.4%–1.5% to 4.3% (Table 4). When patients were categorized in tertiles of nontransformed molecular marker levels, we found a significant association between increased U-CTX-II and S-CRP and pain (Figure 1). As patients in the 2 highest tertiles of either U-CTX-II or CRP had similar

pain levels, we combined patients with marker levels in the 2 highest tertiles. Patients with U-CTX-II and/or S-CRP in the 2 highest tertiles ($n = 336$) had on average a pain VAS score that was 28% higher (45.3 ± 20.0 vs 35.5 ± 18.0 mm; $p = 0.003$) than patients with both U-CTX-II and S-CRP in the lowest tertile ($n = 40$; Figure 1). Patients with urinary CTX-II in the lowest tertile had on average a JSW that was 23% ($p < 0.0001$) greater than patients with U-CTX-II in the highest tertile (Figure 1).

DISCUSSION

This is the first study analyzing concomitantly a panel of 10 different molecular markers measured centrally in a large population of well characterized patients with hip OA. Using principal component analyses, we found that the different markers could be segregated into 5 different clusters that may reflect different pathophysiological processes of

Table 4. Multivariate regression analyses between molecular markers, clinical variables, and radiological signs in 376 patients with hip OA. All marker values were logarithmic transformed before analyses.

Independent Predictor	Joint pain		Patient Global Assessment		Joint Inflammation		Joint Space Width		Subchondral Bone Sclerosis	
	β^*	p	β	p	β	p	β	p	β	p
Sex	4.14	0.06	8.77	0.001	0.57	0.004	0.013	0.89	−0.67	0.009
Age	−0.23	0.12	−0.38	0.037	−0.03	0.015	−0.009	0.18	−0.0035	0.84
BMI	0.79	0.009	0.73	0.040	0.01	0.71	0.013	0.31	−0.032	0.36
r (p) value of model for sex, age, and BMI**	0.20 (0.0008)		0.22 (0.0005)		0.17 (0.011)		0.12 (0.15)		0.12 (0.26)	
U-CTX-II	0.017	0.001	0.017	0.036			−0.001	0.0009	0.0021	0.0072
S-PINP			−0.151	0.048						
S-CRP	1.103	0.046								
S-COMP					0.091	0.012				
r (p) value of global model**	0.27 (< 0.0001)		0.25 (0.0002)		0.22 (0.0014)		0.21 (0.0025)		0.21 (0.021)	

Abbreviations for each marker are given in Table 2. * β is the coefficient of each independent variable included in the regression model and p the corresponding significance level. Only molecular markers that contributed significantly to the model are shown. ** r represents the correlation coefficient (and associated p value) of the model including sex, age, and body mass index (BMI) only, and then when molecular markers are added to the model (global model).

