

EXTENDED REPORT

Procollagen II C propeptide level in the synovial fluid as a predictor of radiographic progression in early knee osteoarthritis

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Objective: To investigate the prognostic value of procollagen type II carboxy-terminal propeptide (PIICP) level in synovial fluid in relation to early tibiofemoral joint osteoarthritis (OA).

Methods: Data were collected on 172 women (age 40 to 59 years) who had knee pain and tibiofemoral joint OA in the early stage. Standing semiflexed knee radiographs were obtained by fluoroscopy at baseline and at four year follow up and a computerised, magnification corrected measurement system was applied to measurement of minimal joint space width in the tibiofemoral compartment. Synovial fluid sampling was performed at baseline and at the four year follow up. Levels of PIICP in the synovial fluid were measured by enzyme immunoassay. The outcome measures were assessed by radiographic joint space narrowing (JSN) in the tibiofemoral joints over four years. Multiple linear regression analyses were used to examine the relation between radiographic JSN and synovial fluid level of PIICP.

Results: The number of women available at both baseline and at four year follow up was 110. The average of radiographic JSN over four years was 0.53 mm (range 0.00–2.01). Body mass index showed a slightly positive association with baseline PIICP level. In multiple linear regression analyses adjusted for age and body mass index, radiographic JSN over four years had a direct positive correlation with baseline PIICP level ($r=0.395$; 95% confidence interval (95% CI) 0.231 to 0.529; $p<0.001$).

Conclusion: In a four year prospective study of women, quantification of synovial fluid PIICP was able to predict subsequent radiographic progression in early tibiofemoral joint OA.

Despite recent progress in technologies for diagnosing knee osteoarthritis (OA), no practical and non-invasive method exists for detecting early loss of articular cartilage. In clinical practice, knee OA is diagnosed by a combination of symptoms, clinical signs, and radiographic findings. Although radiographic changes are regarded as the standard in diagnosis, the problem with radiographic grading is that it only presents a record of past destructive events and does not give information on current disease activity. As pain is the clinical feature that leads most patients with knee OA to seek medical attention, by the time a patient with knee OA presents to a doctor, cartilage breakdown is often advanced. Thus, it is necessary to detect early OA of the knee and predict prognosis of the disease. Attempts have been made to discover specific molecules that are sensitive joint markers for the degeneration and repair processes of the articular cartilage. Although serum levels of cartilage oligomeric matrix protein (COMP)¹ and hyaluronic acid (HA)² have been identified as predictors of disease progression in established knee OA, the doctor is in need of prognostic markers early in the disease to be able to provide adequate care and treatment. However, it remains to be clarified whether biological markers can provide information on variations in the progression of early knee OA.

Osteoarthritis is a slowly progressive degenerative disease that is characterised by early loss of the tensile strength of articular cartilage accompanied by increased denaturation of type II collagen.³ Type II collagen is synthesised as procollagen and contains three identical α chains: a central triple helical domain is flanked by non-helical N-terminal and C-terminal propeptides. These propeptides are removed by specific proteinases before type II collagen is incorporated into collagen fibrils.⁴ Procollagen type II C-propeptide (PIICP) is a product of collagen II synthesis and can be assayed in synovial fluid and serum by a

specific immunoassay.^{5,6} It seems to be absent from tissues other than cartilage and intervertebral discs.⁷ Therefore, it is likely that most of the PIICP in synovial fluid originates from articular cartilage and that PIICP is a marker of type II collagen synthesis in cartilage.^{6,8} In normal cartilage, the degradation and synthesis of matrix molecules become balanced in a stable equilibrium.⁹ During OA development, changes occur not only in the degradation of matrix molecules but also in the rate of synthesis and structure of several matrix components.^{8,10–12} In osteoarthritic cartilage the rate of synthesis and degradation of the matrix is accelerated and the balance between the catabolism and anabolism of the matrix components is disturbed.⁹ The synovial fluid concentration of PIICP has been shown to be significantly higher in patients with early knee OA than in normal controls.⁶ Furthermore, it has been shown that the synovial fluid concentration of PIICP begins to increase at an early stage of cartilage degeneration that can be identified by arthroscopy but not by plain radiography.⁵

We designed the present study to assess the hypothesis that the PIICP concentration in synovial fluid can predict disease outcome in early primary OA of the knee.

