EXTENDED REPORT

# Urinary CTX-II and glucosyl-galactosyl-pyridinoline are associated with the presence and severity of radiographic knee osteoarthritis in men

**K M Jordan, H E Syddall, P Garnero, E Gineyts, E M Dennison, A A Sayer, P D Delmas, C Cooper, N K Arden**

*Ann Rheum Dis* 2006;**65**:871–877. doi: 10.1136/ard.2005.042895

See end of article for authors' affiliations

Correspondence to: Dr N K Arden, Bone and Joint, MRC Epidemiology Resource Centre, Southampton University Hospital, Tremona Road, Southampton SO16 6YD, UK; nka@mrc.soton.ac.uk

Accepted 9 November 2005
**Published Online First 10 November 2006**

**Objective:** To investigate the association between biochemical markers of bone, cartilage, and synovial turnover with the presence and severity of knee osteoarthritis (OA) in men.
**Methods:** 176 men aged 59–70 years from the MRC Hertfordshire Cohort were studied. Weightbearing anteroposterior and lateral semiflexed radiographs were taken of both knees. A lifestyle questionnaire including basic demographic details and a questionnaire detailing knee pain was completed. This random sample was stratified based on the Kellgren and Lawrence (K&L) score, and the following biochemical markers were analysed: serum osteocalcin, serum C-terminal crosslinked telopeptide of type I collagen (CTX-I), urinary C-terminal crosslinked telopeptide of type II collagen (CTX-II), and urinary glucosyl-galactosyl-pyridinoline (Glc-Gal-Pyd).
**Results:** Age, body mass index (BMI), social class, smoking, and alcohol consumption were similar across K&L grades. Only one subject had a grade 4 K&L score, and was amalgamated with grade 3 subjects. A strong significant association was found between the presence of knee OA and urinary CTX-II and urinary Glc-Gal-Pyd ($p = 0.0001$ and $p = 0.009$), which persisted after adjustment for age and BMI. A significant positive association was also found between urinary CTX-II and urinary Glc-Gal-Pyd and the severity of K&L grade, joint space narrowing, and osteophytes scores, which persisted after adjustment for age and BMI. No associations between the presence and severity of knee OA were found for serum CTX-I or serum osteocalcin.
**Conclusions:** Urinary CTX-II and Glc-Gal-Pyd, but not systemic markers of bone turnover, are strongly associated with disease severity and the presence of OA at the tibiofemoral and patellofemoral joints in men.

Osteoarthritis (OA) is the most common form of arthritis in Western populations. OA of the knee, the principal joint affected, results in disabling knee symptoms in an estimated 10% of the UK population older than 55 years, a quarter of whom are severely disabled.[1] The risk of disability attributable to knee OA alone is as great as that from cardiac disease and greater than that from any other medical disorder in the elderly.[2] Radiographic evidence of knee OA in men and women aged over 65 years is reported in 30% of subjects,[3] around one third of whom are symptomatic. A recent World Health Organisation report on the global burden of disease indicates that knee OA is likely to become the fourth most important global cause of disability in women and the eighth most important in men.[4]

Biochemical markers are molecules of connective tissue matrices, which are released into the systemic circulation during tissue turnover. Several biochemical markers have been identified for bone, cartilage, and synovium turnover in humans. It has been suggested that biochemical markers may be useful in identifying those subjects at risk of OA progression and also as instruments to assess therapeutic responses in clinical trials, as radiographic change is often very slow and an unreliable measure of progression.

Many biochemical markers have been investigated in association with both radiographic progression of OA and prevalent disease, often with conflicting results and include collagen type II markers, collagen cross links, hyaluronan, proteoglycan markers, cartilage oligomeric matrix protein (COMP), matrix metalloproteinases, and inflammatory markers.[5–9] COMP, a marker of cartilage destruction has been the subject of most interest and promise; inconsistency in study data with COMP remains and there are controversies about its prognostic use. Some studies have shown a positive association with knee OA progression[10] and prevalent disease,[11 12] whereas others have shown poor correlation with joint space narrowing (JSN)[13] and progression.[14] Furthermore, it has been shown that COMP varies with age,[11 12 15] ethnicity,[15] and is not specific for cartilage, being present in synovium, meniscus, ligament, and tendon.[16]

More recently, a marker which reflects type II collagen turnover, the hallmark of OA, urinary C-telopeptide of type II collagen (CTX-II) and a marker more specific for synovial tissue turnover, glucosyl-galactosyl-pyridinoline (Glc-Gal-Pyd),[17] have been developed. These assays measure specific cross links of collagen that form the framework of cartilage and synovium matrices and are therefore more likely to reflect the destruction of these tissues specifically. Neither of these markers is affected by body weight.[18] Urinary CTX-II and Glc-Gal-Pyd have been shown to be associated with the degree of cartilage loss of the tibiofemoral compartment in patients with knee OA, predominantly in female subjects.[18] However, the associations between these two biochemical markers with other features of joint damage—including

**Abbreviations:** BMI, body mass index; COMP, cartilage oligomeric matrix protein; CTX-I, CTX-II, C-telopeptide of type I or II collagen; CVs, coefficients of variation; IQR, interquartile range; JSN, joint space narrowing; K&L, Kellgren and Lawrence; OA, osteoarthritis; OC, osteocalcin; PF, patellofemoral; TF, tibiofemoral

osteophytes— and at the patellofemoral compartment in men remain to be investigated.

