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Differences in biomarkers of type II collagen in atrophic and hypertrophic osteoarthritis of the hip: implications for the differing pathobiologies

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Summary

Background: Cartilage destruction in osteoarthritis (OA) involves the excessive degradation and increased synthesis of cartilage matrix macromolecules including type II collagen (CII) and proteoglycans. The lack of osteophytes (atrophic form of OA) has been shown to be a disease severity factor in hip OA. Since osteophyte formation involves endochondral ossification and a cartilage intermediate, atrophic OA may also exhibit differences in cartilage turnover compared to hypertrophic OA. Cartilage serum biomarkers may offer an opportunity to identify such differences in patients.

Aim: To determine whether serum levels of cartilage biomarkers can distinguish between the presence and absence of osteophyte formation in patients with atrophic and hypertrophic hip OA.

Patients and methods: Fifty-six patients (mean age/standard deviation (SD): 62/11; mean body mass index (BMI)/SD: 27/11) with symptomatic hip OA (American College of Rheumatology criteria; mean Lequesne index/SD: 8.3/4) were classified as having an atrophic or hypertrophic form of OA, according to the absence or presence, respectively, of any osteophyte on a standard radiograph of the pelvis. Minimum joint space width (minJSW) and angles of dysplasia [centre-edge (CE) and head-neck-shaft (HNS)] were determined by computerized measurements.
The following serum markers were used which are commercial kits from Ibex Diagnostics (Montreal, QC): proteoglycan aggrecans turnover: CS 846; CII synthesis: C-propeptide (CPII), cleavage by collagenase of type II (C2C) and type I and II (C1,2C) collagens.

Statistics: Patients with atrophic and hypertrophic OA were compared for each variable and step to step logistic regression was used to determine the effect of variables on the belonging to each group. Correlations were examined using linear regression or Spearman test.

Results: CPII serum levels were significantly lower in the atrophic OA patients (77.3 vs 117.4 ng/mL). There were no significant differences between groups for C2C, C1,2C and CS 846 . CPII and C2C concentrations were highly correlated in hypertrophic OA ($P = 0.002$) but not in atrophic OA ($P = 0.8$).

Conclusion: Atrophic hip OA is characterized by reduced synthetic activity involving type II collagen synthesis. This could account in part for the absence of osteophyte formation. The highly significant correlation between CPII and C2C in hypertrophic but not in atrophic OA suggests that the physiological coupling between CII formation and degradation may be lost in atrophic OA. These differences may therefore help explain the absence of osteophyte in atrophic OA and its association with more rapid disease progression.
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Key words: Hip osteoarthritis, Type II collagen, Markers, Atrophic osteoarthritis, Type II collagen propeptide.

Osteoarthritis (OA) is anatomically defined as involving focal articular cartilage destruction, combined with osteophyte formation. The latter involves the subchondral bone and the process of endochondral new bone formation at the joint margin. The latter may be a "reparative" response to joint damage and altered mechanical loading and an adaptative reaction of the joint instability.

Nevertheless the onset and the progression of cartilage loss, osteophyte formation can occur at different rates and differ widely among patients and joints[1]. The hip is one of the joints most frequently affected by OA in adults of 55 years and older[2], often leading to the need for total hip arthroplasty in the late stages of the disease. However the rate of progression of hip OA is very heterogenous among patients as demonstrated by number of studies[3–6]. Solomon[7] suggested that the behaviour of coxarthrosis may be determined by three interacting factors such as cartilage degeneration, excessive mechanical stress, and reparative bone

response. When anatomical abnormalities and mechanical features are dominant, cartilage loss is localised, remodelling is good and the hip can stabilise. When inflammatory and degenerative features predominate, reparative new bone formation is minimal and progression is more rapid.

The pattern involving cartilage and bone changes can be classified as hypertrophic or atrophic according to the presence or the absence of osteophytes, respectively[8]. There is strong evidence that the atrophic form is a major factor favouring the severity of hip OA as demonstrated by a faster progression of joint space narrowing (JSN) than in hypertrophic OA[8,9]. This rapid progression may be related to a lack of reparative processes and/or to abnormally elevated levels of cartilage destruction creating an imbalance between synthesis and degradation.

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 study of OA progression. Moreover, it would be important to study type I collagen degradation as well as CII since type I is the predominant collagen of bone, a component of osteophyte formation and structure.