SUBJECTS AND METHODS

Criteria for inclusion in the study

In 1995, a population based survey was conducted in Sakauchi Village and Fujihashi Village, Gifu Prefecture, Japan, to

Abbreviations: BMI, body mass index; CI, confidence interval; COMP, cartilage oligomeric matrix protein; CV, coefficient of variation; HA, hyaluronic acid; JSN, joint space narrowing; JSW, joint space width; NSAIDs, non-steroidal anti-inflammatory drugs; OA, osteoarthritis; PIICP, procollagen type II carboxy-terminal propeptide

research knee OA. The villagers living there did not move much from the area and most of them were small scale farmers. The standing semiflexed and skyline radiographs of the bilateral knee joints were examined according to the methods proposed by Buckland-Wright *et al.*¹³⁻¹⁵ Information about knee pain and duration was collected for each knee separately and each knee joint was examined in a systematic way. Details were collected on whether the person had ever had pain in the knee and the number of episodes of knee pain in the past year.

We selected 172 women aged 40 to 59 who had had knee pain in the past year and had early primary OA of the tibiofemoral joint. Knee pain in the past year was defined as two or more episodes of pain not related to trauma, each lasting for at least 15 days, during the previous year. Women who had one or more small marginal osteophytes (maximum osteophyte length 1.0 to 2.0 mm) in the tibiofemoral compartment were defined as having early OA of the tibiofemoral joint. The inclusion criteria were: (a) osteophytes graded as median, between grade 0 and grade 1, (b) joint space narrowing (JSN) graded as 0, and (c) absence of subchondral sclerosis, bony attrition, and chondrocalcinosis in the tibiofemoral compartment; according to standards of the radiographic atlas published by the Osteoarthritis Research Society.¹⁶ Patients with patellofemoral joint OA were specifically excluded because tibiofemoral and patellofemoral joint OA should be considered as separate disorders; therefore criteria for inclusion were: (a) osteophytes graded as 0, (b) JSN graded as 0, and (c) absence of subchondral sclerosis in the patellofemoral compartment.¹⁶ The diagnosis of early primary OA was made by an orthopaedic specialist (YS). Synovial fluid sampling was performed in the selected 172 women at the baseline examination. Synovial fluids were aspirated as completely as possible from the targeted knee joints using a parapatellar approach by one orthopaedic doctor (SS). More than 1.0 ml of synovial fluid could be obtained for analyses from 128/172 women, and 44 patients had less than 1.0 ml of synovial fluid aspirated at the baseline. We were able to recontact all 128 women after one month and also attempted to obtain samples of synovial fluid. Of the 128, 16 had less than 1.0 ml of synovial fluid aspirated and therefore 112 women, from whom more than 1.0 ml of synovial fluid could be obtained for analyses, were enrolled in the study. By contrast, of 523 healthy control women aged 40 to 59 without knee pain and with normal radiographic findings, more than 1.0 ml of synovial fluid could be obtained for analyses from only 109. The mean (SD) minimal joint space width (JSW) in the tibiofemoral compartment was 3.4 (0.3) mm in the enrolled 112 patients and 3.6 (0.3) mm in the 109 healthy control women. These differences were not significant ($p=0.102$, t test).

