EXTENDED REPORT

Urinary levels of type II collagen C-telopeptide crosslink are unrelated to joint space narrowing in patients with knee osteoarthritis

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Objective: To determine whether urinary concentrations of the cross linked C-telopeptide of type II collagen (CTx-II) distinguish subjects with progressive radiographic or symptomatic knee osteoarthritis from those with stable disease.

Methods: Subjects were 120 obese women with unilateral knee osteoarthritis who participated in a 30 month, randomised, placebo controlled trial of structure modification by doxycycline, in which a standardised semiflexed anteroposterior view of the knee was obtained at baseline and 30 months. Subjects were selected from a larger sample to permit comparisons of urinary CTx-II levels between 60 progressors and 60 non-progressors with respect to medial joint space narrowing. Each group contained 30 subjects who, across five semi-annual assessments, reported on at least two occasions an increase of ⩾20% in 50 ft walk pain (minimum = 1 cm on a 10 cm visual analogue scale), relative to the previous visit. The remainder reported no increases in knee pain. Urine samples were obtained semi-annually for determination of the CTx-II and creatinine concentrations.

Results: In an analysis of the placebo group only, the frequency of radiographic progressors in the upper and middle tertiles (48% and 60%, respectively) of the baseline CTx-II distribution was not significantly different than that in the lower tertile (64%). These results were unchanged after inclusion of data from subjects in the doxycycline group. Furthermore, serial CTx-II levels did not distinguish subjects with progressive radiographic or symptomatic knee osteoarthritis from those with stable disease.

Conclusions: In this pilot study, urinary CTx-II concentration was not a useful biomarker of osteoarthritis progression.

Type II collagen is mostly restricted to cartilages and is, by weight, the most abundant protein in the extracellular matrix of articular cartilage. In osteoarthritis, evidence of increased cleavage of type II collagen in the diseased articular cartilage has been obtained, including cleavage by collagenases of the triple helical domain[1–4] and by collagenases and other proteases of the non-helical telopeptide domain,[5] resulting in loss of fibril structure, tensile strength, and macromolecular organisation of the extracellular matrix.

Short telopeptide domains from type I collagen, cross linked by pyridinoline residues, are excreted in the urine and have proved to be sensitive markers of bone resorption when quantified by immunoassay.[6 7] Type II collagen of cartilage contains equivalent but unique cross linking sequences that are similarly excreted and to which specific antibodies can be raised.[8 9] The type II collagen C-terminal cross linked peptide, herein abbreviated to CTx-II, can be assayed by enzyme linked immunosorbent assay (ELISA) in urine.[9–11] (This analyte was referred to in early reports as col2CTx.) The same peptide epitope (EKGPDP) was later targeted in another urinary immunoassay using a different antibody preparation.[12 13] The same proteolytic epitope (but not necessarily the same peptide fragments) can be assayed in synovial fluid, where its concentration increases soon after joint injury.[14–16] Such markers of structural collagen degradation are potentially useful for assessing disease activity and response to treatment.

We recently conducted a 30 month randomised placebo controlled trial (RCT) of doxycycline in subjects with knee osteoarthritis, in which the serial radiographs used to measure joint space narrowing (JSN) were obtained with a protocol that assured reproducible parallel alignment of the medial tibial plateau with the central *x* ray beam (this has been shown to be superior to conventional radiography for identifying JSN and to have reduced between-subject variability[17]). Doxycycline slowed the rate of JSN and reduced the frequency of clinically important episodes of increased knee pain.[18] Urine samples were obtained from subjects at baseline and every six months thereafter. The pilot study described below was designed to determine whether baseline or serial concentrations of CTx-II distinguish subjects with progressive radiographic and symptomatic knee osteoarthritis from those with stable disease.

METHODS

The doxycycline RCT was approved by institutional review boards affiliated to the six participating clinical research centres (see the acknowledgements at the end of the paper).