In this study we analysed the relationship of these two specific biochemical markers, and also conventional measures of bone turnover (osteocalcin and serum C-telopeptide of type I collagen (CTX-I)), with radiographic knee OA in a cross sectional study using an established cohort of male subjects, the Hertfordshire Cohort.

## PATIENTS AND METHODS

### Patients

The Hertfordshire Cohort Study is a population based cohort study in the United Kingdom. Details of the study design have been published previously.[19] In brief, 498 male subjects who had taken part in a home interview and clinic visit, between January 1999 and April 2001 returned for a dual *x* ray absorptiometry scan and knee radiography (knee radiography was not conducted in three subjects). A detailed lifestyle questionnaire was administered at the home interview, and blood and urine samples were taken at the clinic visit. All subjects who had knee radiography were subsequently mailed a questionnaire about knee pain.

A random sample of 176 of these subjects, stratified by Kellgren and Lawrence (K&L) grade, were selected for biochemical marker analysis. In selecting our subjects for each K&L grade, we attempted to include subjects in each group with concordant K&L scores in each knee and where this was not possible, K&L was graded on the worst knee. Given the larger number of grade 0 and 1 K&L radiographic knee scores within the larger cohort from which the biochemical marker group was drawn, concordance was very good (grade 0: 100%; grade 1: 90%). However, for grade 2 and over, subject numbers were smaller and concordance poorer (grade 2: 33%; grade 3: 28%); for grades 3 and 4, subjects were combined owing to the small numbers available.

### Radiographs

Weightbearing anteroposterior and lateral semiflexed radiographs of both knees were taken at the same hospital using the same radiographic equipment; a standard tube to film distance of 100 cm was used. Radiographs were performed at a median duration of 6 months (interquartile range (IQR) 4.8–7.2) after the clinic visit. Subjects who were taking or had previously had bisphosphonate treatment were excluded.

Radiographs were graded at the tibiofemoral and patellofemoral joints for osteophytes, JSN, and sclerosis individually using a standard atlas[20] and the K&L score was determined.[21]

One trained reader (PB) graded the radiographs. A grade of ⩾2 osteophyte score or JSN score was defined as definite osteophyte or definite JSN and K&L grade ⩾2 was defined as definite OA.

A summative score for total osteophytes was calculated by adding the individual osteophyte scores together for each subject (right and left lateral tibiofemoral, right and left medial tibiofemoral, and right and left patellofemoral joints). The minimum score obtainable was 0 and the maximum 18.

A summative score for total JSN was also calculated by summing the individual JSN scores for each subject (right and left lateral tibiofemoral, right and left medial tibiofemoral, and right and left patellofemoral joints). The minimum score obtainable was 0 and the maximum 18.

### Biochemical markers

Fasting blood samples and early morning urine samples were obtained from all subjects at clinic visit. All biological samples were frozen at −80℃ until assayed.