Major reasons for exclusion were regular use of non-steroidal anti-inflammatory drugs (NSAIDs) during the previous six months, intra-articular injection of corticosteroid or hyaluronate within the previous six months, pregnancy or nursing, valgus or varus knee deformity, recent knee injury, and post-traumatic or rheumatoid arthritis,

Radiographic technique

All radiographs were obtained by one radiological technician using a single x ray machine (Sire Graph C, Siemens, Munich, Germany) at a public clinic of the Sakauchi and Fujihashi villages during the whole study. We determined protocols defining the precise positioning of the knee joints, standard criteria for x ray beam alignment, and allowance for inherent radiographic magnification to ensure that we would obtain comparable radiographs at subsequent visits.

Protocol for the tibiofemoral compartment

Each knee was radiographed separately. The x ray tube was positioned so that the central ray of the x ray beam was horizontal and parallel to the floor, with the film to focus distance

set to 1 m. A 5 mm diameter metal sphere encased in a block of Plexiglas was placed on the head of the fibula of each knee to determine the degree of radiographic magnification. The precise position of the knee was confirmed visually by fluoroscopy. With the subject standing straight, the knee was flexed until the tibial plateau was horizontal and perpendicular to the x ray film. The tibial plateau was horizontal when the anterior and posterior margins of the medial compartment were superimposed. With the heel fixed, the foot was internally or externally rotated until the tibial spines appeared centrally placed relative to the femoral notch. The radiograph was taken immediately after this position was obtained. The outline of the foot was drawn on a large sheet of paper taped to the floor to facilitate joint repositioning at the follow up study.

Protocol for the patellofemoral compartment

Each knee was radiographed separately. The x ray tube was positioned so that the x ray beam was directed vertically downwards and the film to focus distance was set to 1.5 m. A 5 mm diameter metal sphere encased in a block of Plexiglas was placed on the anterior surface of the patella. With the subject standing straight, the knee was flexed to 30 degrees and the leg was positioned so that it was aligned in the vertical plane. The subject's stability was maintained by a walker frame. The radiographic plate was placed on a box positioned below the knee joint. With the aid of the tube's positioning light, the central ray of the x ray beam was directed so as to project through the patellofemoral joint space. The radiograph was taken immediately after the desired position was obtained.

Baseline examination and follow up

The study period was four years. The 112 patients enrolled were first examined in 1995, at which time demographic and clinical data including age, weight, height, and clinical symptoms of the knee joints were recorded. Body mass index (BMI) was calculated as weight (kg) divided by height (m)². None of the enrolled patients were receiving NSAIDs, except for the occasional use of these drugs as rescue treatment during the study period (maximum use <four times a month). The four year follow up study was performed in 1999, and the standing semiflexed and skyline radiographs of the bilateral knee joints were re-examined by the radiological technician according to our protocols. Synovial fluid was aspirated from the targeted knee joints by the same orthopaedic doctor (SS) in the same manner as for the baseline examination.

Assessment of radiographs

The radiograph of the targeted knee was digitised with an image analyser (Mediscan, Hologic, Massachusetts, USA) at a resolution of 1024×768 pixels/inch² and 256 grey levels. The digitised images of the radiographs were prepared with a specially programmed computer system (VAIO, Sony, Tokyo, Japan). The computer was correctly calibrated through repeated measurements of a line segment of known length until the coefficient of variation (CV) of the measurement was less than 0.3%. The 5 mm dimension of the metal sphere was used to determine the degree of radiographic magnification. The digitised image was modified by the computer software using edge detection and magnification methods to obtain a very clear outline of the tibiofemoral joint space. The joint space contours were delineated using the computer mouse on the margin of the femur condyle and the margin of the tibial plateau according to the methods proposed by Buckland-Wright.¹⁵ On the digitised images of the radiographs at the baseline and at the four year follow up, the minimal JSW was measured automatically by the computer as the interbone distance at the narrowest points of the tibiofemoral joint. All assessments were carried out by one observer (SS). The

Table 1 Epidemiological data of 110 women with complete data at baseline

	Subjects (mean, SD (range))
Age (years)	50.2 (6.0 (40–59))
Body mass index (kg/m ²)	24.7 (3.3 (19.2–35.0))
Minimal joint space width (mm)	3.4 (0.3 (2.6–4.3))
Synovial fluid volume (ml)	2.9 (3.0 (1.0–16.0))
PIICP (ng/ml)	2.09 (0.86 (0.69–5.10))