Subjects

Subjects were 120 women aged 45 to 64 years, all of whom had unilateral knee osteoarthritis at baseline, based upon Kellgren and Lawrence (K&L) criteria.[19] All subjects were in the upper tertile of the age, race, and sex appropriate norms for body mass index (BMI) established by the Second National Health and Nutrition Examination Survey.[20]

Abbreviations: BMI, body mass index; CTx-II, type II collagen C-terminal cross linked peptide; JSN, joint space narrowing; JSW, joint space width; RCT, randomised controlled trial; uCr, urinary creatinine concentration

Procedures

The subjects were selected from a larger sample (n = 431) in order to permit comparisons of urinary CTx-II levels between osteoarthritis progressors and non-progressors. Sixty subjects (21 doxycycline and 39 placebo) were radiographic progressors; all had JSN ⩾0.33 mm in the index knee (mean (SD), 0.97 (0.75) mm), as determined by manual measurement of magnification-corrected joint space width (JSW)[21] in semi-flexed anteroposterior knee radiographs[22] taken at baseline and 30 months later. Among the 60 radiographic non-progressors (30 doxycycline, 30 placebo), 30 month JSN in the index knee was ⩽0.22 mm (−0.03 (0.17) mm, $p<0.0001$).

Pain assessments were conducted after a washout (five half lives) of all non-steroidal anti-inflammatory drugs and analgesics taken by the subject. Because most subjects in the RCT were recruited from the community rather from than among patients seeking consultation for knee pain, baseline pain scores were low, affording little opportunity for improvement, and remained low in both treatment groups throughout the trial. Nonetheless, progressor and non-progressor groups were constituted so as to contain 30 subjects (60 total) who reported increases of ⩾20% in 50 ft walk pain relative to their previous examination, with a minimum increase of 1 cm on a 10 cm visual analogue scale (VAS), on at least two of their five semi-annual pain assessments. The remainder (30 subjects/group, 60 in all) reported no such increase in knee pain during any follow up visits. These groups are referred to as symptomatic progressors and symptomatic non-progressors, respectively. The magnitude of increase in knee pain used in our definition of symptomatic progression is consistent with the minimum clinically important difference defined in a recent analysis of RCTs in hip and knee osteoarthritis.[23 24]

Urinary CTx-II concentration

A second morning void urine sample was obtained from each subject at the baseline visit and at each semi-annual follow up visit. After centrifugation, the samples were kept at −70℃ until shipped on solid CO_2 for assay at the University of Washington. Urinary levels of CTx-II were measured by an ELISA,[9–11 15] based upon a murine monoclonal antibody (mAb) 2B4, which was raised against the synthetic peptide, EKGPDP. This sequence, which was originally isolated from human urine, is found in the C-telopeptide domain of type II collagen and is presumably generated from human cartilage collagen by proteolysis, for example by the action of matrix metalloproteinases.[25] Various experimental findings and theoretical considerations suggest that calcified cartilage degraded by osteoclasts may be a primary source of the urinary metabolite.[15]

Microtitre plates (MaxiSorp; Nunc, Naperville, Illinois, USA) were coated overnight at 4℃ with matrilysin digested human type II collagen. Matrilysin is a particularly efficient generator of the C-terminal–GPDP proteolytic epitope in vitro.[26] Plates were washed and then blocked overnight at 4℃ with 0.2% (wt/vol) bovine serum albumin (BSA) in 50 mM HEPES, 0.2% (vol/vol) Tween 20, and 0.002% (wt/vol) thimerosal, pH 7.4. Before assay, samples were thawed (stored at −20℃) then diluted 1:4 with assay buffer (50 mM HEPES, 0.2% (wt/vol) BSA, 0.2% Tween 20, 0.1 M NaCl, and 0.5 M urea, pH 7.4. The assay standard was also a matrilysin digest of human type II collagen. Wells were loaded with 80 µl of assay buffer (the same as the sample diluent), followed by 30 µl of sample or calibrator. Finally, 90 µl of mAb 2B4 conjugated with horseradish peroxidase (1:50 K in 50 mM HEPES; 0.2% (wt/vol) BSA; 0.2% (vol/vol) Tween 20, 50 mM NaCl, 2 mM $CaC1_2$, pH 7.8) were added to the wells. Plates were incubated for 16 hours at 4℃ with gentle shaking. After washing, 200 µl of the peroxidase substrate tetramethylbenzidine was added to the wells and the plates were incubated on a shaker at room temperature for 15 minutes. The peroxidase reaction was stopped with 1.0 M sulphuric acid (100 µl per well) and the optical density was measured at 450 nm.