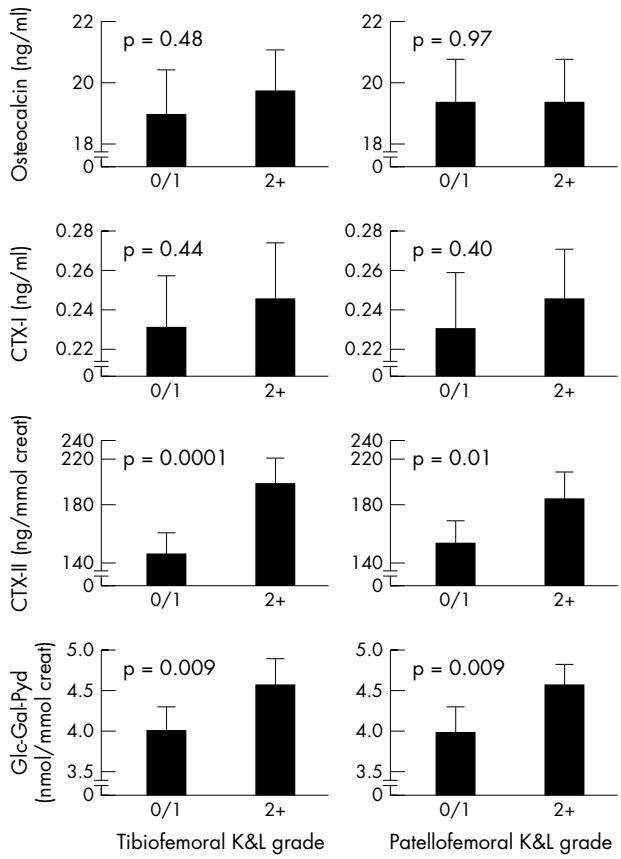


**Figure 1** Biochemical markers and absence/presence of tibiofemoral and patellofemoral OA.

**Table 1** Baseline characteristics of biochemical marker subset

| Characteristic | All subjects in cohort (n = 176) | Knee K&L grade 0 (n = 48) | 1 (n = 49) | 2 (n = 49) | 3/4 (n = 30) | p Value |
|---|---|---|---|---|---|---|
| Age (years)* | 64.8 (2.5) | 64.7 (2.5) | 64.3 (2.6) | 65.1 (2.6) | 65.0 (2.6) | 0.64 |
| BMI (kg/m$^2$)† | 27.4 (1.1) | 27.1 (1.2) | 27.1 (1.1) | 27.6 (1.1) | 27.9 (1.1) | 0.32 |
| Smoker‡ | | | | | | |
| Never | 57 (32) | 13 (27) | 16 (33) | 17 (35) | 11 (37) | 0.58 |
| Ex | 92 (52) | 23 (48) | 27 (55) | 25 (51) | 17 (57) | |
| Current | 27 (15) | 12 (25) | 6 (12) | 7 (14) | 2 (7) | |
| Alcohol (units/wk)* | 16.2 (14.9) | 17.2 (14.8) | 17.6 (16.7) | 13.0 (11.0) | 17.8 (17.1) | 0.39 |
| Social class‡ | | | | | | |
| Non-manual | 62 (35) | 19 (40) | 19 (39) | 16 (33) | 8 (27) | 0.87 |
| Manual | 104 (59) | 26 (54) | 26 (53) | 31 (63) | 21(70) | |

*Mean (SD); †geometric mean (SD); SDs calculated from $\log_e$ transformed distribution; ‡number (percentage).

### Markers of bone turnover

Serum total osteocalcin (OC), a specific marker of bone formation, was measured by a two site assay of both intact and N-mid-peptide using an automatic system (Elecsys; Roche Diagnostic, Manheim, Germany). Measuring N-mid-peptide—the main proteolytic fragment of OC—allows for the potential degradation of OC in vitro and the determination of precise measurements. Intra- and interassay coefficients of variation (CVs) are <2.5% and <3%, respectively.

Serum β isomerised C-terminal crosslinking telopeptide of type I collagen (CTX-I) was determined with an automated system (Serum Crosslaps, Elecsys; Roche Diagnostic). This serum resorption marker assay uses two monoclonal antibodies raised against a synthetic 8 amino acid peptide with an amino acid sequence specific for a part of the C-telopeptide of the α1 chain of type I collagen (Glu-Lys-Ala-His-βAsp-Gly-Gly-Arg). Intra-and interassay CVs are <8%.

### Markers of cartilage turnover

Urinary CTX-II was measured by enzyme linked immunosorbent assay (ELISA) based on a monoclonal antibody raised against a linear six amino acid epitope of CTX-II. Intra- and interassay CVs are <8% and <10%, respectively.[22]

### Markers of synovitis

Urinary Glc-Gal-Pyd, a specific marker of degradation of type I and type III collagens found in the synovium tissue, was measured on non-hydrolysed samples by high performance liquid chromatography. Intra- and interassay variations are <8% and <11%, respectively.[17]

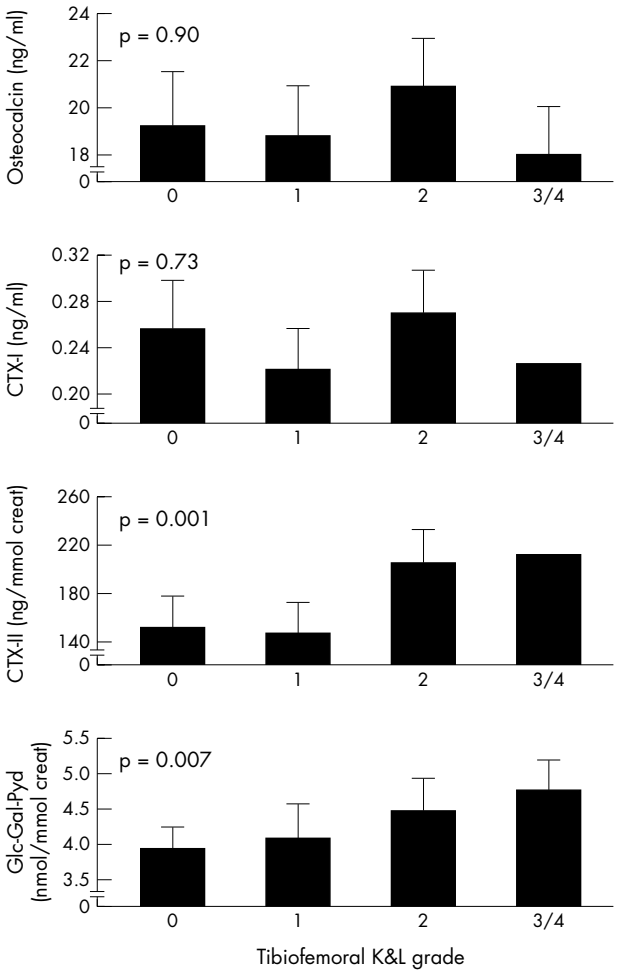


**Figure 2** Biochemical markers and increased severity of radiographic tibiofemoral OA.

### Statistical analysis

The STATA statistical software package, release 8.0, was used for the analyses.