Table 2 Minimal joint space width and PIICP concentration at baseline and at four year follow up

	Subjects available at the baseline n=110 (mean (SD (range)))	Subjects available at the follow up n=110 (mean (SD (range)))	Healthy controls* n=109 (mean (SD (range)))
Minimal joint space width (mm)	3.4 (0.3 (2.6–4.3))	2.9 (0.5 (1.6–4.2))	3.6 (0.3 (2.8–4.4))
PIICP (ng/ml)	2.09 (0.86 (0.69–5.10))	2.40 (1.35 (0.88–8.58))	0.68 (0.24 (0.20–1.44))
Ln PIICP (units)	0.66 (0.38 (–0.36–1.63))	0.76 (0.47 (–0.11–2.15))	–0.39 (0.17 (–1.61–0.37))

*Taken at the baseline of the study subjects.

decrease in the minimum JSW (mm) over four years was calculated, enabling the determination of radiographic JSN.

The reproducibility of the standing semiflexed x ray film technique and of the computerised, magnification corrected measurement of minimal JSW was assessed by the CV. For 20 randomly selected patients from the enrolled 112 patients, three standing semiflexed radiographs were performed by the radiological technician on different days to assess the reproducibility for minimal JSW measurement. The minimal JSW was measured 10 times by one observer (SS) for each radiograph in 10 randomly selected patients to assess the reproducibility for repeat measures.

Measurement of PIICP concentration

Synovial fluid samples collected from each subject were kept frozen at –80°C until used for analyses. The synovial fluid was digested with 0.005 units of hyaluronidase SD (Seikagaku KK, Tokyo, Japan; purified from the culture broth of *Streptococcus dysgalactiae*) per 1.0 ml fluid for 30 minutes at 25°C. Hyaluronidase SD catalyses the eliminative cleavage of N-acetylhexosaminide linkages of HA and does not act on chondroitin, dermatan, heparan, or keratan sulphate. The concentration of PIICP in the synovial fluid was measured by a sandwich enzyme immunoassay using commercially available kits (Teizin KK, Osaka, Japan). The synovial assay used rabbit polyclonal antibodies raised against bovine PIICP and then immobilised on polystyrene balls.⁶ These antibodies have been shown to cross react significantly with human and bovine PIICP and not to cross react with type I and II collagen, hyaluronate, osteocalcin, procollagen type III aminoterminal propeptide, or procollagen type II carboxyterminal propeptide.⁶ Values were reported as equivalents of PIICP purified from fetal bovine growth plate cartilage. All determinations were made in duplicate. The sensitivity of the assay was 0.2 ng/ml for human PIICP. The within assay and between assay CVs were 3.0% and 4.1%, respectively.

Statistical analyses

The statistical analyses were performed with SAS programs.¹⁷ When the distribution of variables was not normal, a logarithmic transformation (natural log, ln) was applied for statistical analyses. A *t* test was performed on each set of variables. Linear regression analyses were used to examine the relations between continuous variables such as age, BMI, synovial fluid

volume, minimal JSW, PIICP level, and radiographic JSN. The regression coefficient was shown as *r*. To account for possible intercorrelations, multiple linear regression analyses were undertaken with radiographic JSN entered as the dependent variable and baseline PIICP level entered along with the other variables as one of the independent variables. Differences at *p*<0.05 were considered significant and confidence intervals (CIs) were stated at 95%.

RESULTS

Baseline data and follow up

The response rate of the originally enrolled 112 patients for the four year follow up study was 110/112 (98.2%) because two were lost to follow up during the study period. The lost patients were not significantly different from the enrolled 112 patients for age, BMI, minimal JSW, PIICP level, and synovial fluid volume. At the four year follow up study more than 1.0 ml of synovial fluid could be obtained from all 110 patients for analyses. All cross sectional analyses were carried out on the 110 patients for whom complete data were available. Table 1 gives the epidemiological data of the 110 patients at baseline.