The concentration of CTx-II in the unknown samples was determined by constructing a standard curve from measurements of known concentrations of matrilysin digested type II collagen. The results were then standardised to the urinary creatinine concentration (uCr) of the sample, which was quantified using a commercial kit (Sigma, St Louis, Missouri, USA), with an analysis based on the interaction of creatinine with picric acid to form a Janovski complex. The units for CTx-II concentration are ng/mg creatinine, calibrated by comparing a reference standard of the synthetic peptide EKGPDP, cross linked to BSA by glutaraldehyde, with the matrilysin digested type II collagen as operating standard. Samples were run in duplicate as a coded series in which progressors and non-progressors were randomly dispersed. Random samples from each assay plate were selected for repeat analysis. For those showing poor reproducibility, the entire plate of samples was repeated and the mean value used.

Dilution of standard and samples of urine generated parallel curves. Based on the results of analyses of 30 duplicate pairs of urine samples which were randomly dispersed among the aliquots from the subjects in the RCT, interassay reproducibility of the CTx-II/creatinine result was 27.7%. No explanation for this higher than usual coefficient of variability was found.

Statistical analysis

The ability of baseline levels of urinary CTx-II to predict radiographic progression of osteoarthritis in the index knee

Table 1 Characteristics of progression subgroups at baseline

	Radiographic progression		Symptomatic progression	
Variable	**Yes**	**No**	**Yes**	**No**
Number of subjects	60	60	60	60
Age (years)	55.2 (5.6)	54.4 (5.6)	54.5 (5.7)	55.1 (5.5)
Body mass index (kg/m^2)	36.7 (6.6)	36.3 (5.6)	37.7 (6.4)*	35.3 (5.7)*
Postmenopausal	40 (67%)	38 (63%)	42 (70%)	36 (60%)
Minimum JSW (mm)	3.42 (1.35)	3.78 (1.16)	3.74 (1.33)	3.47 (1.19)
30 Month index knee JSN (mm)	0.97 (0.75)	−0.03 (0.17)	0.53 (0.88)	0.41 (0.57)
Frequency of pain increases ⩾20%, relative to previous visit (% of 5 semi-annual assessments)	23 (24)	22 (23)	45 (9)	0 (0)
CTx-II (ng/mg uCr)	65.0 (24.0)	65.3 (24.6)	66.8 (25.6)	63.5 (22.8)

Values are mean (SD) or n (%).
*$p<0.05$ for comparison of symptomatic progressors *v* non-progressors.
CTx-II, type II collagen C-terminal cross linked peptide; JSN, joint space narrowing; JSW, joint space width; uCr, urinary creatinine concentration.

Table 2 Frequency of progression of joint space narrowing by tertile of baseline CTx-II and adjusted odds ratios for prediction of progressors versus non-progressors at 30 months

Baseline CTx-II tertile (ng/mg uCr)*	Placebo group (n = 69)				Combined treatment groups (n = 120)			
	n	Progressors	OR†	95% CI	n	Progressors	OR†‡	95% CI
Upper (73.2 to 137.4)	27	13 (48%)	0.52	0.16 to 1.70	40	20 (50)	0.92	0.37 to 2.31
Middle (53.0 to 73.1)	20	12 (60%)	0.89	0.24 to 3.27	40	19 (48)	0.94	0.37 to 2.34
Lower (12.6 to 52.9)	22	14 (64%)	1.00	n/a	40	21 (53)	1.00	n/a