Biological biomarkers are now available to study types I and II collagen turnover *in vivo*. To assess CII degradation, we measured serum concentrations of the neo-epitopes C2C (type II) and C1,2C (type I) generated by collagenases[10,11]. CII biosynthesis was evaluated by measuring the C-propeptide of type II collagen[12] (CPII) whereas the turnover of the cartilage proteoglycan aggrecan was measured using the proteoglycan CS 846 assay[13,14].

The aim of the present study was to determine whether these biomarkers reflect metabolic differences between atrophic and hypertrophic patterns of hip OA which may then help in our understanding of the pathophysiology of the disease.

Subjects and methods

PATIENTS

This prospective cross-sectional case–control study was conducted in 56 patients (34 women, 22 men) of mean age [standard deviation (SD)]: 62 [11.1] years (range: 44–95 years) who met the American College of Rheumatology criteria for primary hip OA[15]. All patients but two were Caucasians. Patients with hip OA secondary to alternative arthropathies were excluded (i.e., infectious or inflammatory arthritis, aseptic osteonecrosis, Paget disease and major congenital abnormalities such as congenital dislocation of the hip). All patients underwent a full clinical examination to record the following information: height, weight, body mass index (BMI), disease duration, polyarticular OA involvement (Heberden or Bouchard's nodes, radiological – (if X-rays available) – spine OA or knee OA). Pain was self-assessed by the patients on a 100 mm visual analog scale (VAS) and disability relative to hip OA was evaluated using the Lequesne algofunctional index[16]. None of the patients presented with any other clinically detectable disease that may influence biomarker levels. None of the patients received steroid or hyaluronic acid intra-articular injection in the previous 3 months. For each patient, all clinical radiological and laboratory data were obtained on a single day.

RADIOGRAPHIC DATA

All patients underwent radiographs of the pelvis, using a standardized procedure. Antero-posterior (AP) radiographs of the pelvis were performed in standing position with 20° of internal rotation of the lower limbs. The focus-film distance was 100 cm and the beam was aligned to the top of pubic symphysis. Lequesne "faux-profil" (FP) of the two hips was also performed using the standardized procedure[17].

Morphological evaluation: Radiographic changes were graded according the modified Altman score[18,19] using a five point scale (0–4) for JSN, a four point scale (0–3) for osteophytes, a three point scale (0–2) for cysts and a two point scale (0–1) for sclerosis. The selected score was the highest one obtained from the two radiological views (AP or FP). Inter-observer kappa value (95% CI) was 0.91 (0.88–0.94), 0.79 (0.71–0.87), 0.77 (0.67–0.87) and 0.76 (0.68–0.85) for each item, respectively[19].

Each hip was then classified as hypertrophic or atrophic according to presence or absence of osteophytes. To be classified as atrophic, the hip had to be of grade 0 for osteophyte and >1 for JSN. To be classified as hypertrophic it must be of grade >1 for both JSN and osteophyte.

The intra-class coefficient of correlation (95% CI) between repeated evaluations was 0.96 (0.91–0.99)[9]. The pattern of femoral head migration within the acetabulum was classified as supero-lateral, supero-intermediate, supero-medial or inferior as proposed by Ledingham *et al.*[8].

JOINT SPACE MEASUREMENT

Joint space width (JSW) was measured using a novel version of a software whose results were previously published[20]. The new version (Holy's software-ß16™, Lyon, France) uses an edge-based algorithm that automatically detects the joint space contours. Standing AP hip X-rays were digitized in a BMP format at a resolution of 300 ppi (giving a pixel size of 0.08 mm). The region in which the measurement was performed was delineated within a 60° angle whose summit was the centre of the femoral head (automatically given by the computer from three peripheral points drawn by the reader using the mouse) and whose landmarks are the internal boundary and the non-osteophytic external edge of the acetabulum (obtained by a single click by the reader).

Within this angle, the joint space contours detection was automatically performed by the gradient algorithm and both minimum and mean JSW (minJSW, meanJSW) were automatically calculated. When the algorithm failed to delineate the contours the reader could also correct the computer-drawn contours as appropriate, by drawing them with the mouse.

