Markers of Bone Formation and Resorption Identify Subgroups of Patients with Clinical Knee Osteoarthritis Who Have Reduced Rates of Cartilage Loss

PATRICIA A. BERRY, ROSE A. MACIEWICZ, FLAVIA M. CICUTTINI, MARK D. JONES, CAROLINE J. HELLAWELL, and ANITA E. WLUKA

ABSTRACT. ***Objective.*** To determine whether serum markers of bone formation and resorption, used individually or in combination, can be used to identify subgroups who lose cartilage volume at different rates over 2 years within a knee osteoarthritis (OA) population.

Methods. Changes in cartilage volume over 2 years were measured in 117 subjects with knee OA using magnetic resonance imaging. We examined relationships between change in cartilage volume and baseline serum markers of bone formation [intact N-terminal propeptide of type I procollagen (PINP) and osteocalcin] and resorption [N-telopeptide of type I collagen (NTX-I), C-telopeptide of type I collagen (CTX-I), and C-telopeptide of type I collagen (ICTP).

Results. The baseline markers of bone formation, PINP and osteocalcin ($p = 0.02$, $p = 0.01$, respectively), and the baseline markers of bone resorption, CTX-I and NTX-I ($p = 0.02$ for both), were significantly associated with reduced cartilage loss. There were no significant associations between baseline ratios of bone formation to resorption markers and cartilage loss. However, when subjects were divided into subgroups with high or low bone formation markers (based on levels of marker ≥ mean or < mean for the population, respectively), in the subgroup with high PINP there was a significant association between increasing bone resorption markers CTX-I and NTX-I and reduced cartilage loss ($p = 0.02$, $p = 0.001$, respectively). Similarly, in the subgroup with high osteocalcin, there was a significant association between increasing CTX-I and NTX-I and reduced cartilage loss ($p = 0.02$, $p = 0.003$, respectively). In contrast, in subgroups with low bone formation markers, no significant associations were obtained between markers of bone resorption and cartilage loss.

Conclusion. Overall, the results suggest that higher bone remodeling (i.e., higher serum levels of bone formation and resorption) is associated with reduced cartilage loss. Considering markers of bone formation and resorption together, it is possible to identify subgroups within the OA population who have reduced rates of cartilage loss. (First Release April 15 2010; J Rheumatol 2010;37:1252–9; doi:10.3899/jrheum.091055)

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From the Department of Epidemiology and Preventive Medicine, Monash University Medical School, Alfred Hospital, Prahran, Victoria; Respiratory and Inflammation Research Area; and Baker Heart Research Institute, Melbourne, Victoria, Australia.

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P.A. Berry, BBio Med Sci (Hons), Department of Epidemiology and Preventive Medicine, Monash University Medical School; R.A. Maciewicz, BA (Hons), MA, PhD, Respiratory and Inflammation Research Area; F.M. Cicuttini, MBBS, FRACP, PhD, Department of Epidemiology and Preventive Medicine, Monash University Medical School; M.D. Jones, BSc (Hons); C.J. Hellawell, BSc (Hons), Respiratory and Inflammation Research Area; A.E. Wluka, MBBS, FRACP, PhD, Department of Epidemiology and Preventive Medicine, Monash University Medical School, Baker Heart Research Institute.

Address correspondence to Dr. A. Wluka, Department of Epidemiology and Preventive Medicine, Monash University, Central and Eastern Clinical School, Alfred Hospital, Melbourne, Victoria 3004, Australia. E-mail: anita.wluka@med.monash.edu.au

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Osteoarthritis (OA) is a complex disease that affects tissues of the entire joint, including articular cartilage and subchondral bone simultaneously. In those with OA, skeletal size and composition is abnormal, even distant to affected joints[1,2]. Increased osteoblastic activity, demonstrated by bone scans, has been linked to increased progression of knee OA[3]. Recently, the presence of bone marrow lesions, occurring in the subchondral region, has been linked to pain and disease progression[4-6]. These bony abnormalities may be revealed by serum markers of bone metabolism.