Assessment of radiographs

The standing semiflexed radiographic technique showed good reproducibility. The mean CV (SD) was 4.1 (1.8)% for minimum JSW measurement on the standing semiflexed radiograph. The computerised, magnification corrected method of JSW measurement also had good reproducibility with a mean CV (SD) of 1.5 (0.7)% for repeat measures.

All cross sectional analyses were carried out on the 110 patients available at both baseline and at four year follow up. Table 2 summarises the results. The mean (SD) minimal JSW in the targeted knee was 3.4 (0.3) mm at the baseline and 2.9 (0.5) mm at the follow up study. Minimal JSW significantly decreased during the study period (*p*<0.001, *t* test). The mean (SD) radiographic JSN over four years was 0.53 (0.43) mm (median 0.40, range 0.00–2.01).

Analyses of synovial fluids

All cross sectional analyses were carried out in the 110 patients available at both baseline and at four year follow up. Table 2 summarises the results. The mean (SD) PIICP level was 2.09 (0.86) ng/ml (median 1.85, range 0.69–5.10) and 2.40

Table 3 Results of multiple linear regression analyses of radiographic joint space narrowing and other variables

	Crude		Age and body mass index adjusted	
	Regression coefficient (95% CI)	p Value	Regression coefficient (95% CI)	p Value
Age	0.109 [-0.067 to 0.284]	0.238	-	-
Body mass index	0.260 [0.084 to 0.419]	0.005	-	-
Minimal joint space width	-0.107 [-0.278 to 0.072]	0.265	-0.084 [-0.228 to 0.114]	0.372
Synovial fluid volume*	0.005 [-0.171 to 0.180]	0.964	0.004 [-0.172 to 0.180]	0.972
PIICP*	0.440 [0.282 to 0.575]	<0.001	0.395 [0.231 to 0.529]	<0.001

*Logarithmically transformed values.

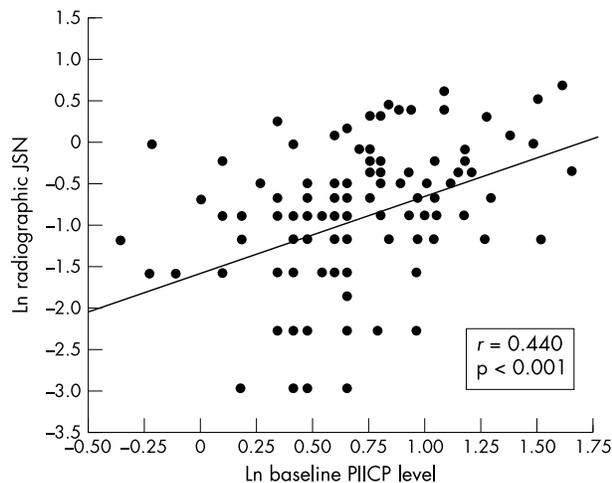


Figure 1 Correlation between baseline PIICP level (ln transformed data) and radiographic JSN over four years (ln transformed data) in 110 patients with early knee OA.

(1.35) ng/ml (median 1.98, range 0.88–8.58) at the baseline and at the follow up, respectively. The mean (SD) change in PIICP levels over four years was 0.31 (0.90) ng/ml (median 0.15, range -1.93–5.02) and the change was significant ($p < 0.001$ by t test; 95% CI 0.16 to 0.50). The mean (SD) PIICP level in the 109 healthy controls, taken at the baseline of the study patients, was 0.68 (0.24) ng/ml (median 0.70, range 0.20–1.44). The mean (SD) synovial fluid volume at baseline in the 110 patients was 2.9 (3.0) ml (median 2.0, range 1.0–16.0). Because the distributions of PIICP level and synovial fluid volume were positively skewed (not distributed normally) at any time during the study period, ln transformed data were used for further analyses. Baseline PIICP level (mean (SD), 0.66 (0.38) units) in the 110 patients was significantly higher than in the 109 healthy controls (mean (SD) -0.39 (0.17) units; $p < 0.001$, t test).