Values are n (%).
*Based on the distribution of baseline CTx-II values of subjects in both treatment groups.
†Adjusted for baseline joint space width, age, and menopausal status.
‡Adjusted also for treatment group.
CI, confidence interval; CTx-II, type II collagen C-terminal cross linked peptide; n/a, not applicable; OR, odds ratio; uCr, urinary creatinine concentration.

was evaluated with multiple logistic regression analysis. Separate analyses were carried out on data from the placebo group (n = 69) and from subjects in the combined treatment groups (n = 120). Logistic regression models calculated the change in odds of showing progression of JSN associated with CTx-II levels in the upper and middle tertiles of the baseline distribution in comparison with the lower tertile. Odds ratios were adjusted for baseline JSW. The parallel analysis carried out on data from all subjects adjusted the odds ratios for treatment group. Interactions of baseline CTx-II concentration and treatment group were examined in all models.

The degree to which serial CTx-II values reflected post-randomisation differences between treatment groups in the DMOAD trial or longitudinal differences between progressors and non-progressors with respect to JSN or pain was evaluated using a mixed effects linear model. This repeated measures comparison of mean change in adjusted CTx-II values over each of the six month intervals in the 30 month trial included a random subject effect and fixed effects for treatment group, radiographic progression, symptomatic progression, time (six months, 12 months, and so on), the baseline CTx-II value, and the treatment group×time interaction.

RESULTS

Subjects were, on average, 54.8 years old and had a mean BMI of 36.5 kg/m^2. Radiographic progressor and non-progressor groups did not differ at baseline with respect to mean age, BMI, menopausal status, urinary CTx-II level, or medial tibiofemoral compartment JSW (table 1). Because of the stratified selection process, radiographic progressors and non-progressors were similar with respect to the frequency with which they reported an increase of ⩾20% in 50 ft walk pain over successive semi-annual pain assessments (23% *v* 22%). Baseline CTx-II concentrations were only slightly higher in post-menopausal than in pre-menopausal women (mean (SD), 62.2 (21.4) *v* 66.7 (25.6) ng/mg uCr, p = 0.181).

Predictive value of the baseline CTx-II Level

The results of logistic regression analyses to determine the extent to which the CTx-II concentration at baseline predicted progression of JSN in the index knee are shown in table 2. In the analysis of the placebo group, the frequency of radiographic progressors in the upper and middle tertiles (48% and 60%, respectively) was not significantly different than that in the lower tertile (64%). These results were unchanged after inclusion of data from subjects in the doxycycline group and controlling for treatment group (table 2). The results of these regression analyses were unchanged also after control for concurrent JSN in the contralateral knee.

Association between JSN, joint pain, and change in CTx-II concentrations

Adjusted (least squares) means from the mixed model analysis of changes in CTx-II concentrations are shown in table 3. After controlling for the baseline CTx-II level and time, the model indicated that serial changes in CTx-II values failed to distinguish subjects with respect to treatment group (p = 0.777), progression of JSN (p = 0.415), or progression of osteoarthritis pain (p = 0.872).

DISCUSSION

The search for a molecular biomarker of osteoarthritis that may identify subjects likely to undergo rapid loss of articular cartilage has been under way for more than 20 years. Approximately 15 years ago, we reviewed a variety of scientific and clinical issues that may confound the interpretation and affect the utility of biomarker measurements. We concluded that no marker was available at that time which could confidently be used to monitor the response of an osteoarthritis patient to treatment.[27] Among the issues we raised were the absence of controls for sources of molecular markers other than the index joint (that is, articular cartilage in other joints, non-articular cartilage) and the limitations of conventional radiography for detecting the progression of osteoarthritis.

