Serum concentrations of type II collagen biomarkers (C2C, C1, 2C and CPII) suggest different pathophysiologies in patients with hip osteoarthritis

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Abstract

Background
Cartilage destruction in osteoarthritis (OA) involves excessive degradation and increased synthesis of cartilage matrix macromolecules including type II collagen and proteoglycans. Cartilage biomarkers exist for the measurement of cartilage matrix turnover and may reveal differences in patients with OA.

Objective
To determine whether there are detectable differences in and relationships between biomarkers of type II collagen (CII) degradation (C2C, C1, 2C) and synthesis (CP II) in patients with only hip OA (OHOA) and those suffering from multiple sites OA (MSOA).

Patients and methods
Fifty-six patients classified as MSOA or OHOA. Minimum hip joint space width (Min JSW) measured by computer from standard radiographs. Serum measurement of CII synthesis C-propeptide (CPII) and cleavage of type II (C2C) and types I and II (C1, 2C) collagens. Aggrecan metabolism was assessed by serum CS 846 assay. Step to step logistic regression to determine the effect of the quantitative data on the assignment to each subgroup.

Results
Twenty-four subjects were classified with MSOA. Among the 32 OHAO patients, 15 had bilateral hip OA and 17 had unilateral hip OA. The latter were classified with "Isolated hip OA" (IHOA). CPII levels were significantly lower in patients with MSOA than in those with OHOA (99.9 ± 50.3ng/mL versus 141.9 ± 81.2ng/mL, $p=0.04$. OR= 0.18 for CPII >120 ng/mL, $p<0.005$). C2C levels were also lower in MSOA (9.7 ± 2.3ng/mL) versus OHOA (11.4 ± 3.2ng/mL, $p=0.03$. OR= 0.26 for C2C >10 ng/mL, $p=0.02$). There was an inverse correlation between min JSW and C2C only in patients with IHOA ($r=0.50$, $p= 0.02$).

Conclusion
Hip OA, in patients with MSOA, might be related to alteration in CII metabolism which may result in a deficient type II collagen repair process. The significant relationship between C2C and JSW in IHOA suggests that this marker is of value in assessing cartilage degradation patients with involvement of a single joint.

Key words
Hip, osteoarthritis, biological markers, collagen, radiography, CP II, C2C.

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Introduction

The hallmark of osteoarthritis (OA) is focal articular cartilage loss, combined with osteophyte formation at the margin of the joint. Articular cartilage destruction results from increased degradation of cartilage matrix molecules including type II collagen (CII) and proteoglycans (PGs) (1). Osteophyte formation involves the subchondral bone and new endochondral new bone formation at the joint margin. Although these features are nearly always present in an OA joint, the clinical and radiological presentation of the disease varies widely between patients and joints. OA can be primary or idiopathic (no detectable cause) or secondary (*i.e.*, caused by trauma, rheumatic or metabolic disease or congenital abnormalities). The extent of OA is also very variable among individuals, since it can be limited to one joint or may affect multiple joints. The rate of progression is also very heterogeneous among patients as demonstrated by number of studies (2-5).

The anatomical severity of OA is usually assessed by joint imaging using standard radiographs, ultrasonography, CT scan and magnetic resonance imaging. Another way of detecting structural changes in OA may be by measurement of molecular markers in serum and urine.

Because type II collagen (CII) is the most abundant protein of cartilage matrix, the assessment of CII synthesis and degradation may be of value in the assessment of OA severity and progression. Moreover, it would be important to study type I collagen (CI) degradation as well as CII, since type I is the predominant collagen of bone, a component of osteophyte formation and structure. A number of surrogate markers of CI and CII metabolism have been studied such as urine CI and CII C telopeptides (6). CII degradation can also be assessed by measuring serum concentrations of the neoepitopes C2C (of CII) and C1, 2C (of CI and CII) generated by collagenases (7, 8). CII biosynthesis can be evaluated by measuring the C-propeptide of type II procollagen (9) (CPII). The turnover of the cartilage proteoglycan aggrecan, the other major macromolecule of cartilage matrix, can be measured using the proteoglycan aggrecan CS 846 assay (10, 11).