The mean (SD) synovial fluid volume in the 110 patients was 0.70 (0.72) and 0.69 (0.73) units at the baseline and at the one month review, respectively. The mean (SD) PIICP level in the 110 patients was 0.66 (0.38) and 0.65 (0.38) units at the baseline and at the one month review, respectively. Over a one month period, the CV for synovial fluid volume was 78% on average; however, the CV for PIICP levels was only 15% on average.

Of the continuous variables, BMI showed a slight positive association with baseline PIICP level ($r = 0.220$, $p = 0.017$). There was no difference in baseline PIICP level according to age ($r = 0.133$, $p = 0.147$), synovial fluid volume ($r = 0.070$, $p = 0.443$) or minimal JSW at the baseline ($r = -0.105$, $p = 0.266$).

Correlations between radiographic JSN and the other variables

Because the distribution of radiographic JSN was positively skewed, ln transformed data were used for further analyses in

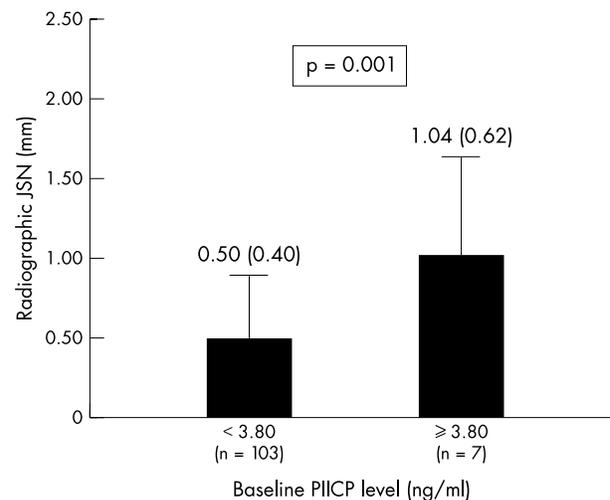


Figure 2 Radiographic JSN over four years (mm) according to the baseline PIICP level (ng/ml) (cut off value of 3.80 ng/ml) in 110 patients with early knee OA.

the 110 patients. Linear regression analyses showed significant positive correlations between radiographic JSN and baseline PIICP level ($r = 0.440$, 95% CI 0.282 to 0.575, $p < 0.001$) and baseline BMI ($r = 0.260$, 95% CI 0.084 to 0.419, $p = 0.005$, fig 1). There was no difference in radiographic JSN according to age ($r = 0.109$, $p = 0.238$), synovial fluid volume at the baseline ($r = 0.005$, $p = 0.964$) or minimal JSW at baseline ($r = -0.107$, $p = 0.265$, table 3).

A cut off value of 3.80 ng/ml for PIICP level corresponding to the value of the mean + 2 SD PIICP level at the baseline was chosen. Figure 2 shows that patients with baseline PIICP level equal to or above 3.80 ng/ml had a rate of radiographic JSN about twice as high as those with baseline PIICP level below 3.80 ng/ml ($p = 0.001$, t test). As BMI and PIICP levels were intercorrelated, the correlations between radiographic JSN and baseline PIICP level might have been due to confounding factors such as BMI. To assess this possibility, multiple linear regression techniques were used with radiographic JSN entered as the dependent variable and baseline PIICP level entered along with age and BMI at the baseline as one of the independent variables. The multiple linear regression models indicated a significant positive correlation between radiographic JSN and baseline PIICP level ($r = 0.395$, 95% CI 0.231 to 0.529, $p < 0.001$, table 3). Thus, even after age and BMI were taken into account, baseline PIICP level was directly associated with radiographic JSN. These results indicate that an increase of one unit in the ln transformed PIICP value results in an increase of 0.395 units of ln transformed JSN value. For example, if baseline PIICP level is equal to 1.00, 3.00, 5.00, and 7.00 ng/ml, radiographic JSN over four years is calculated to be 0.21, 0.64, 0.98, and 1.28 mm, respectively.