Currently there is limited information about the relationship between bone biomarkers and articular cartilage loss. It is unclear whether OA is characterized by increased or decreased bone turnover. Subjects with knee OA were found to have lower markers of bone formation (osteocalcin) and resorption [C-telopeptide of type I collagen (CTX-I)] compared to controls[7]. However, others have suggested that OA

is associated with a higher rate of bone turnover[8]. Previous studies examining the relationship between markers of bone metabolism and structural progression of OA also yielded inconsistent results[8-12]. These studies examined the relationship between structural change and markers of bone formation and resorption individually.

It is possible that considering markers of bone formation and resorption together may be used to identify subgroups within an OA population that show different rates of progression. A number of methods may be used to do this. In osteoporosis, ratios of bone formation and resorption have been used[13]. Similarly, imbalances in markers of cartilage synthesis and degradation have also been considered in the progression of knee OA[14-16]. Use of an uncoupling index of cartilage synthesis and degradation markers strengthened the ability to identify subjects at risk of rapid disease progression[15], and using a combination of cartilage markers has been shown to be more sensitive than using markers in isolation[17]. Despite this, the balance between bone formation and resorption processes has yet to be examined in knee OA.

The aim of our study was to examine whether serum markers of bone formation and resorption, used individually or in combination, identify subgroups who lose cartilage volume at different rates over 2 years within a population of subjects with knee OA.

MATERIALS AND METHODS

Study participants. A total of 132 subjects aged > 40 years with knee OA were recruited by advertising through local newspapers, the Victoria branch of the Arthritis Foundation of Australia, and through general practitioners, rheumatologists and orthopedic surgeons[18]. The study was approved by the ethics committee of the Alfred and Caulfield hospitals, Melbourne, Australia. All patients gave informed consent.

All subjects fulfilled the American College of Rheumatology clinical and radiographic criteria for knee OA [pain in at least 1 pain dimension of the Western Ontario and McMaster Universities Osteoarthritis index (WOMAC) > 20% and osteophytes present][18,19]. The exclusion criteria were any other form of arthritis, contraindication to magnetic resonance imaging (MRI), knee replacement planned, or inability to cooperate with study requirements. A list of medications was collected and only one woman reported use of bisphosphonate therapy.

Data collection. At baseline, weight was measured to the nearest 0.1 kg and height to the nearest 0.1 cm[18]. Body mass index (BMI; weight/height^2, kg/m^2) was calculated. Knee pain (0 to 500 scale), stiffness (0 to 200), and function (0 to 1700) were assessed with the WOMAC at baseline, where 0 represents no symptoms[20].

MRI examination. An MRI scan was performed on the symptomatic knee at baseline, and about 2 years later. Baseline and followup MRI scans were performed at a similar time of day for all study subjects. Where both knees were symptomatic the knee with least severe radiographic change was imaged, to minimize loss to followup from joint replacement. Knees were imaged in a sagittal plane on the same 1.5-Tesla whole-body MR unit (Signa Advantage HiSpeed, GE Medical Systems, Milwaukee, WI, USA) using a commercial receive-only extremity coil as described[18].

Cartilage volume measurement. Tibial cartilage volume was measured with image processing on an independent workstation using the Osiris program (University of Geneva)[18]. Two trained observers measured cartilage volume on each MRI, blinded to the patient's identification and study sequences[18]. Their results were compared and the average used if the results were within ± 20% of each other. If results were outside this range, the measurements were repeated blind to the previous results. The coefficient of variation (CV) for cartilage volume measures were 3.4% for medial and 2.0% for lateral tibial cartilage[18].

Bone area measurement. Medial and lateral tibial plateau cross-sectional areas were directly measured from images, reformatted in the axial plane, using Osiris[18]. The CV were 2.3% for medial, 2.4% for lateral tibial plateau area[18,21].

Measurement of biomarkers. Nonfasted blood samples were obtained in the morning for all subjects via direct venepuncture and centrifuged (1000 g at 4°C for 10 min) within 30 min of blood sampling. Serum was aliquoted and frozen upright at –80°C.