Osteoarthritis of the hip is one of the most common causes of pain and functional disability in subjects aged 55 years and older. Monitoring of the disease usually employs standard radiographs, including both a standing anteroposterior (AP) view of the pelvis and an oblique view (Lequesne "faux profil) (12), using various assessment methods of which the most used are the Kellgren-Lawrence grading scale (13), the OARSI grade for joint space narrowing and osteophyte (14), and the quantitative measurement of the joint space width, at the site of maximum narrowing (15). The latter can be achieved by direct (using a graduated magnifying lens) or computer-assisted methods. Reproducibility of joint space width measurement is good for all these methods (16, 17). However standard radiology requires frequently follow-up of 2-3 years duration to accurately detect significant changes in joint structure. The imaging techniques show a "snapshot" image of the joint and do not give information on the current metabolism of the affected joint. It is likely that OA evolves by sequences of cartilage degradation (chondrolysis) interrupted by relative remissions. Thus radiography, that can show the spreading of the disease, is not able to give valuable information on disease activity at a given time. Immunoassays have been developed to assess osteoarthritic cartilage metabolism and a number of studies have investigated the value of various biological markers for the assessment of hip OA progression (18-23) However, the levels of the markers may wax and wane over time with activity of disease and a single sample might not be adequate to characterize disease progression. Obviously, biomarkers are also related to both the index OA joint and the presence of OA at other sites. However the influence of OA at multiple sites, generally termed generalized OA (GOA) was rarely considered (23). GOA generally affects knees, hand, feet and spine but not the hip. However a proportion of patients with hip OA was found with OA at other sites and could represent a subset of

GOA (24). Pathophysiological mechanisms in patients with only hip OA and in patients with hip OA and GOA could be different.

The aim of the present study was to determine whether CI, CII and aggrecan biomarkers reflect metabolic differences according to the radiological patterns and the extent of the disease in patients with hip OA. The finding of differences in type II collagen and /or proteoglycans metabolism, according to the different kinds of hip OA, might then help in our understanding of differences in the pathophysiology of these conditions.

Subjects and methods
Patients
This cross-sectional study was conducted in 56 patients (34 women, 22 men) who met the American College of Rheumatology criteria for primary hip OA (25). All of them were prospectively recruited in the out-patients' department of rheumatology. Patients with hip OA secondary to alternative arthropathies were excluded (*i.e.*, infectious or inflammatory arthritis, aseptic osteonecrosis, Paget's disease).

To be selected for the study, patients must have undergone a full clinical examination, performed by a senior rheumatologist. Height, weight, body mass index (BMI) and hip OA date of diagnosis were recorded. A careful joints examination was performed to clinically detect any symptomatic and asymptomatic OA joint (Heberden or Bouchard's nodes, trapezometacarpal and metatarsophalangeal joints deformation, knee swelling or crepitus, range of motion of any joints). Previous x-rays were collected if available (spine, chest, knees). Radiographs of the painful or clinically abnormal joints were performed. No other radiographs (except x-rays of the pelvis and hips) were taken if patients did not report pain and if joints examination was normal.

Patients were then classified in 2 groups: Patients with clinical and/or radiological OA in 2 or more other joints than hip were classified as having "multiple site OA" (MSOA). Patients with only hip OA (no other detectable OA joints with the exception of spine OA) were classified as having "only hip OA" (OHOA). These patients could have unilateral or bilateral hip OA. Among them, those with unilateral hip OA (and of course no other detectable osteoarthritic joint) constituted a particular subgroup which was defined as isolated hip OA (IHOA).

None of the selected patients presented with any other clinically detectable disease that may influence biomarker levels. None of the patients received steroid or hyaluronic acid intraarticular injection in the previous three months. For each patient, all clinical radiological and laboratory data were obtained on a single day.

Radiographic data
All patients underwent anteroposterior radiographs of the pelvis (AP) and Lequesne "faux-profil" of the two using standardized procedures (12).

Morphological evaluation
Radiological grade was determined by two observers with both the Kellgren-Lawrence (KL) grading scale (13) and the Altman score (14) for joint space narrowing (JSN) and osteophytes. For each grade the selected score was the highest obtained from the 2 radiological views (AP or oblique). Inter-observer kappa value (95% CI) was 0.89 (0.87-0.93), 0.91 (0.88-0.94), 0.79 (0.71-0.87), for KL grade and each item of the Altman score respectively (18).