Reproducibility of JSW measurement was assessed from the repeated measurement of 50 hip radiographs. The root-mean-square SD (RMS-SD) was 0.013 and 0.006 for minJSW and meanJSW, respectively, giving a coefficient of variation (CV) for minJSW and meanJSW of 0.57% and 0.27%, respectively. The intra-class coefficient of correlation was 0.99. The SD for repeated measurements of the same film was 0.17 and 0.12 mm so that the smallest detectable difference was 0.34 and 0.24 mm (2SD) for minJSW and meanJSW, respectively. 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 re-frozen. Then they were thawed again to assay them.

The Enzyme linked immunosorbent assay biomarker assays were obtained from IBEX (Montreal, Canada). Their use and reproducibility for serum has been described in detail[21].The following assays were used. The C2C assay employs a mouse monoclonal antibody that recognizes the carboxy-terminal neo-epitope generated by the cleavage of CII by collagenases[10].

The C1,2C assay uses a polyclonal rabbit antibody that recognizes the carboxy-terminal neo-epitope generated by the cleavage of collagenases in both I and II collagens[11].

The CS 846 assay measures an epitope on the chondroitin sulphate chains of aggrecan molecules.

The CPII assay measures the synthesis of type II collagen[12]. Results for all assays are expressed as ng/ml.

The intraassay reproducibility of measurements of concentrations of C2C, CPII, C1,2C and CS 846 was 9.7%, 6.4%, 10% and 11.5%, respectively.

STATISTICS

A computer database containing all measured data was created in StatView© 5.0 (SAS Institute Inc) format. Statistical analyses were performed using the following procedures: patients with atrophic and hypertrophic OA were compared for each variable using the chi-squared test, Student's *t* test or Mann and Whitney test as appropriate. Step to step logistic regression (including sex, age, BMI and the variables that were statistically related to a group in the univariate analysis) was used to determine the effect of quantitative data on the belonging to each group. Correlations between quantitative data were studied using linear regression or Spearman test as appropriate. *P* values <0.05 were considered statistically significant.

Results

Among the 56 patients, 14 fulfilled the criteria for atrophic OA and 42 for hypertrophic OA. In the total population no correlation was found between biomarkers and clinical or demographic data except for BMI and C1,2C that were weakly correlated ($P = 0.05$).

None of the biomarkers was related to the radiological severity (minJSW, meanJSW or any score), nor to any other clinical parameters.

Table I summarizes the differences between the two subgroups in both univariate and multivariate analyses.

In the univariate analysis, there was no significant difference between the two groups regarding age, sex ratio, pain and disability, bilaterality, generalized OA and any of the biomarkers. BMI and CE angle were higher in the hypertrophic group whereas meanJSW (but not minJSW) and HNS angle were significantly larger in the atrophic group. However logistic regression revealed that serum concentrations of CPII were significantly higher in the hypertrophic group ($P < 0.004$) (Fig. 1). The logistic regression estimates in Table I represent the significant variables from a single stepwise model including all variables, and show that meanJSW and CE angle were also significantly and independently related to the pattern of bone response.

In the hypertrophic group CPII and C2C were correlated (Spearman test: Rho = 0.48, $P = 0.002$) whereas there was no correlation in the atrophic group (Rho = 0.07, $P = 0.78$) (Fig. 2). C2C and C1,2C were correlated in both groups (Rho = 0.69 and 0.38; $P = 0.01$ and 0.02 for atrophic and hypertrophic OA, respectively) (Fig. 3) while CS 846 and CPII were correlated only in atrophic OA (Rho = 0.7, $P = 0.01$). There were no associations of the CS 846 epitope with other biomarkers or clinical parameters.

Discussion

The present data suggest that the lack of osteophyte formation and the more rapid progression that distinguishes a particular subgroup called atrophic hip OA may result from a deficiency in the synthetic (reparative) processes involving CII, in view of the selective reduction in the level of CPII, a marker of type II collagen synthesis. Cartilage proteoglycan turnover was unchanged demonstrating the selectivity of the collagen differences. The distinctive pathology of atrophic OA therefore may be due in part to the inability for the chondrocytes to synthesize new CII in sufficient amount in response to increased cartilage breakdown. The coupling between CII cleavage by collagenases and synthesis which was observed in hypertrophic OA was absent in atrophic hip OA. This no doubt contributes to the differences. On the other hand we did not find that the biomarkers of collagen degradation were different compared with the common form hypertrophic OA. Also, the C2C and C1,2C epitopes were correlated in both groups. Whether this indicates that overall bone and cartilage collagen cleavage remain coupled irrespective of other changes remains to be established with other more specific assays for bone resorption. The deficiency in cartilage collagen synthesis and lack of coupling with