Nonetheless, recent studies of another version of the urinary collagen type II C-telopeptide assay (CTx-II) have been the source of some optimism that a useful biomarker of articular cartilage degradation may be forthcoming. Mean urinary levels were related to disease burden (number of

Table 3 Least square means of change in CTx-II levels by treatment group and among radiographic and symptomatic progressors and non-progressors after adjustment for time and baseline urinary CTx-II concentration

Subgroup	No of subjects	Change in urinary CTx-II (ng/mg uCr)*
Doxycycline treatment group	51	1.14 (1.93)
Placebo group	69	0.53 (1.75)
Radiographic progressors†	60	−0.03 (1.88)
Radiographic non-progressors	60	1.69 (1.78)
Symptomatic progressors‡	60	1.11 (2.90)
Symptomatic non-progressors	60	0.56 (1.39)

Values are mean (SEM).
*Changes in CTx-II levels did not differ significantly between any of the paired subgroups examined.
†Based on 30 month joint space narrowing in the index knee.
‡Based on two or more increases ⩾20% in semi-annual assessments of 50 ft walk pain over 30 months.
CTx-II, type II collagen C-terminal cross linked peptide; uCr, urinary creatinine concentration.

involved joints) in patients with knee and hand osteoarthritis.[11] Mean urinary CTx-II was also found by Garnero *et al*[28] to be increased in patients with knee osteoarthritis compared with healthy controls. Reijman *et al*[29] showed that the risk of progression of radiographic osteoarthritis of the hip (mean interval 6.6 years) increased more than eightfold among subjects whose baseline CTx-II level was in the highest quartile in comparison with those in the lowest quartile.

The results of the present study do not support the above findings—at least for obese women. We failed to find an association between raised levels of baseline CTx-II and JSN at 30 months. It should be acknowledged that our ability to detect a valid biomarker may have been limited by the procedures used for the collection of urine samples. Smith *et al*[30] showed that levels of collagen crosslinks (pyridinoline, deoxypyridinoline, and N-telopeptide of type II collagen) were less variable when measured in a 24 hour urine sample than in a second morning void, and that the increased variability of single void collections appeared to be confounded by normalising to the urinary creatinine concentration. In the present study, second morning void samples were obtained when feasible. The error associated with this limitation may have precluded accurate prediction of JSN in a small pilot study. Alternatively, the prognostic significance of urinary CTx-II may be apparent only over intervals longer than 30 months. The minimum interval to predict JSN based on raised CTx-II levels may be three years.[31]

Garnero *et al*[32] showed that a change in the ratio of urinary CTx-II (presumably a marker of cartilage collagen degradation) to the serum level of the N-propeptide of type IIA procollagen (PIIANP) (a marker of collagen synthesis), reflecting an uncoupling of type II collagen synthesis and degradation, identified patients at high risk for rapid progression of radiographic knee osteoarthritis more effectively than either measurement alone. The difference in the conclusions reached by Garnero *et al*[32] and those of the present study may reflect the choice of protocols for knee radiography. The former study used the standing AP view, and even though it employed fluoroscopy to achieve radioanatomical alignment of the medial tibial plateau and central *x* ray beam, JSN measured in serial standing AP views can be confounded by longitudinal changes in knee pain.[33] Also, the definition of radiographic progression used by Garnero *et al*[29]—that is, ⩾0.5 mm within one year—may have identified subjects who had more rapidly progressive knee osteoarthritis than those in the present study (that is, ⩾0.33 mm within 2.5 years).

More recently, urinary CTx-II concentrations have been shown to be markedly decreased after treatment with the antiresorptive drug risedronate in patients with knee osteoarthritis.[33 34] However, the effect of risedronate on CTx-II was not accompanied by a slowing of JSN in knee radiographs acquired with a positioning protocol identical to that used in the present study.