Joint space width (JSW)
JSW was measured using an edge-based algorithm that automatically detects the joint space Contours as previously described (16). Patterns of hip joint dysplasia: head-neck-shaft angle (HNS), acetabular depth (AD) and centre-edge (CE) angle were also obtained using the computer.

Biochemical measurements
Blood samples were obtained from each subject on the day the radiographs were taken and the clinical evaluation was made. The serum was immediately frozen and stored at -25°C. The samples were thawed once to aliquot them and then refrozen. Then they were thawed again to assay them.

The ELISA biomarker assays (C2C, C1, 2C, CP II, CS 846) were obtained from IBEX (Montreal, Canada).Their use and reproducibility for serum have been described in detail (7, 8, 26). The intraassay reproducibility of measurements of concentrations of C2C, CPII, C1, 2C and CS846 was 9.7%, 6.4%, 10% and 11.5% respectively.

Statistics
A computer data base containing all measured data was created in StatView© 5.0 (SAS Institute Inc.) format. Statistical analyses were performed using the following procedures: Subgroups of patients were compared for each quantitative variable using Student's *t*-test or Mann and Whitney test according to the distribution of the variable. A step by step logistic regression was carried-out to determine the effect of the quantitative data (biomarkers and clinical or demographic variables) on the assignment to each subgroup. Correlations between quantitative data were studied using linear regression or Spearman test according to the distribution of the variables. P-values <0.05 were considered statistically significant.

Results
All the 56 patients suffered from symptomatic hip OA (mean Lequesne index/SD: 8.3/4). Twenty-two patients had bilateral and 34 unilateral hip OA. Twenty-four subjects were classified as having MSOA. The OHOA group had 32 patients. Among them, 15 had bilateral hip OA and IHOA was observed in 17 patients. The mean age was 62 years (range: 44-95 years) and the mean BMI was 26.6 (range 18-47).

Ten, 11, 19 and 16 patients had KL grade I, II, III and IV, respectively. The average min JSW was 2.23±1.25 mm (median 1.92 mm). The median of CP II, C2C, C1, 2C and CS846 levels were respectively 120 ng/mL (range 31-457), 10 ng/mL (range 6.4-22.5), 123 ng/mL (range 20.6-506.9) and 229 ng/mL (range 27-729).

In the total population, no correlation was found between any biomarker and any clinical or demographic data except for BMI and C1, 2C that were weakly correlated (r=0.27; p=0.05).

None of the biomarkers was related to the radiological severity (minJSW, mean JSW or any score), nor to any other clinical parameters. C2C and CPII were significantly correlated in the most anatomically advanced cases (KL III-IV, and min JSW<1.92mm; r=0.34 and r=0.48, p=0.03, respectively) but not in the moderate cases (KL I-II: r=0.20; p=0.47; min JSW>1.92mm: r=0.27; p=0.11). C2C and C1, 2C were highly correlated in the total population and in all sub-groups (all p<0.0003). In the sub-populations (Table I and II), patients with MSOA were slightly but not significantly older than those with OHOA (median 64 vs. 58 years, p=0.07). CPII levels were significantly lower in patients with MSOA than in those with OHOA (99.9±50.3ng/mL vs. 141.9±81.2 ng/mL, p=0.04) (Table II and Fig. 1). C2C levels were also significantly lower in the MSOA group than the OHOA group (9.7±2.3ng/mL vs. 11.4±3.2ng/mL, p=0.04). By contrast no statistically significant difference was found between these groups for C1, 2C and CS846.

C2C levels were inversely correlated with minJSW in patients with IHOA (Spearman test: Rho=-0.57, p=0.027) (Fig. 2), but neither in MSOA, nor in the 15 OHOA patients with bilateral involvement. CS 846 neoepitope and C1, 2C were found to be correlated in patients of the OHOA group (r=0.42, p=0.02), particularly in patients with IHOA. (r=0.59, p=0.01). In contrast this correlation was lacking in patients with MSOA.

Interestingly, the ratios of CPII/C2C, CPII/C1, 2C and C2C/C1, 2C were not statistically different between MSOA and OHOA. The ratio CPII/C1, 2C was higher in the OHOA group than in the MSOA group (1.64±1.64 vs.1.09±0.81), but the difference did not reach statistical significance. Step by step logistic regression also revealed that a decrease of CE angle was independently associated with risk of OHOA. Furthermore, CE angle was inversely related to CPII (Rho=-0.41, p=0.02 and Rho=-0.39; p=0.03) in OHOA but not in MSOA (Rho=-0.03, p=0.87 and Rho=0.02; p=0.89).