Analysis of variance and tests for linear trend were used to explore the associations between the continuously distributed biochemical markers and osteophytes or JSN, with and without adjusting for the confounders detailed below. Multivariate logistic regression analysis was used, with and without adjusting for confounders, to examine the association between the binary knee OA variable and biochemical markers and adult lifestyle variables.

The biochemical markers osteocalcin, CTX-I, and CTX-II had skewed distributions and were $\log_e$ transformed for analysis; geometric means and standard deviations are therefore displayed for these assays.

Potential confounders investigated were sex, age, weight, height, body mass index (BMI), smoking habit, alcohol consumption, and social class (according to standard occupational classification determined by economic activity status, and occupation status in employment and industry).[23]

Power calculations were carried out to assess the numbers required within each group to have sufficient power to detect a difference. Assuming a sample size of 176, there was 84% power at the 5% statistical significance level to declare a difference between K&L groups 0/1 (n = 62) and K&L 2–4 (n = 114) for Glc-Gal-Pyd and 97% power at the 5% statistical significance level for CTX-II.

## RESULTS

### Clinical characteristics

The prevalence of K&L grade in the complete knee radiograph cohort (using the worst knee as the index knee) was: grade 0, n = 99 (19.9%); grade 1, n = 188 (37.8%); grade 2, n = 177 (35.5%); grade 3, n = 30 (6%); grade 4, n = 1 (0.2%).

Demographic details of the biochemical markers subsets did not differ from those of the whole group with a knee radiograph. Across the different grades of K&L in the study group, there were no statistical differences for age, weight, height, BMI, smoking habit, alcohol intake, or social class (table 1).

Assessing individual characteristics, we found the correlation between osteophytes and JSN using Spearman's correlation was $r_s = 0.6$. The correlation between CTX-II and Glc-Gal-Pyd was also strongly significant with Pearson's correlation coefficient $r = 0.31$.

### Biochemical markers and K&L scores

Urinary CTX-II and Glc-Gal-Pyd were significantly associated with presence of knee radiographic OA as graded by tibiofemoral (TF) K&L score (fig 1). Both markers were significantly increased with escalating severity of TF grade also (fig 2). All associations were independent of age and BMI. No associations were found between OC or CTX-I and presence or severity of TF knee OA.

Urinary CTX-II and Glc-Gal-Pyd were also significantly associated with presence of knee radiographic OA as graded by patellofemoral (PF) K&L score. These associations persisted after adjustment for age and BMI (fig 1). No associations were found between OC or CTX-I and the presence of PF knee OA.

Both CTX-II and Glc-Gal-Pyd were found to have independent effects, of strikingly similar magnitude, on the odds of having a high K&L grade. A mutually adjusted logistic model for K&L score on these two variables yielded odds ratios of 1.6 per SD increase in CTX-II (p = 0.01) and 1.58 per SD increase in Glc-Gal-Pyd (p = 0.02).