DISCUSSION

Identification of a biological marker that would predict the evolution of knee OA is of great interest. In this four year prospective study, only PIICP level in the synovial fluid and BMI were identified as risk factor variables for radiographic JSN of the tibiofemoral joint. Quantification of PIICP in the synovial fluid was shown to provide prognostic information in women with early tibiofemoral joint OA. The findings that an increase of PIICP level in the synovial fluid is predictive of progressive loss of joint space in early disease may be of practical relevance to clinicians, because intra-articular injection of hyaluronate has been widely used in the treatment of knee OA and it has become easier for clinicians to obtain synovial fluid. A few studies on biological markers have been performed in patients with knee OA. In a five year prospective study in established knee OA, it was shown that serum levels of HA at the baseline and increases in serum levels of COMP during the first year were helpful in identifying patients with more rapid disease progression.^{1,2} Serum levels of C reactive protein were identified as predictors of disease progression in early knee OA.¹⁸ By contrast, a study on synovial fluid markers has shown that chondroitin sulphate, keratan sulphate, HA, and total glycosaminoglycans in the synovial fluid could not predict radiographic progression in established knee OA.¹⁹ No marker present in the synovial fluid has been previously shown to be useful as a prognostic marker in knee OA. Whereas serum markers represent an integrated measure of turnover activity in all body cartilage, a marker present in the synovial fluid may be representative of the cartilage turnover in a single joint.²⁰ If one knee shows progression and the other shows no change in a patient with bilateral knee OA, the serum level should legitimately be applied to both knees, presenting difficulties in interpretation. We therefore examined the PIICP level in the synovial fluid to determine any prognostic value. The marker level in the synovial fluid is likely to be affected by a synovial fluid volume that varies with the degree of synovitis. Our results indicate that the CVs of PIICP levels were relatively low by contrast with those of synovial fluid volumes over a one month period in the same patients. Slight spontaneous variation in PIICP levels in individual joints over a short period will enhance its value for clinical use as a marker.

Longitudinal studies of tibiofemoral OA yielded annual rates of JSN varying from 0.06 to 0.60 mm/year, and the median annual JSN rate was 0.26 mm/year in clinic based patients with knee OA.²¹ By contrast, the annual rates of JSN in patients with knee OA recruited from the community were about 0.08 mm/year in the population based studies such as the Bristol OA500 study²² and the Framingham study.²³ The average of radiographic JSN over four years was 0.53 mm and the average rate of annual radiographic JSN was equivalent to 0.13 mm/year in our study. The variance of radiographic JSN depends on differences in radiographic technique, error variation in JSW measurement method, and true biological variability among patients. Buckland-Wright showed that reproducibility of JSW measurement was greatest in the standing semiflexed view, compared with the tunnel view and the fully extended knee view.¹⁵ Therefore, the standing semiflexed view was obtained with the aid of fluoroscopy to standardise knee flexion and foot rotation, and the computerised, magnification corrected measurement system was applied to JSW measurement on the radiograph. A good degree of reproducibility for minimum JSW measurement and for repeat measures was shown by our method (CV=4.1% for minimal JSW measurement, CV=1.5% for repeat measures); however, it was slightly inferior to that of Buckland-Wright *et al*¹⁴ (CV=3.2% for minimal JSW measurement, CV=1.0% for repeat measures).

