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

Kelsey M Jordan¹, Holly E Syddall¹, Patrick Garnero²,³, Evelyne Gineyts²
Elaine M Dennison¹, Avan Aihie Sayer¹, Pierre D Delmas², Cyrus Cooper¹,
Nigel K Arden¹

¹ Bone and Joint, MRC Epidemiology Resource Centre, Southampton University, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, GB
² INSERM Research unit 403, Lyon, France
³ Molecular Markers, Synarc, Lyon, France

Correspondence and reprint requests to:
Dr. Nigel K Arden
Bone and Joint, MRC ERC
Southampton University Hospital
Tremona Road
Southampton SO16 6YD, UK
E-mail nka@mrc.soton.ac.uk

Keywords: knee osteoarthritis; biochemical markers; CTX-II; Glc-gal-pyd; radiographic osteoarthritis
Abstract

Objective: To investigate the association between biochemical markers of bone, cartilage and synovial turnover with the presence and severity of knee osteoarthritis in men

Methods: 176 men aged 59-70 years from the MRC Hertfordshire Cohort were studied. Weight bearing AP and lateral semi-flexed radiographs were taken of both knees. A lifestyle questionnaire including basic demographic details and a questionnaire detailing knee pain was completed. This stratified random sample based on Kellgren Lawrence (K/L) score had analysis of the following biochemical markers: 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, BMI, social class, smoking and alcohol consumption were similar across K/L grades. There was only one subject with grade 4 K/L score, who was amalgamated with grade 3 subjects. There was a strong statistically significant association between presence of knee osteoarthritis and urinary CTX-II and urinary Glc-Gal-Pyd (p=0.0001 and p=0.009), which persisted after adjustment for age and BMI. There was also a significantly positive association between urinary CTX-II and urinary Glc-Gal-Pyd and the severity of K/L grade, joint space narrowing and osteophytes scores which also persisted after adjustment for age and BMI. No associations between presence and severity of knee osteoarthritis 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 presence of osteoarthritis at the tibio-femoral and patello-femoral joints in men.
**Background**

Osteoarthritis (OA) is the most common form of arthritis in Western populations. The knee, the principal joint affected by OA, 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 due to cardiac disease and greater than that due to any other medical disorder in the elderly (2). Radiographic evidence of knee osteoarthritis 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 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 osteoarthritis disease 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 osteoarthritis and prevalent disease, often with conflicting results and include collagen type II markers, collagen crosslinks, hyaluronan, proteoglycan markers, cartilage oligomeric matrix protein (COMP), matrix metalloproteinases and inflammatory markers (5), (6),(7),(8),(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 regarding its prognostic use. Some studies have shown a positive association with knee OA progression (10) and prevalent disease (11),(12) while others have shown poor correlation with joint space narrowing (13) and progression (14). Furthermore, it has been shown 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 tibio-femoral compartment in patients with knee OA, predominantly in female subjects (18). However, the associations between these two biochemical markers with other joint damage features -including osteophytes- and at the patello-femoral 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 osteoarthritis 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 DXA scan and knee radiographs (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 knee radiograph subjects were subsequently mailed a questionnaire regarding knee pain.

A random sample of 176 of these subjects, stratified by Kellgren and Lawrence grade were selected to have biochemical marker analysis performed. In selecting our subjects for each Kellgren and Lawrence grade, we attempted to include subjects into 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 that the biochemical marker group were drawn from, concordance was very good (grade 0: 100%; grade 1: 90%). However, for grade 2 and over, subject numbers were fewer and concordance poorer (grade 2: 33%; grade 3: (28%); for grade 3 and 4, all subjects were combined due to the small numbers available.

Radiographs
Weight-bearing antero-posterior and lateral semi-flexed radiographs of both knees were taken at the same hospital using the same radiographic equipment; a standard tube to film distance of 100cm was used. Radiographs were performed at a median duration of 6 months after the clinic visit (Interquartile range 4.8-7.2 months). Subjects who were taking or had previously taken bisphosphonate treatment were excluded.

Radiographs were graded at the tibio-femoral and patello-femoral joints for osteophytes, joint space narrowing and sclerosis individually using a standard atlas (20) and Kellgren and Lawrence score (K/L) was determined (21).

One trained reader graded the radiographs (PB). A grade of ≥ 2 osteophyte score or joint space narrowing score was defined as definite osteophyte or definite joint space narrowing and K/L grade ≥ 2 was defined as definite osteoarthritis.

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

A summative score for total joint space narrowing score was also calculated by summing the individual JSN scores for each subject (right and left lateral tibio-femoral, right and left medial tibio-femoral and right and left patello-femoral joints). The minimum score obtainable being 0 and 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°C until assayed.

Markers of bone turnover
Serum total osteocalcin (OC), a specific marker of bone formation, was measured by a two-site assay measuring 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 variations (CV) are lower than 2.5% and 3% respectively.