The failure of this pilot study to find an association between urinary levels of CTx-II and JSN in knees with established osteoarthritis is not the result of limited power. With 50% of the 120 subjects selected because they had incontrovertible evidence of progressive knee osteoarthritis, logistic regression analysis of data from both treatment groups had 80% power to detect a 35% difference between the highest and lowest tertiles of baseline CTx-II in the frequency of progression of JSN (that is, 67.5% *v* 32.5%). The observed frequencies of JSN in the highest and lowest tertiles were very similar (48% and 64%, respectively), and the difference was clinically insignificant. Likewise, even given the degree of error variance in CTx-II values suggested by the interassay reproducibility estimate, the repeated measures analysis of serial CTx-II concentrations still had 80% power to detect a mean six month increase in CTx-II in the 60 progressors that was 2.87 ng/mg uCr greater than that among the 60 non-progressors. However, the mean six month change in CTx-II levels among progressors was *smaller* than that measured in non-progressors (table 3), and the magnitude of the difference was only 60% of the minimum detectable difference.

It should also be noted that the assay we used is based on a different antibody preparation from that used in the studies of Garnero *et al*[12 13 28 32 33] and Reijman *et al*,[29] although the target epitope (C-terminus of EKGPDP where K is the lysine involved in cross linking) is the same. The tissue sources and metabolic pathways leading to the excretion of this peptide fragment in adult urine have not been clearly defined, but high levels in children and other considerations implicate growth plates in children and resorption of mineralised tissue in adults.[8 15 25] The suppression of levels by bisphosphonate,[33 34] higher levels in postmenopausal women than in men, and doubling of the concentration over the period of menopause[35] all support an origin in osteoclastic resorption.

In summary, the present study was a pilot investigation to ascertain whether baseline or serial measures of urinary CTx-II could distinguished between subjects undergoing radiographic or symptomatic progression of knee osteoarthritis, or both, as evaluated with a radiographic protocol for fluoroscopically standardising the position of the knee in serial examinations.[22] We found that baseline levels of urinary CTx-II did not accurately predict which subjects would undergo radiographic JSN, nor did serial assays of CTx-II distinguish radiographic or symptomatic progressors from subjects with stable disease. As in previous studies, the potential sources of CTx-II in urine are the index knee and all other joints, and type II collagen containing tissues. We have shown, for example, that the contralateral knee in these subjects is a common source of osteoarthritis that is not apparent in the conventional standing AP knee radiograph.[36] However, adjustment for concurrent JSN in the contralateral knee did not improve the prediction of JSN. We conclude that urinary CTx-II is not a useful biomarker of progression of knee osteoarthritis.

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REFERENCES

1 **Poole AR**. Biochemical/immunochemical biomarkers of osteoarthritis: utility for prediction of incident or progressive osteoarthritis. *Rheum Dis Clin North Am* 2003;**29**:803–18.
2 **Billinghurst RC**, Dahlbert L, Ionescu M, Reiner A, Bourne R, Rorabeck C, *et al*. Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. *J Clin Invest* 1997;**99**:1534–45.