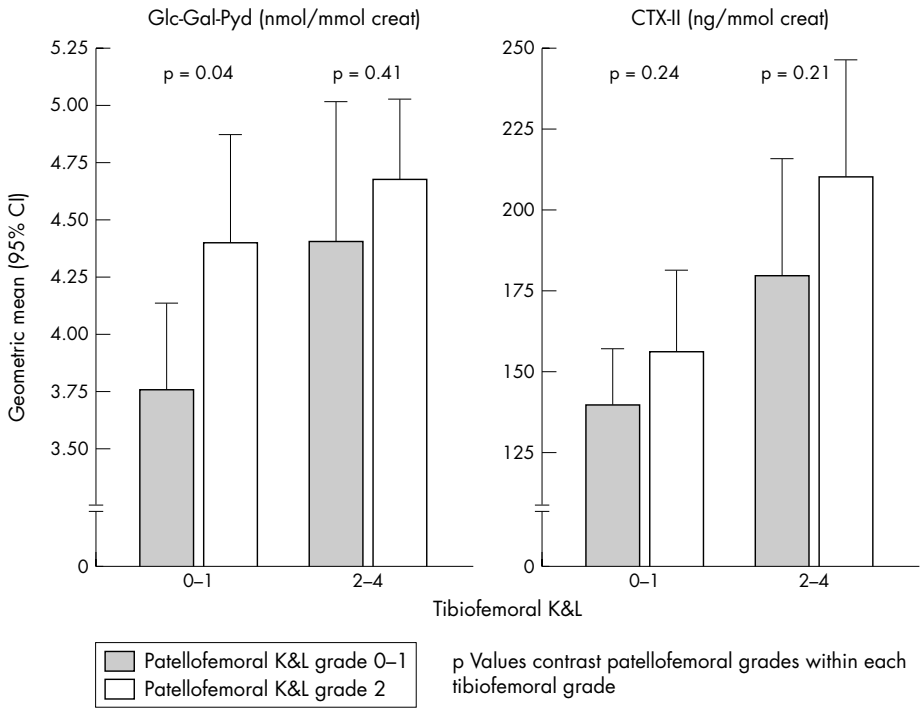


**Figure 3** Tibiofemoral OA adjusted for patellofemoral OA and CTX-II and Glc-Gal-Pyd.

There was significant overlap between the presence of TF and PF OA: 62.5% were concordant for the absence/presence of OA at these two sites, 19.9% had isolated PF OA, and 16% had isolated TF OA. In those subjects with isolated TF OA, the increase in CTX-II and Glc-Gal-Pyd reached borderline significance ($p = 0.02$ and $p = 0.06$, respectively) and a similar magnitude of effect was seen for osteophytes in isolated TF disease ($p = 0.02$ and $p = 0.10$ respectively). In subjects with isolated PF OA, Glc-Gal-Pyd was significantly increased ($p = 0.04$) (fig 3).

### Biochemical markers and osteophytes

Urinary CTX-II and Glc-Gal-Pyd were significantly raised in subjects with radiographic knee osteophytes; associations remained after adjustment for age and BMI (table 2). No associations were found between OC and CTX-I and the presence of osteophytes at the knee.

The summative score for osteophytes at the knee ranged from 0 to 15 (median 4, IQR 0–6). There were significant positive associations for CTX-II and Glc-Gal-Pyd with increasing osteophyte summative score ($p<0.0001$ for both) and this was independent of age and BMI (fig 4A). No associations between summative osteophyte score and OC or CTX-I were found.

### Biochemical markers and JSN

Urinary CTX-II and Glc-Gal-Pyd were significantly increased in subjects with radiographic knee JSN, and significance remained after adjustment for confounders (table 2). No associations were found between OC and CTX-I.

The summative score for JSN at the knee ranged from 0 to 11 (median 2, IQR 0–3). There were significant positive associations between CTX-II and Glc-Gal-Pyd with increasing summative JSN score ($p = 0.004$ and $p<0.0001$, respectively) (fig 4B), which were independent of age and BMI.

There was a suggestion of a plateau effect with no apparent increase and a possible decrease in CTX-II level above a summative JSN score of 6. Neither OC nor CTX-I were associated with the summative JSN score.

## DISCUSSION

In this study of men, we found that new, specific biochemical markers of cartilage degradation (CTX-II) and synovial turnover (Glc-Gal-Pyd) were significantly associated with both the presence and severity of knee OA as measured by osteophyte formation, JSN, and also, overall K&L grade at the tibiofemoral and patellofemoral joints. However, we found no significant association between any measure of knee OA and biochemical markers of bone metabolism.

**Table 2** Biochemical marker according to presence/absence of osteophytes and joint space narrowing

| | Osteophyte score | | | JSN score | | |
|---|---|---|---|---|---|---|
| **Biochemical marker** | **0/1** | **2–3** | **p Value** | **0/1** | **2–3** | **p Value** |
| Osteocalcin (ng/ml)* | 19.21 (1.43) | 19.25 (1.35) | 0.89<br>0.89‡ | 19.18 (1.32) | 19.02 (1.33) | 0.91<br>0.85‡ |
| CTX-I (ng/ml)* | 0.24 (1.67) | 0.24 (1.62) | 0.92<br>0.68‡ | 0.24 (1.66) | 0.24 (1.62) | 0.98<br>1.0‡ |
| CTX-II (ng/mmol Cr)* | 150.0 (1.6) | 203.0 (1.7) | 0.0002<br><0.0001‡ | 151.8 (1.61) | 216.39 (1.96) | 0.007<br>0.01‡ |
| Glc-Gal-Pyd (nmol/mmol Cr)† | 4.25 (1.5) | 4.72 (1.3) | 0.002<br>0.007 | 4.14 (1.49) | 4.96 (1.04) | 0.01<br>0.01‡ |

*Geometric mean (SD); SDs calculated from log(natural) transformed distribution; †mean (SD); ‡adjusted for age and BMI.