Serum β isomerised C-terminal cross-linking telopeptide of type I collagen (CTX-I) using an automated system (Serum Crosslaps, Elecsys, Roche Diagnostic, Manheim, Germany). 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 lower than 8%.

Markers of cartilage turnover
Urinary C-terminal cross linking telopeptide of type II collagen (CTX-II) was measured by ELISA based on a monoclonal antibody raised against a linear six amino acid epitope of the type II collagen C telopeptide. Intra- and interassay CVs are lower than 8% and 10%, respectively (22).

Markers of Synovitis
Urinary Glucosyl –galactosyl –pyridinoline (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 below 8% and 11% respectively (17).

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 osteoarthritis variable with biochemical markers and adult lifestyle variables.

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

Potential confounders investigated were gender, age, weight, height, BMI, smoking habit, alcohol consumption, 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 (36.1%); grade 3, n=30 (6%); grade 4, n=1 (0.2%).
Demographic details of the biochemical markers subset did not differ to that of the whole knee radiograph group. 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].

Table 1 Baseline characteristics of biochemical marker subset

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All subjects in cohort (n= 498)</th>
<th>Knee K/L Grade</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (n=48)</td>
<td>1 (n=49)</td>
<td>2 (n=49)</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>64.8 (2.5)</td>
<td>64.7 (2.5)</td>
<td>64.3 (2.6)</td>
</tr>
<tr>
<td>BMI (kg/m²)**</td>
<td>27.4 (1.1)</td>
<td>27.1 (1.2)</td>
<td>27.1 (1.1)</td>
</tr>
<tr>
<td>Smoker***</td>
<td>Never</td>
<td>Ex</td>
<td>Current</td>
</tr>
<tr>
<td></td>
<td>57 (33)</td>
<td>92 (52)</td>
<td>27 (15)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>16.2 (14.9)</td>
<td>17.2 (14.8)</td>
<td>17.6 (16.7)</td>
</tr>
<tr>
<td>Social class</td>
<td>Non-manual</td>
<td>Manual</td>
<td></td>
</tr>
<tr>
<td></td>
<td>62 (35)</td>
<td>104 (59)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Mean (SD)</td>
<td>** Geometric mean (SD); SDs calculated from logₑ transformed distribution</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*** Number (percentage)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Assessing individual characteristics, we found the correlation between osteophytes and joint space narrowing using Spearman’s correlation was r=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 Kellgren/Lawrence scores

Urinary CTX-II and Glc-gal-pyd were significantly associated with presence of knee radiographic osteoarthritis as graded by tibio-femoral (TF) K/L score [Figure 1]. Both markers were significantly increased with escalating severity of TF grade also [Figure 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 osteoarthritis as graded by patello-femoral (PF) K/L score. These associations persisted after adjustment for age and BMI [Figure 1]. No associations were found between OC or CTX-I and 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).

There was significant overlap between the presence of TF and PF OA: 62.5% are concordant for 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
CTX-II and Glc-gal-pyd reached borderline statistical significance (p=0.02 and p=0.06 respectively) and a similar magnitude of effect was seen for osteophytes in isolated tibiofemoral disease (p=0.02 and p=0.10 respectively). In subjects with isolated PF OA, Glc-gal-pyd was significantly increased (p=0.04) [Figure 3].

Biochemical markers and osteophytes
Urinary CTX-II and Glc-gal-pyd were significantly elevated 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 presence of osteophytes at the knee.

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

<table>
<thead>
<tr>
<th>Biochemical Marker</th>
<th>Osteophyte Score</th>
<th>JSN Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0/1</td>
<td>2-3</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td></td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)*</td>
<td>19.21 (1.43)</td>
<td>19.25 (1.35)</td>
</tr>
<tr>
<td></td>
<td>0.89*</td>
<td>0.89#</td>
</tr>
<tr>
<td>CTX-1 (ng/ml)*</td>
<td>0.24 (1.67)</td>
<td>0.24 (1.62)</td>
</tr>
<tr>
<td></td>
<td>0.92</td>
<td>0.68#</td>
</tr>
<tr>
<td>CTX-2 (ng/mmol Cr)*</td>
<td>150.0 (1.6)</td>
<td>203.0 (1.7)</td>
</tr>
<tr>
<td></td>
<td>0.0002</td>
<td>&lt;0.0001#</td>
</tr>
<tr>
<td>Glc-gal-pyd (nmol/mmol Cr)**</td>
<td>4.25 (1.5)</td>
<td>4.72 (1.3)</td>
</tr>
<tr>
<td></td>
<td>0.002</td>
<td>0.007#</td>
</tr>
</tbody>
</table>

* Geometric mean (SD); SDs calculated from log(natural)-transformed distribution
** Mean (SD)
# Adjusted for age and BMI

The summative score for osteophytes at the knee ranged from 0-15 (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 [Figure 4a]. No associations between summative osteophyte score and OC or CTX-I were found.

Biochemical markers and joint space narrowing
Urinary CTX-II and Glc-gal-pyd were significantly elevated in subjects with radiographic knee JSN, which remained after adjustment for confounders [Table 2]. No associations were found between OC and CTX-I.