3 **Dahlbert L**, Billinghurst C, Manner P, Ionescu M, Reiner A, Tanzer M, *et al.* Collagenase-mediated cleavage of type II collagen is selectively enhanced in osteoarthritis cartilage and can be arrested with a synthetic inhibitor which spares collagenase-1 (MMP-1). *Arthritis Rheum* 2000;**43**:673–82.
4 **Squires G**, Ionescu M, Okouneff S, Poole AR. The pathobiology of focal lesions development in aging human articular cartilage and molecular matrix changes characteristic of osteoarthritis. *Arthritis Rheum* 2003;**48**:1261–70.
5 **Poole AR**. Cartilage in health and disease. In:Koopman W, editor.*Arthritis and allied conditions.A textbook of rheumatology*, 14th edition. Philadelphia: Lippincott, Williams & Wilkins, 2001:226–84.
6 **Hanson DA**, Weis MA, Bollen AM, Maslan SL, Singer FR, Eyre DR. A specific immunoassay for monitoring human bone resorption: quantitation of type I collagen cross-linked N-telopeptides in urine. *J Bone Miner Res* 1992;**7**:1251–8.
7 **Eyre DR**. Biochemical basis of collagen metabolites as bone turnover markers. In:Raisz L, Rodin G, Bilezikian JP, editors. *Principles of bone biology*. London: Academic Press, 1996:143–53.
8 **Eyre DR**, Shao P, Vosberg-Smith K, Weis M, Shaffer K, Yoshihara P. Cross-linked telopeptides from collagenase types I, II and III in human urine. *J Bone Miner Res* 1996;**11**(suppl):S193.
9 **Norlund LL**, Shao P, Yoshihara P, Eyre DR. Markers of bone type I and cartilage type II collagen degradation in the Hartley guinea pig model of osteoarthritis. *Trans Orthop Res Soc (San Francisco)* 1997;**22**:313.
10 **Moskowitz RW**, Holderbaum D, Atley LM, Eyre DR. Type II collagen C-telopeptide 2B4 epitope is a marker for cartilage degradation in familial osteoarthritis (abstract). *Arthritis Rheum* 1998;**41**:S352.
11 **Atley LM**, Sharma L, Clemens JD, Shaffer K, Pietka TA, Riggins JA, Eyre DR. The collagen II CTx degradation marker is generated by collagenase 3 and in urine reflects disease burden in knee OA patients (abstract). *Trans Orthop Res Soc* 2000;**25**:168.
12 **Christgau S**, Garnero P, Fledelius C, Moniz C, Ensign M, Gineyts E, *et al.* Collagen type II C-telopeptide fragments as an index of cartilage degradation. *Bone* 2001;**29**:209–15.
13 **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.
14 **Lohmander LS**, Atley LM, Pietka TA, Eyre DR. The release of crosslinked peptides from type II collagen into human synovial fluid is increased soon after joint injury and in osteoarthritis. *Arthritis Rheum* 2003;**48**:3030–9.
15 **Matyas JR**, Atley L, Ionescu M, Eyre DR, Poole AR. Analysis of cartilage biomarkers in the early phases of canine experimental osteoarthritis. *Arthritis Rheum* 2004;**50**:543–52.
16 **Lindhorst E**, Wachsmuth L, Kimmig N, Raiss R, Aigner T, Atley L, Eyre D. Increase in synovial fluid collagen type II degradation products after meniscectomy. *Osteoarthritis Cartilage* 2005;**13**:139–45.
17 **Mazzuca SA**, Brandt KD, Dieppe PA, Doherty M, Katz BP, Lane KA. Effect of alignment of the medial tibial plateau and x-ray beam on apparent progression of osteoarthritis in the standing anteroposterior knee radiograph. *Arthritis Rheum* 2001;**44**:1786–94.
18 **Brandt KD**, Mazzuca SA, Katz BP, Lane KA, Buckwalter KA, Yocum DE, *et al.* Effect of doxycycline on progression of osteoarthritis. *Arthritis Rheum* 2003;**52**:2015–25.
19 **Kellgren JH**, Lawrence JS. Radiographic assessment of osteoarthritis. *Ann Rheum Dis* 1957;**16**:494–502.
20 **Najjar MF**, Rowland M. Anthropometric reference data and prevalence of overweight—United States, 1976–80. Hyattsville, Maryland: National Center for Health Statistics, 1987; Department of Health and Human Services publication No (PHS) 87–1688 (Vital and Health Statistics, Series 11, No 238).