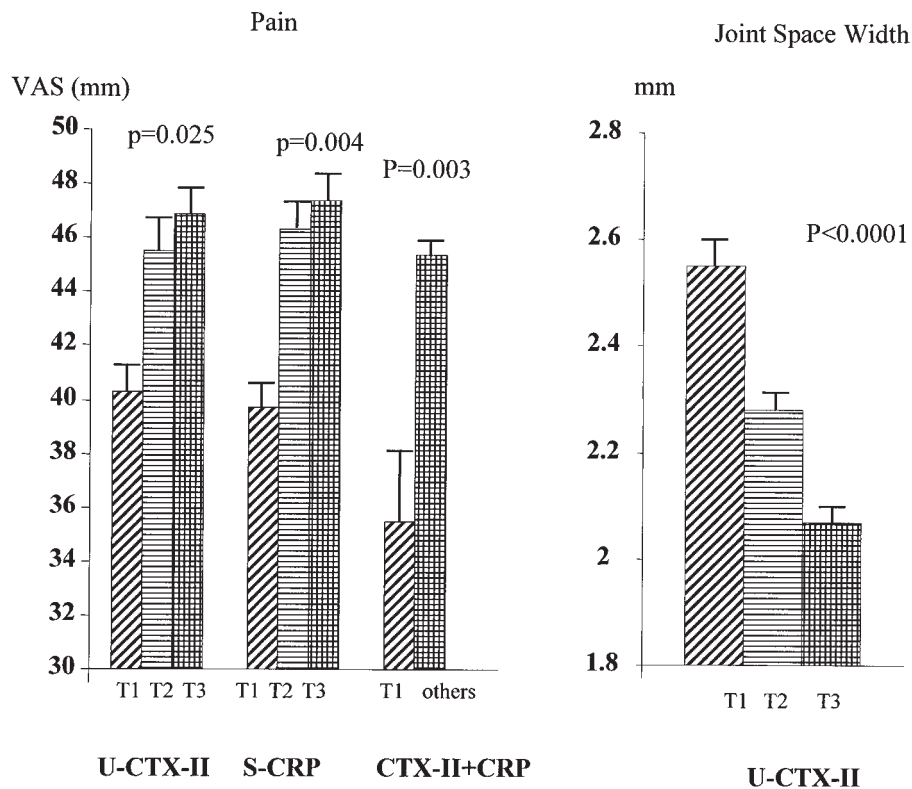


Figure 1. Relationships between concentrations of biochemical markers of joint tissue metabolism, joint pain, and joint space width (JSW) in 376 patients with hip OA. Patients were categorized in tertiles (T1 to T3, from lowest to highest) of levels of molecular markers. Intensity of pain, evaluated by 100 mm VAS, and JSW measured on hip radiographs were calculated in each tertile. For pain, when the 2 markers were combined, the VAS was calculated in those patients with levels of both markers in the lowest tertile (T1) versus all other patients, as there was no difference in VAS between tertile 2 and tertile 3 for both urinary C-terminal crosslinking telopeptide of type II collagen (CTX-II) and serum ultrasensitive CRP. P values refer to the difference in pain and JSW across tertiles of markers.

OA. Among the markers we analyzed, U-CTX-II showed the most consistent association with both symptoms and joint damage of hip OA, although the contribution of this marker to explain variability in these parameters was minor.

One of the main roles of principal component analysis is to generate mechanistic hypotheses. We found that S-YKL-40 segregated together with S-CRP, a well established non-specific molecular marker of systemic inflammation. YKL-40 has been proposed as a specific product of chondrocytes and consequently as a marker of cartilage turnover in OA and rheumatoid arthritis^{17,22}. However, recent studies have described expression of this protein by synovial cells, osteoblasts, and other tissues, and increased concentrations have been reported in different conditions associated with systemic inflammation^{23,24}. Our data suggest that in OA, serum YKL-40, similarly to CRP, may reflect mainly systemic inflammation. Serum COMP, initially proposed as a cartilage-specific molecule²⁵, has subsequently been shown to be synthesized by ligament, tendon, synovial fibroblasts, and osteoblasts⁴. Thus high circulating COMP levels may indicate increased cartilage breakdown and/or inflammation

of the synovial membrane. Our results in hip OA showing that COMP segregated together with serum HA and PIIINP, 2 nonspecific markers of synovial activity, suggest that this marker could in part reflect joint inflammation in hip OA, in agreement with a recent study in knee OA showing that serum COMP was associated with clinical synovitis²⁶. Principal component analysis showed that the structural markers of bone turnover (PINP and CTX-I) and cartilage degradation (urinary CTX-II) segregated in the same factor, suggesting that in hip OA, alterations of cartilage and bone metabolism are associated. However, it should be noted that because the dynamics of each biochemical marker may differ between separate body compartments, i.e., serum and urine, the results of our principal component analyses should be interpreted with caution, and should be confirmed by other studies using measurements of all markers in the same biological fluid.

Another goal of our study was to analyze the relationships between the levels of these biological markers and the clinical and structural signs of disease. Although previous small cross-sectional studies reported some associations