Table I

Biological, demographics and radiological differences between atrophic and hypertrophic hip OA. The logistic regression estimates represent the significant variables from a single stepwise model including all variables

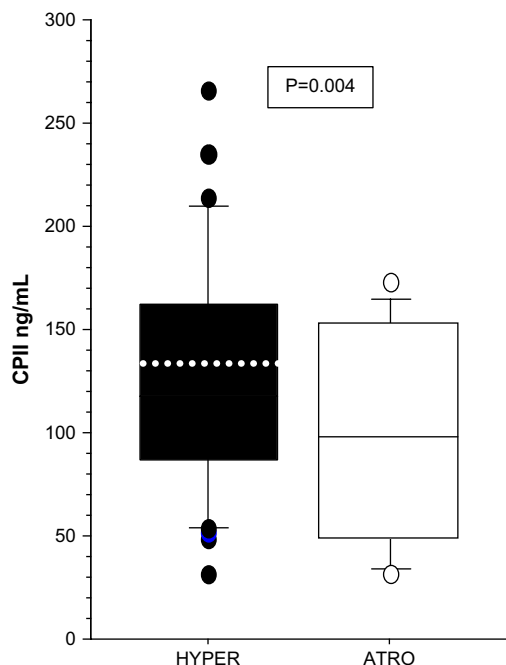| Variables	Units	N	Atrophic [median (mean/SD)]	N	Hypertrophic [median(mean/SD)]	Univariate analysis (*P*)	Logistic regression (*P*)
C2C	ng/mL	14	9.9 (10.1/2.9)	42	10.6 (10.9/2.9)	0.43	
C1,2C	ng/mL	14	85 (110/89.8)	42	123 (157.6/108.6)	0.15	
CPII	ng/mL	14	77.3 (93.7/50.7)	42	117.4 (132.6/75.2)	0.07	0.004
CS 846	ng/mL	14	196.1(225.2/144.6)	42	232.9 (301/192.5)	0.19	
Age	years	14	57 (58.6/11.8)	42	64 (61.9/10)	0.23	
BMI	kg/m^2	13	23 (23.7/3)	41	26 (27.5/5.5)	0.03	
MeanJSW	mm	14	3.5 (3.4/1.15)	42	2.5 (2.68/1.13)	0.06	0.04
CE angle	°	14	26.7 (26.8/7.3)	42	36.5 (36.6/11.4)	0.01	0.002
HNS angle	°	13	139.5 (138.9/6.4)	41	128.5 (128.7/7.7)	0.0009	

Fig. 1. Difference of collagen II propeptide (CPII) serum concentrations between atrophic and hypertrophic hip OA.

Two other markers of CII degradation were previously demonstrated to be reflective of the progression of hip OA. Urinary C telopeptide of type II collagen (CTX-II) and Helix II[23,24] are increased in rapidly destructive OA (also frequently associated with the absence of osteophytes), suggesting that a sustained increased rate of CII destruction, as assessed by these specific urinary biomarkers, would lead to more rapid destruction of cartilage. Other studies have shown that of different molecular markers of bone, cartilage and synovium turnover, urinary CTX-II was the most predictive for assessing the progression of knee OA[25] and that an uncoupling of CII synthesis and degradation was also predictive of joint damage progression[26]. The present biomarkers were not examined in this study.

It has also been reported that a combination of a biochemical marker of type II collagen synthesis (serum type IIA collagen N-propeptide) and degradation (CTX-II) was more effective than one of these two markers alone to predict disease progression in knee OA[23] and that combining a marker of synovitis (serum hyaluronic acid) with CTX-II improved prediction of disease progression in hip OA[27]. Thus, it may be useful to combine markers of different metabolic processes (e.g., synthesis and degradation of cartilage CII) and/or markers of turnover of different joint tissues (synovium and cartilage), and/or markers of different molecular mechanisms of cartilage degradation, to better understand the mechanisms of progression in OA.