The summative score for joint space narrowing at the knee ranged from 0-11 (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) [Figure 4b] and this was 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 novel, specific biochemical markers of cartilage degradation (CTX-II) and synovial turnover (Glc-gal-pyd) were significantly
associated with both presence and severity of knee osteoarthritis as measured by osteophyte formation, joint space narrowing and also overall Kellgren Lawrence grade at the tibio-femoral and patello-femoral joints. However, we found no significant association between any measure of knee osteoarthritis and biochemical markers of bone metabolism.

Our results demonstrating an association between CTX-II and joint space narrowing are consistent with other published work for the knee. In a study of 67 women and men with knee OA defined by the ACR 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 an MRI 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 elevated in patients with spinal disc degeneration although no significant association was found with spinal osteophytosis (25). More recently CTX-II has been demonstrated to be increased in patients with joint space narrowing at the hip and in those with rapidly progressive hip osteoarthritis (26),(27). Although there is little existing data on the association of these markers with osteophytosis, our data suggest that both markers are significantly increased in patients with osteophytes.

Our study does differ from others investigating biochemical markers in several important ways however: only men were investigated; individual osteophyte grade, joint space narrowing grade and a cumulative, or summative, score was determined; patellofemoral disease was evaluated; subjects with more severe osteoarthritis were evaluated and the association with Galactosyl-glucosyl-pyridinoline was explored.

There is a considerable overlap between the presence of osteophytes and joint space narrowing in patients with knee OA making it difficult to dissect out 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 osteoarthritis increases, determined by a summary osteophyte, summary joint space narrowing or Kellgren & Lawrence grade, then both CTX-II and Glc-Gal-Pyd increase in a dose dependent manner. The one exception to this is that with increasing severity of joint space narrowing there appears to be a plateau effect for production of urinary CTX-II, in that once a certain level of joint space narrowing has been reached, CTX-II production plateau’s 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 joint space narrowing summative scale were few 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 osteoarthritis 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 osteoarthritis and in the biochemical markers of bone turnover used. There is good evidence of increased sub-chondral bone turnover in patients with knee osteoarthritis from studies using scintigraphy which could explain an increase in markers of bone turnover (29). However, patients with osteoarthritis 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 osteoarthritis will depend on the balance between the magnitude of the subchondral bone response (a combination of the number and intensity of joints involved) 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 osteoarthritis.

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. Weight bearing radiographs were used to examine the tibio-femoral joint and there are potential limitations using this technique as compared with semi-flexed or metatarsophalangeal views when measuring joint space narrowing but not for scoring of osteophytes and Kellgren and Lawrence grade which is heavily dependent on osteophytes as demonstrated by the similar magnitude of effects seen for K/L and osteophytes in isolated tibiofemoral disease. It has several strengths in that we investigated a large group of men, randomly selected from a large cohort study, with a full range of joint disease severity, which included isolated patello-femoral disease, areas which have previously been under researched. We also analysed individual osteophyte and joint space narrowing 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 markers in assessing the presence and severity of osteoarthritis in both the patello-femoral and tibio-femoral joints in men.

Acknowledgements
We would like to 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.

Ethical Approval
The study received ethical approval from the North and East Hertfordshire Local Ethics Research Committee, and all subjects gave written informed consent.
Legends for Figures
Figure 1
Biochemical markers and absence/presence of tibiofemoral and patellofemoral osteoarthritis
Figure 2
Biochemical markers and increased severity of radiographic tibiofemoral osteoarthritis
Figure 3
Tibiofemoral osteoarthritis adjusted for patellofemoral osteoarthritis and CTX-II and Gly-gal-pyd
Figure 4a
Biochemical markers and summative osteophyte (OP) score
Figure 4b
Biochemical markers and summative joint space narrowing (JSN) score
Reference List


Copyright: The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd to permit this article (if accepted) to be published in ARD and any other BMJPGL products and sublicences such use and exploit all subsidiary rights, as set out in our licence (http://ard.bmjournals.com/misc/ifora/licenceform.shtml)."

Conflict of interest: All authors would like to declare no conflict of interest
Figure 1
Biochemical markers and absence/presence of tibiofemoral and patellofemoral osteoarthritis

Tibiofemoral K&L grade  Patellofemoral K&L grade
Figure 2
Biochemical markers and increasing severity of radiographic tibiofemoral osteoarthritis

Osteocalcin (ng/ml)

p=0.90

0
1
2
3/4

CTX-I (ng/ml)

p=0.73

0
1
2
3/4

CTX-II (ng/ml creat)

p=0.001

0
1
2
3/4

Glyco-gal-pyd (nmol/mmol creat)

p=0.007

0
1
2
3/4

Tibiofemoral K&L grade
Figure 4a
Biochemical markers and summative osteophyte (OP) scores

Figure 4b
Biochemical markers and summative Joint space narrowing (JSN) scores