21 **Mazzuca SA**, Brandt KD, Buckwalter KA, Lequesne M. Pitfalls in the accurate measurement of joint space narrowing in semiflexed anteroposterior radiographs of the knee. *Arthritis Rheum* 2004;**50**:2508–15.
22 **Buckland-Wright JC**, Macfarlane DG, Williams SA, Ward RJ. Accuracy and precision of joint space width measurements in standard and macroradiographs of osteoarthritic knees. *Ann Rheum Dis* 1995;**54**:872–80.
23 **Strand V**, Kelman A. Outcome measures in osteoarthritis: randomized controlled trials. *Curr Rheum Rep* 2004;**6**:20–30.
24 **Tubach F**, Ravaud P, Baron G, Falissard B, Logeart I, Bellamy N, *et al.* Evaluation of clinically relevant changes in patient reported outcomes in knee and hip osteoarthritis: the minimally clinically important improvement. *Ann Rheum Dis* 2005;**64**:29–33.
25 **Eyre DR**, Atley LM, Shao P, Vosberg-Smith K, Shaffer K, Ochs V, *et al.* Biochemical markers of bone and cartilage collagen degradation. In: Goldberg M, Boskey A, Robinson C, eds. *Chemistry and biology of mineralized tissues*. Rosemont, IL: American Academy of Orthopaedic Surgeons, 2000:347–50.
26 **Atley LM**, Shao P, Ochs V, Shaffer K, Eyre DR. Matrix metalloproteinase-mediated release of immunoreactive telopeptides from cartilage type II collagen (abstract). *Trans Orthop Res Soc* 1998;**23**:850.
27 **Brandt KD**. A pessimistic view of serologic markers for diagnosis and management of osteoarthritis. Biochemical, immunologic and clinicopathologic barriers. *J Rheum* 1989;**16**(suppl 18):39–42.
28 **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.
29 **Reijman M**, Hazes JMW, Bierma-Zeinstra SMA, Koes BW, Christgau S, Christiansen C, *et al.* A new marker for osteoarthritis. Cross-sectional and longitudinal approach. *Arthritis Rheum* 2004;**50**:2471–8.
30 **Smith SM**, Dillon EL, DeKerlegand DE, Davis-Street JE. Variability of collagen crosslinks: Impact of sample collection period. *Calcif Tissue Int* 2004;**74**:336–41.
31 **Maziéres B**, Garnero P, Gueguen A, Abbal M, Berdah L, Freiburghaus C, *et al.* Molecular markers of cartilage breakdown and synovitis are strong independent predictors of structural progression of hip osteoarthritis (abstract). The ECHODIAH cohort. *Arthritis Rheum* 2003;**48**(9 suppl):S683.
32 **Garnero P**, Ayral X, Rousseau J-C, Christgau S, Sandell LJ, Dougados M, *et al.* Uncoupling of type II collagen synthesis and degradation predicts progression of joint damage in patients with knee osteoarthritis. *Arthritis Rheum* 2002;**46**:2613–24.
33 **Garnero P**, Bingham C, Aronstein W, Cohen S, Conaghan P, Cline G, *et al.* Treatment with risedronate reduced urinary CTX-II, a specific biochemical marker of cartilage type II collagen degradation, in a 24-month study of knee OA (abstract). *Arthritis Rheum* 2004;**50**(suppl 9):S656.
34 **Bingham C**, Aronstein W, Adami S, Cohen S, Conaghan P, Dougados M, *et al.* Treatment with risedronate reduced urinary CTX-II, a specific biochemical marker of cartilage type II collagen degradation, in a 24-month study of knee OA (abstract). *Osteoarthritis Cartilage* 2004;**12**(suppl B):S9.
35 **Mouritzen U**, Christgau S, Lehmann H-J, Tankó LB, Christiansen C. Cartilage turnover assessed with a newly developed assay measuring collagen type II degradation products: influence of age, sex, menopause, hormone replacement therapy, and body mass index. *Ann Rheum Dis* 2003;**62**:332–6.
36 **Mazzuca SA**, Brandt KD, German C, Buckwalter KA, Lane KA, Katz BP. Development of radiographic changes of osteoarthritis in the ''Chingford knee'' reflects progression of disease or non-standardised positioning of the joint, rather than incident disease. *Ann Rheum Dis* 2003;**62**:1061–5.