It is reasonable to consider biological variability among patients, as patients have different degrees of risk factors for OA progression. Many factors have been reported to affect the

incidence and progression of knee OA. Examples are sex,²⁴ age,²⁴ obesity,^{25,26} valgus or varus knee deformity,²⁶ knee injury,²⁶ occupational physical activity,²⁷ and intake of NSAIDs.²⁵ For this reason, the patients with knee deformity and recent knee injury were specifically excluded at enrolment. Inclusion criteria for our study differed from those of the previously published studies in many respects: age (40 to 59 years old), sex (female), living area (rural), occupation (small scale farmer), presence of synovial effusion (>1.0 ml), NSAID intake (no regular use), and severity of OA (early stage). Farmers would be expected to have specific occupational activities such as kneeling and squatting, which have been identified as risk factors for progression of knee OA.²⁷ Moreover, the presence of synovial effusion has been shown to be one of the risk factors.²⁵ Our results indicate that baseline BMI was positively correlated with progression of radiographic JSN. It is well recognised that obesity is an important factor that exacerbates OA of the tibiofemoral joint.^{25,26} In agreement with the previously reported findings from the study,²⁸ we showed that PIICP levels had a slightly positive association with BMI but no association with age. High PIICP levels may be associated with activated collagen synthesis because of increased mechanical stress such as being overweight; however, the pathogenic pathway needs to be studied further.

Recent studies have shown that the cleavage and degradation of type II collagen involve collagenases belonging to the matrix metalloproteinase family and are excessive in osteoarthritic articular cartilage compared with non-arthritic articular cartilage.¹⁰⁻¹² Nelson *et al* have reported that the synthesis of type II collagen is higher in osteoarthritic cartilage than in normal cartilage.⁸ They also showed that the content of PIICP in cartilage can be used as an index of the synthesis of type II collagen because the half life of PIICP in cartilage is relatively short (range 14 to 16 hours).⁸ Therefore, PIICP in the synovial fluid is a putative marker of type II collagen synthesis in the cartilage. In osteoarthritic cartilage, both synthesis and degradation of type II collagen are increased^{8,11,12}; however, the balance between the catabolism and anabolism of the matrix components is disturbed.⁹ Interestingly, immunohistological studies in osteoarthritic cartilage have shown that both synthesis and degradation of type II collagen increased in the middle and deep zones; however, in the more superficial zone, although the degradation was most pronounced, synthesis did not increase.^{8,11,12} The collagen fibrils provide the tensile strength and maintain the integrity of articular cartilage by forming an elaborated fibrillar network.²⁹ In early osteoarthritic cartilage, there is a loss of the tensile properties, indicative of damage to the fibrillar network that is made up of primarily type II collagen.^{3,29} Damage to the collagen fibrillar network, accompanied by the degradation of type II collagen, is thought to be the key process leading to cartilage destruction in animal models of spontaneous OA.³⁰

One of the main findings of the present study, a direct positive correlation between radiographic JSN and baseline PIICP level, was confirmed by multiple linear regression analyses. Our findings suggest that increased PIICP levels show accelerated degradation as well as activated synthesis of matrix collagen and indicate progressive cartilage loss. Another explanation for these findings may be that PIICP levels reflect cartilage degeneration that was not visualised on plain knee radiographs at enrolment but yielded subsequent radiographic JSN at follow up. A study in traumatic arthritis of the knee has documented a positive correlation between PIICP level in the synovial fluid and the degree of cartilage damage evaluated by arthroscopy.³¹ Synovial fluid levels of PIICP have been shown to increase at an early stage of cartilage degeneration that can be identified by arthroscopy, but which shows no or only minimal radiographic changes.⁵ The JSN in weight bearing knee radiographs correlates imperfectly with cartilage degeneration as seen by arthroscopy.³² The possibility

that the patients with a more rapid rate of JSN had moderate cartilage degeneration in the tibiofemoral joints at enrolment cannot be excluded because arthroscopy had not been performed at the baseline examination. In conclusion, the PIICP level in synovial fluid, which is a putative marker of the repair response of chondrocytes in cartilage, provides prognostic information in women with early tibiofemoral joint OA.

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