Sample handling. All samples were thawed at 4°C, aliquoted, relabeled, and refrozen. Prior to assaying, samples were defrosted at room temperature for 4 h. Previously analyte stability had been assessed over 24 h. Replicates of samples stored at +4°C for 24 h had %CV < 5 and recovery within < 18%, which is within the recognized limits of %CV < 25 and percentage recovery within 30%. The intra- and interassay CV for each of the biomarkers were intact N-terminal propeptide of type I procollagen (PINP) (2.2%, 2.3%), osteocalcin (14.4%, 16.4%), N-telopeptide of type I collagen (NTX-I) (8.3%, 10.8%), CTX-I (18.9%, 15.5%), and C-telopeptide of type I collagen (ICTP) (7.3%, 8.1%), respectively.

Biomarker assays. All ELISA were run using duplicate wells for each sample, and the mean biomarker concentration was reported in each case. Bone formation was assessed with serum levels of PINP (UniQ™ PINP RIA; Orion Diagnostica, Espoo, Finland), expressed as μg/l. Bone mineralization was assessed using osteocalcin (N-MID® Osteocalcin ELISA; IDS Nordic, Boldon Colliery, UK), expressed as ng/ml[22]. Methods were performed as per the manufacturer's instructions with the inclusion of a standard curve on each assay plate. A single batch number was used for both the quality assessment of the kit and the sample analysis.

Bone resorption was assessed using serum levels of NTX-I [Serum Osteomark NTx, Inverness Medical, Stockport, UK; expressed as nmole/l (nM) of bone collagen equivalents (BCE)], CTX-I (Serum CrossLaps® ELISA, IDS Nordic; expressed as ng/ml), and ICTP (UniQ™ ICTP RIA, Orion Diagnostica; expressed as ng/ml).

Biomarker quality assessment. A quality assessment of the biomarker kits was completed prior to assessment of the samples to ensure robustness of these data. This was undertaken using serum from 8 male/female healthy volunteers/donors aged 30–60 years, and included assessment of the intra- and interassay precision and accuracy (all CV < 25%), data drift across the plate, lower and upper limit of quantitation (LLOQ and ULOQ, respectively), linearity of the response, and spiking accuracy (recovery values fall within a range of 70%–130%).

All kits met approval when healthy volunteers' samples were analyzed. All sample datapoints of the monitored participants fell within the low to middle range of the standard curves and none of the samples approached the ULOQ for any of the analytes. The LLOQ was also recorded for each assay and data below this limit were set to the LLOQ for the analysis; 36% of the CTX-I data were below the LLOQ, as defined in the healthy volunteer samples. Since similar results were obtained including and excluding these subjects, we chose to use all the data to reduce biasing our population. Table 1 describes the median and range of the baseline data for each assay.

Statistical analyses. Descriptive statistics of subject characteristics were tabulated. Unpaired 2-sample t tests were used to compare means in baseline characteristics between study completers and those lost to followup. The Mann-Whitney U test was used for comparison of medians, and the chi-square test for comparison of proportions. All biomarker data were log-transformed to ensure these data approximated the normal distribution, as the raw data were skewed. Annual change in cartilage volume was calculated by subtracting followup cartilage volume from initial cartilage volume, divided by time between MRI scans. The distribution of this outcome variable approximated the normal distribution.

To examine the interrelated effect of the processes of bone formation and resorption, ratios were obtained by dividing a marker of bone formation (PINP, osteocalcin) by a marker of bone resorption (CTX-I, NTX-I,

Table 1. Characteristics of study population at baseline comparing those who completed 2 year followup and those lost to followup. Values reported as mean (SD) at baseline unless otherwise stated. T tests were used for comparison of means.