Our study has, however, some limitations. This cross-sectional study did not directly examine progression of hip OA and the association of progression with these markers. The levels of these markers may wax and wane over time with activity of disease and a single sample may not be adequate to characterize progression. Indeed the atrophic CPII measurements appear to have a bimodal distribution in the atrophic population. This suggests that relative periods of quiescence would have lower levels in the serum.

The number of patients is also limited, and the status of other potential sources of CII markers, such as the knees, hands and spine, was not fully evaluated and could have confounded the results[28]. Furthermore the design of the study made us unable to take into account the potential effects of treatments and physical activity. Either variable can affect biomarker levels. A final point requires some discussion. It is unclear whether the serum measurements we have made involving these biomarkers reflect cartilage turnover as a consequence of hip pathology or a reflection of
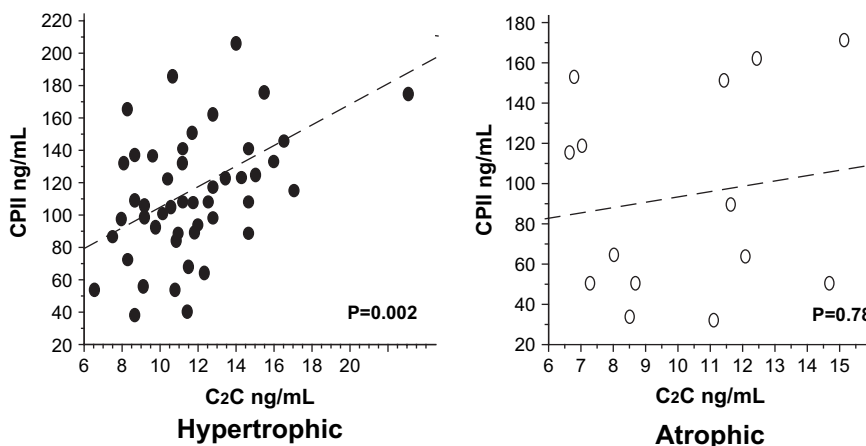
degradation may, however, contribute to the overall pathology including the lack of osteophytes. These are formed through a process of endochondral bone formation that involves a cartilage intermediate and is therefore dependant upon active cartilage collagen synthesis, coupled to cartilage resorption[22].

Very little has been published on this topic. In two previous studies we found that serum bone sialoprotein (BSP) serum concentrations (reflective of bone turnover) were inversely correlated with osteophyte score[19] but we did not observe any difference between atrophic and hypertrophic hip OA or associations with these conditions when serum hyaluronic acid, cartilage oligomeric matrix protein, collagenase, type I collagen, C-terminal cross linking telopeptide of type I collagen, tissue inhibitor of metalloproteases-1 and C reactive protein were examined[9].

Hypertrophic

Atrophic

Fig. 2. Correlation between collagen II propeptide (CPII) and collagen II neo-epitope C2C serum concentrations in atrophic and hypertrophic hip OA.
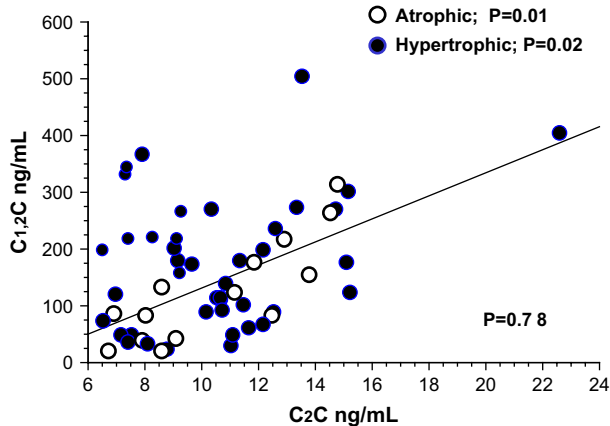
Fig. 3. Correlation between collagen II neo-epitope C2C and C1,2C serum concentrations in atrophic and hypertrophic hip OA.

systemic differences in cartilage metabolism that predispose towards these differences in pathology. It may well be the latter but this remains to be seen.

In summary, atrophic pathology in hip OA might be due to a lack of a deficiency in synthetic process involving CII and a lack of coupling between the synthesis and degradation of this molecule. Prediction of phenotype and progression based on this combination of markers will need to be formally assessed in larger groups preferably over a period of years.

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