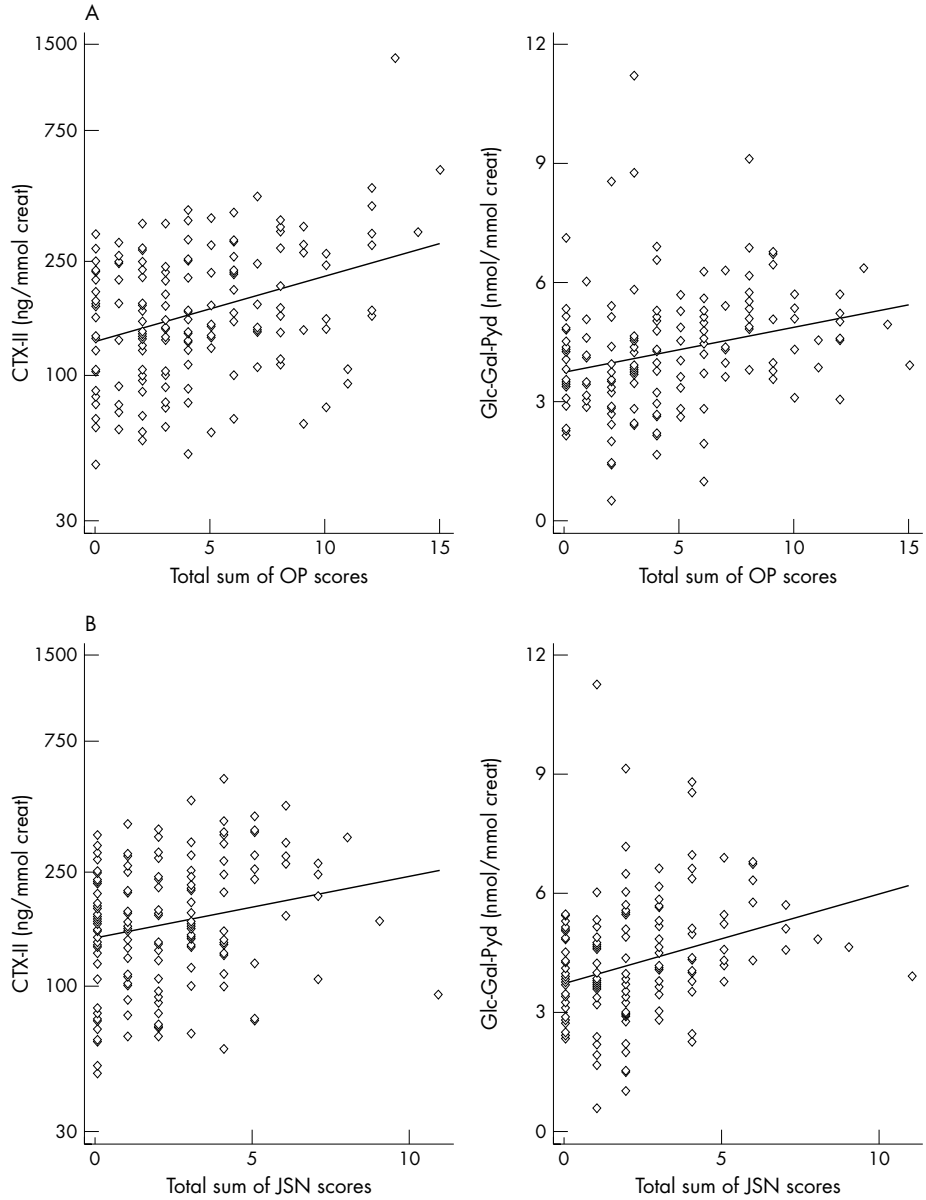


**Figure 4** (A) Biochemical markers and summative osteophyte (OP) score; (B) biochemical markers and summative joint space narrowing (JSN) score.

Our results demonstrating an association between CTX-II and JSN are consistent with other published work for the knee. In a study of 67 women and men with knee OA defined by the American College of Rheumatology criteria, both CTX-II and Glc-Gal-Pyd were increased and correlated with minimum joint space width and joint surface area[18]; men and women were not analysed separately. In a magnetic resonance imaging study of men and women, CTX-II was associated with the severity, but not presence, of cartilage defects in women, but no association was found in men.[24] Neither of these studies reported an association with osteophytosis. CTX-II is also raised in patients with spinal disc degeneration, although no significant association was found with spinal osteophytosis.[25] More recently, it has been shown that CTX-II is increased in patients with JSN at the hip and in those with rapidly progressive hip OA.[26 27] Although there is little information on the association of these markers with osteophytosis, our data suggest that both markers are significantly increased in patients with osteophytes.

Our study, however, does differ from others investigating biochemical markers in several important ways: only men were investigated; individual osteophyte grade, JSN grade, and a cumulative or summative score was determined; patellofemoral disease was evaluated; subjects with more severe OA were evaluated and the association with Glc-Gal-Pyd was explored.

There is a considerable overlap between the presence of osteophytes and JSN in patients with knee OA, making it difficult to determine the relative importance of each. We have performed multivariate and stratified analyses (data not shown), which suggest that both radiographic features are associated with CTX-II and Glc-Gal-Pyd, with effects of a similar magnitude.

We have also demonstrated, for the first time to our knowledge, that as the severity of OA increases, determined by a summative osteophyte score, summative JSN score or K&L grade, both CTX-II and Glc-Gal-Pyd increase in a dose dependent manner. The one exception to this is that with increasing severity of JSN there appears to be a plateau effect

for production of urinary CTX-II, such that once a certain level of JSN has been reached, CTX-II production reaches a plateau and possibly starts to decline. Although this pattern was statistically significant in piecewise regression models (results not shown), the numbers of subjects at the severe end of the JSN summative scale were small and therefore this effect would need to be demonstrated in a larger group in order to be scientifically robust. This pattern of association is intuitive as once severe cartilage loss has occurred, there is minimal cartilage left to produce CTX-II regardless of the rate of cartilage destruction or turnover.