Table I. Demographics of patients with "multiple site"(MSOA) or "only hip" osteoarthritis (OHOA)

Variables	Units	n	OHOA Median (mean/SD)	n	MSOA Median (mean/SD)	p
Gender	F/M	32	19/13	24	14/10	0.12
Age	years	32	58 (58.6/11.8)	24	64 (61.9/10)	0.07
Weight	kg	32	75 (73.7/14.8)	24	76.9 (74.2/11.5)	0.8
BMI	kg/m²	32	25 (23.7/3)	24	26 (27.5/5.5)	0.16
CE angle	°	32	30,5 (26.8/7.3)	24	36 (36.6/11.4)	0.04
AD angle	°	32	5.6 (138.9/6.4)	24	5.9 (128.7/7.7)	0.16
Joint space width	mm	32	3.0 (2.9/1.03)	24	2.84 (2.7/1.0) 2	0.5
Lequesne index	pts	32	6.7 (8.7/4)	24	7.5 (8.2/4.7)	0.6

Table II. Biological data of patients with "multiple site"(MSOA) or "only hip" osteoarthritis (OHOA).

Variables	Units	n	OHOA Median (mean/SD)	n	MSOA Median (mean/SD)	p
C2C	ng/mL	32	10.9 (11.4/3.2)	24	9.0 (9.7/2.3)	0.04
CPII	ng/mL	32	134.8 (141.9/81.2)	24	102.2 (99.9/50.3)	0.04
C1, 2C	ng/mL	32	149.4 (162.4/112.7)	24	138.9 (140.5/98.8)	0.82
CS 846	ng/mL	31	305.2 (337.2/209.7)	24	263 (260.9/181.5)	0.14

Discussion

In this study, in which about half of patients with hip OA had polyarticular involvement, significant differences in type II collagen biomarkers were found between patients with hip OA alone and patient with hip OA and GOA. Thus, systemic measurement of CII biomarkers suggests possible different pathogenesis of hip OA.

Cartilage biomarkers are expected to reflect cartilage changes in patients having OA in a unique OA joint. A significant correlation between joint space narrowing, assessed by JSW measurement, and C2C was observed in patients with isolated hip OA. Hip dysplasia is a known risk factor for both isolated hip OA and OA progression. Interestingly, hip dysplasia, assessed by CE angle,
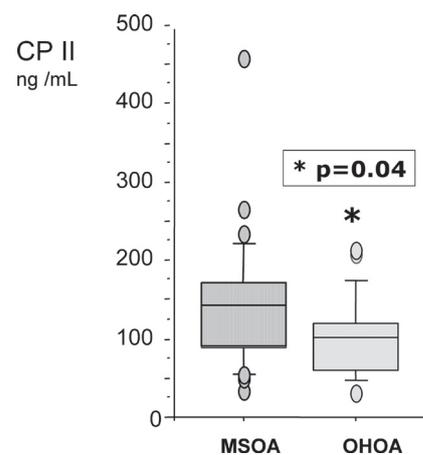

Fig. 1. Difference of collagen II propeptide (CPII) serum concentrations between patients with "Multiple Sites" (MSOA) and "Only" Hip Osteoarthritis (OHOA).
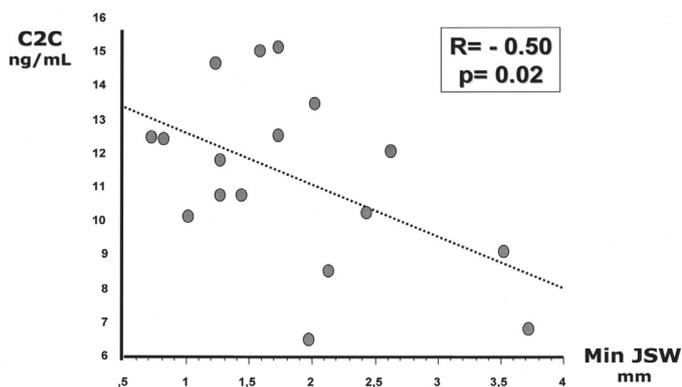
Fig. 2. Correlation between collagen II neo-epitope C2C serum concentrations and radiological minimum joint space width (min JSW) in 17 patients with isolated hip osteoarthritis (IHOA).
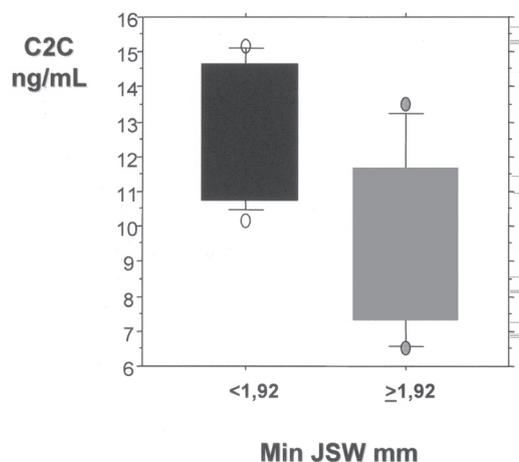