with one or a few markers, mainly in patients with knee OA (as reviewed^{4,27,28}), to our knowledge this is the first comprehensive study of a panel of different markers in a large and well characterized population of patients with hip OA. Regarding the clinical variables, we selected 4 different domains that in addition to pain in the joint and functional impairment also included an evaluation of patient global assessment and joint inflammation. Indeed, in the field of rheumatology, including in OA²⁹, it is common to differentiate inflammatory and mechanical pain. The Lequesne index has been validated only as a global composite index consisting of 12 questions, and thus using only the 4 questions relating to physical function apart from the whole questionnaire may or may not reflect physical function alone. Nevertheless, the expert panel enlisted recently by the Osteoarthritis Research Society International to define responder criteria for OA clinical trials selected these 4 questions as an index of physical function³⁰. We used the presence of night pain and the duration of morning stiffness as a clinical index of inflammation. Although there is no published evidence indicating that these 2 markers adequately reflect inflammation in OA, rheumatology texts commonly distinguish pain related to inflammation from mechanically driven pain using morning stiffness and night pain. In multivariable analyses, we found that increased cartilage degradation assessed by urinary CTX-II was significantly associated with joint pain and patient's global assessment after adjustment for age, sex, and BMI, factors that have been shown to be associated with CTX-II levels in postmenopausal women³¹. In addition to urinary CTX-II, pain was also associated with systemic inflammation as assessed by serum CRP, as one would expect and in agreement with the findings of Otterness, *et al*⁶. Joint inflammation was not significantly related to markers of bone or cartilage structural damage, but only to serum COMP after adjustment for age, sex, and BMI. This finding is in agreement with the outcome of the principal component analysis and with a recent study showing that serum COMP was associated with stiffness of the hip in the absence of radiographic signs of OA³².

One of the main potential uses of biochemical markers would be to identify patients at high risk for rapid progression of joint damage, which remains a challenge for the clinician. To assess radiological joint damage we quantitatively measured JSW and also collected the information on the presence or absence of subchondral bone sclerosis, 2 of the main structural features of OA. Our multivariate analysis showed that among the 10 different biochemical markers, only urinary CTX-II was significantly associated with both JSW and bone sclerosis after adjustment for age, sex, and BMI. These data suggest that increased rate of type II collagen destruction, as detected by urinary excretion of CTX-II, is associated with greater joint damage in hip OA. This finding is in agreement with data obtained from smaller popula-

tions of patients with knee^{16,33} and hip³⁴ OA, and suggests that this marker is probably one of the most useful to assess joint damage.

Our study has strengths and some limitations. This is the largest study investigating biochemical markers in patients with hip OA. We evaluated most of the available molecular markers for bone, synovium, and cartilage metabolism including a specific marker of type II collagen breakdown. However, this was a cross-sectional study and we could not assess the potential predictive value of the biochemical markers to predict disease progression. Participants in the ECHODIAH study were patients who agreed to participate in a 3-year clinical investigation and thus may not be representative of the general population with hip OA. Indeed, it is expected that these patients have more severe disease, as suggested by the higher rate of total hip replacement in the cohort compared to population based studies³⁵. We did not perform radiographic evaluation of the knees, spine, and hands and thus could not determine the contribution of non-hip joints to systemic concentrations of biochemical markers and their potential influence on the relationships between markers and hip OA. In a recent study, it was found that knee OA, spine disc degeneration, and hand OA contribute significantly and independently of each other to increased urinary CTX-II levels in postmenopausal women³⁶. We were not able to measure biochemical markers in sex and age matched controls with no OA and similar comorbidities as in this hip OA cohort. Consequently, we could not compare the sensitivity of the different markers to differentiate patients with hip OA compared to healthy individuals and assess their specificity for the hip OA process. Indeed, some of the markers, including CRP, can be affected by various inflammatory conditions common in the elderly, such as myocardial disease. However, several small cross-sectional studies using one or a few of these markers have shown that a large proportion of patients with OA had values within the normal range, suggesting that they have limited diagnostic value^{28,34}. Our results are based on a single determination of biochemical markers, which may actually underestimate the true association between these variables and joint damage/symptoms because of the variability of biochemical marker measurements²⁸.

This large study of biochemical markers may provide evidence of different molecular mechanisms involved in the pathophysiology of hip OA. Among the markers investigated, urinary CTX-II, a biochemical marker of type II collagen degradation, was the one most consistently associated with clinical and radiological indices of hip OA. Although the contribution of molecular markers to explain the variability of disease activity and joint damage was minor, combinations of some of them may prove to be useful for clinical investigation of patients with hip OA. Longitudinal studies are required to confirm this.

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