The previous studies examining the association between biochemical markers of bone turnover and OA have provided conflicting results; showing either increased[27] or decreased markers[18] in subjects with OA or no association.[26 28] These studies have varied in the site and definition of OA and in the biochemical markers of bone turnover used. There is good evidence of increased subchondral bone turnover in patients with knee OA from studies using scintigraphy, which might explain an increase in markers of bone turnover.[29] However, patients with OA tend to have higher bone mineral density throughout the skeleton and lower rates of bone loss, which may account for a reduction in markers of bone turnover.[30 31] It is likely therefore that the overall concentration of biochemical markers in a patient with OA will depend on the balance between the magnitude of the subchondral bone response (a combination of the number and intensity of joints affected) and the rates of skeletal bone turnover. Consequently, it is unlikely that these markers of overall skeletal turnover will prove to be useful in the diagnosis or monitoring of OA.

Our study has several strengths and potential limitations. It is a cross sectional design, which limits our interpretation of biochemical markers in predicting joint progression. Weightbearing radiographs were used to examine the tibiofemoral joint. This technique has potential limitations in comparison with semiflexed or metatarsophalangeal views when measuring JSN, but not for scoring of osteophytes and K&L grade. The latter is heavily dependent on osteophytes as demonstrated by the similar magnitude of effects seen for K&L grade and osteophytes in isolated tibiofemoral disease. It has several strengths in that we investigated a large group of men, randomly selected form a large cohort study, with a full range of joint disease severity, which included isolated patellofemoral disease, areas which have previously been underresearched. We also analysed individual osteophyte and JSN grades and produced summative scores for each to assess the total burden of disease at the knee.

In conclusion, our study proposes that urinary CTX-II and Glc-Gal-Pyd, but not bone markers, are useful for assessing the presence and severity of OA in both the patellofemoral and tibiofemoral joints in men.

## ACKNOWLEDGEMENTS

We thank the Arthritis Research Campaign, the Medical Research Council, and the NHS Research and Development Departments for funding the study, and Patricia Byng for grading the radiographs.

### Authors' affiliations

**K M Jordan, H E Syddall, E M Dennison, A A Sayer, C Cooper, N K Arden,** Bone and Joint, MRC Epidemiology Resource Centre, Southampton University, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK
**P Garnero, E Gineyts, P D Delmas,** INSERM Research Unit 403, Lyon, France
**P Garnero,** Molecular Markers, Synarc, Lyon, France

**Conflict of interest:** None.

Ethical approval: The study received ethical approval from the North and East Hertfordshire Local Ethics Research Committee, and all subjects gave written informed consent.