Fig. 3. Differences of collagen II neo-epitope C2C serum concentrations according to the threshold of 1.92 mm for the radiological minimum joint space width (min JSW) in 17 patients with isolated hip osteoarthritis (IHOA).

was correlated with CPII and C2C in patients with OHOA. Thus, results suggest a progressively increased synthesis and degradation of type II collagen with progression of OA, in patient with only hip OA. This would be in agreement with reported studies of collagen degradation and synthesis in human OA cartilage and in experimental OA. In patients with MSOA type II collagen biomarkers were expected to be more elevated than in patients with OHOA. Unexpectedly CPII serum concentrations were significantly lower in patients with MSOA than in those with OHOA. Patients with serum CPII <120 ng/mL had a 5 fold higher risk to have MSOA than those with CPII levels>120 ng/mL. C2C was also significantly lower in MSOA than in OHOA resulting in a statistically not significantly different CPII/C2C ratio in both groups. CS846 did not differ between the two groups. Thus, the results suggest that hip OA in patients with GOA might be have a specific alteration in type II collagen metabolism consisting of an reduced collagen turnover which might translate into a deficient type II collagen repair process.

The present results do not confirm previous studies performed with urinary CTX-II, another marker of type II collagen degradation (23, 27) and with serum type IIA procollagen N propeptide (29). CTX-II was found to be increased in patients with generalized OA and to correlate with the severity and the number of the OA joints. Type IIA procollagen N propeptide alone, and in combination with CT-II mainly, was found to correlated with knee OA progression (28). Discrepancies could be variously explained. CTX-II and type IIA procollagen N-propeptide studies were based in patients with knee OA while the present study selected patients consulting for hip OA.

Thus the difference might result from index joint differences or from different subset of GOA patients. Interestingly, C2C, CPII, CS846, and C1, 2C were not found to be significant predictors of progression of JSN in patients with knee OA (26). Furthermore, the native COL2- 3/4C fragment, present in the perichondrocyte matrix and the synovial fluid of patients with advanced hip OA, was also found to be correlated with the OA macroscopic and microscopic grades (30). Thus, most probably C2C and CTX-II, as well as CPII COL2-3/4C and type IIA procollagen N propeptide, may reflect the activity of different enzymes that may also play a critical role at different stages of the disease. These enzymes may be working in different tissue sites.

This present cross sectional study is mainly limited by the relatively small number of patients and by the fact the classification of patients in MSOA or OHOA was based on clinical examination. It was indeed not ethical to perform full skeletal radiographs to be sure that patients had no asymptomatic OA localisations. Nevertheless, the very careful examination of all joints by experienced senior rheumatologists, with radiograph for any OA suspected joint, made unlikely a wrong classification of patients. Another possible limitation was that we did not take into account the presence or absence of spine OA. Nevertheless the presence of CII in annulus fibrosus and nucleus pulposus of the spinal discs and in the cartilage of the facet joints may not contribute to the serum levels of CII biomarkers since previous studies have failed to detect evidence for type II collagen synthesis in the lumbar spine (31). Lastly, we did not take into account the potential effects of treatments and physical activity variables that can affect biomarker levels.

Despite these limitations the present work suggests that, in patients consulting for hip OA, the unexpected decreased in II collagen biomarkers in relation with the presence of OA at other sites could be due to an alteration in type II collagen metabolism.

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