## REFERENCES

1 **Peat G**, McCarney R, Croft P. Knee pain and osteoarthritis in older adults: a review of community burden and current use of primary health care. *Ann Rheum Dis* 2004;**60**:91–7.
2 **Guccione AA**, Felson DT, Anderson JJ, Anthony JM, Zhang Y, Wilson PWF, *et al*. The effects of specific medical conditions on the functional limitations of elders in the Framingham study. *Am J Public Health* 1994;**84**:351–8.
3 **Cooper C**. Epidemiology of osteoarthritis. In: Klippel JH, Dieppe PA, eds. *Rheumatology*.2nd ed. London: Mosby, 1998:1–20.
4 **Murray CJL**, Lopez AD. *The global burden of disease*. Geneva: World Health Organisation, 1997.
5 **DeGroot J**, Bank RA, Tchetverikov I, Verzijl N, TeKoppele JM. Molecular markers for osteoarthritis: the road ahead. *Curr Opin Rheumatol* 2002;**14**:585–9.
6 **Wollheim FA**. Serum markers of articular cartilage damage and repair. *Rheum Dis Clin North Am* 1999;**25**:417–49.
7 **Otterness IG**, Weiner E, Swindell AC, Zimmerer RO, Ionescu M, Poole AR. An analysis of 14 molecular markers for monitoring osteoarthritis. Relationship of the markers to clinical end-points. *Osteoarthritis Cartilage* 2001;**9**:224–31.
8 **Garnero P**, Rousseau JC, Delmas PD. Molecular markers and clinical use of biochemical markers of bone, cartilage and synovium in joint diseases. *Arthritis Rheum* 2000;**43**:953–68.
9 **Sowers M**, Jannausch M, Stein E, Jamadar D, Hochberg M, Lachance L. C-reactive protein as a biomarker of emergent osteoarthritis. *Osteoarthritis Cartilage* 2002;**10**:595–601.
10 **Vilim V**, Olejarova M, Machacek S, Gatterova J, Kraus VB, Pavelka K. Serum levels of cartilage oligomeric matrix protein (COMP) correlate with radiographic progression of knee osteoarthritis. *Osteoarthritis Cartilage* 2002;**10**:707–13.
11 **Clark AG**, Jordan JM, Vilim V, Renner JB, Dragomir AD, Luta G, *et al*. Serum cartilage oligomeric matrix protein reflects osteoarthritis presence and severity: the Johnston County osteoarthritis project. *Arthritis Rheum* 1999;**42**:2356–64.
12 **Vilim V**, Vytasek R, Olejarova M, Machacek S, Gatterova J, Prochazka B, *et al*. Serum cartilage oligomeric matrix protein reflects the presence of clinically diagnosed synovitis in patients with knee osteoarthritis. *Osteoarthritis Cartilage* 2001;**9**:612–18.
13 **Bruyere O**, Collette JH, Ethgen O, Rovati LC, Giacovelli G, Henrotin YE, *et al*. Biochemical markers of bone and cartilage remodeling in prediction of longterm progression of knee osteoarthritis. *J Rheumatol* 2003;**30**:1043–50.
14 **Conrozier T**, Saxne T, Fan CS, Mathieu P, Tron AM, Heinegard D, *et al*. Serum concentrations of cartilage oligomeric matrix protein and bone sialoprotein in hip osteoarthritis: a one year prospective study. *Ann Rheum Dis* 1998;**57**:527–32.
15 **Jordan JM**, Luta G, Stabler T, Renner JB, Dragomir AD, Vilim V, *et al*. Ethnic and sex differences in serum levels of cartilage oligomeric matrix protein. The Johnston County Project. *Arthritis Rheum* 2003;**48**:675–81.
16 **Cooper C**, Walker-Bone K, Arden N, Dennison E. Novel insights into the pathogenesis of osteoporosis: the role of intrauterine programming. *Rheumatology (Oxford)* 2000;**39**:1312–15.
17 **Gineyts E**, Garnero P, Delmas PD. Urinary excretion of glucosyl-galactosyl-pyridinoline: a specific biochemical marker of synovium degradation. *Rheumatology (Oxford)* 2001;**40**:315–23.
18 **Garnero P**, Piperno M, Gineyts E, Christgau S, Delmas PD, Vignon E. Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: relations with disease activity and joint damage. *Ann Rheum Dis* 2001;**60**:619–26.
19 **Aihie Sayer A**, Syddall H, Dennison EM, Gilbody HJ, Duggleby SL, Cooper C, *et al*. Birth weight, weight at 1 year and body composition in older men: findings from the Hertforshire Cohort Study. *Am J Clin Nutr* 2004;**80**:199–203.
20 **Spector TD**, Cooper C, Cushnaghan J, Hart DJ, Dieppe PA. *A radiographic atlas of knee osteoarthritis*. London: Springer, 1992.
21 **Kellgren JH**, Lawrence JS. Radiological assessment of osteoarthritis. *Ann Rheum Dis* 1957;**16**:494–502.
22 **Christgau S**, Garnero P, Fledelius C, Moniz C, Ensig M, Gineyts E, *et al*. Collagen type II C-telopeptide fragments as an index of cartilage degradation. *Bone* 2001;**29**:209–15.
23 **Government statistical service**. *Standard occupational classification*. Office of population censuses and surveys II: Employment department group, London:HMSO, 1990.
24 **Ding C**, Garnero P, Cicuttini F, Scott F. Knee cartilage defects: association with early radiographic osteoarthritis, decreased cartilage volume, increased joint surface area and type II collagen breakdown. *Osteoarthritis Cartilage* 2005;**13**:198–205.
25 **Garnero P**, Sornay-Rendu E, Arlot M, Christiansen C, Delmas PD. Association between spine disc degeneration and type II collagen degradation in postmenopausal women: the OFELY study. *Arthritis Rheum* 2004;**50**:3137–44.
26 **Garnero P**, Conrozier T, Christgau S, Mathieu P, Delmas PD, Vignon E. Urinary type II collagen C-telopeptide levels are increased in patients with rapidly destructive hip osteoarthritis. *Ann Rheum Dis* 2003;**62**:939–43.
27 **Garnero P**, Mazieres B, Gueguen A, Abbal M, Berdah L, Lequesne M, *et al*. 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. *J Rheumatol* 2005;**32**:697–703.

28 **Hunter DJ**, Hart DJ, Snieder H, Bettica P, Swaminathan R, Spector TD. Evidence of altered bone turnover, vitamin D and calcium regulation with knee osteoarthritis in female twins. *Rheumatology (Oxford)* 2003;**42**:1311–16.
29 **Dieppe P**, Cushnaghan J, Young P, Kirwan J. Prediction of the progression of joint space narrowing in osteoarthritis of the knee by bone scintigraphy. *Ann Rheum Dis* 1993;**42**:1378–85.
30 **Arden NK**, Nevitt MC, Lane NE, Gore R, Hochberg MC, Scott JC, *et al.* Osteoarthritis and risk of falls, rates of bone loss, and osteoporotic fracture. *Arthritis Rheum* 1999;**42**:1378–85.
31 **Sowers M**, Lachance L, Jamadar D, Hochberg MC, Hollis B, Crutchfield M, *et al.* The associations of bone mineral density and bone turnover markers with osteoarthritis of the hand and knee in pre- and perimenopausal women. *Arthritis Rheum* 1999;**42**:483–9.