Characteristic	Completers, n = 117	Lost to Study, n = 15	p
Age, yrs	63.7 (10.3)	58.4 (11.4)	0.07
No. female (%)*	68 (58)	5 (33.3)	0.07
BMI, kg/m^2	28.9 (5.1)	28.3 (4.6)	0.68
Kellgren-Lawrence, n ≥ grade 2* (%)	84 (72)	7 (47)	0.09
WOMAC			
Pain	80.9 (43.9)	102.7 (18.6)	0.01
Stiffness	39.6 (22.2)	49.2 (19.2)	0.21
Function	306.6 (169.8)	399.4 (54.3)	0.12
Total	427.1 (225.5)	551.3 (171.6)	0.11
Tibial cartilage volume, ml			
Medial	1.74 (0.5)	1.82 (0.5)	0.53
Lateral	1.92 (0.6)	2.20 (0.6)	0.08
Tibial plateau bone area, cm^2			
Medial	20.8 (3.9)	21.5 (4.2)	0.49
Lateral	13.6 (2.6)	14.0 (1.9)	0.58
Markers of bone formation**			
PINP, μg/l[a]	40.6 (11.2–177.4)	38.7 (26.0–49.4)	0.67
Osteocalcin, ng/ml[b]	16.8 (6.2–48.9)	18.7 (6.2–39.4)	0.83
Markers of bone resorption**			
CTX-I, ng/ml[c]	0.29 (0.03–1.12)	0.27 (0.02–1.41)	0.57
ICTP, ng/ml[b]	3.5 (2.01–11.68)	3.5 (2.2–3.9)	0.45
NTX-I, nM BCE[d]	19.8 (7.8–78.13)	21.3 (12.5–28.4)	0.30

** Values are median (range). Mann-Whitney U test for comparison of medians due to skewed distribution of raw data. * Chi-square tests for comparison of proportions. Biomarker data available at baseline for 122 subjects[a], 124 subjects[b], 123 subjects[c], 120 subjects[d]. Of 15 subjects lost to followup, 9 had biomarker data at baseline. PINP: intact N-terminal propeptide of type I procollagen; NTX-I: N-telopeptide of type I collagen; CTX-I: C-telopeptide of type I collagen; ICTP: C-telopeptide of type I collagen; BMI: body mass index; WOMAC: Western Ontario and McMaster Universities Osteoarthritis index.

ICTP). However, as a major limitation of using marker ratios is that they cannot differentiate populations where both formation and resorption markers were either high or low, we performed analyses where the population was considered in subgroups of subjects in whom the level of the formation markers was high or low. Those with high formation marker levels (PINP or osteocalcin) were defined as those in whom the level was greater than the mean for the population, and low was defined as less than the mean for the population. The mean marker level was used as these marker data used in the analysis approximated the normal distribution, so the mean and median levels were similar.

Linear regression models were used to examine the relationship between baseline levels of individual bone formation and resorption markers with annual change in cartilage volume. In multiple regression analysis, adjustment was made for potential confounders including age, sex, BMI, baseline cartilage volume, and tibial plateau area. These relationships were also examined in men and women separately. Further, the independent samples z-test was used to compare the rate of cartilage loss between men and women. In the Results, a positive regression coefficient indicates higher levels of biomarker were associated with disease progression as measured by an increased rate of cartilage loss. A negative regression coefficient indicates higher levels of biomarkers were associated with a reduction in the rate of cartilage loss.

The relationship between markers of bone resorption (CTX-I, NTX-I, ICTP) and annual change in cartilage volume was also examined in subgroups with high and low bone formation markers (PINP and osteocalcin). Pearson's correlation coefficient was used to examine the relationship between individual bone markers. A p value < 0.05 (2-tailed) was regarded as statistically significant. All analyses were performed using the SPSS statistical package (standard version 14.0; SPSS, Chicago, IL, USA).

RESULTS

Subject characteristics. The characteristics of the study population are shown in Table 1. Of the original 132 participants, 117 (89%) completed followup with both interpretable MRI scans and sera. Fifteen subjects were lost to followup: 2 moved overseas or interstate; 3 were too busy to continue in the study; 2 had knee surgery; 1 died of complications related to diabetes mellitus and chronic obstructive airways disease; 1 subject was too ill to continue due to multiple sclerosis; and 6 subjects did not provide baseline serum specimens. Subjects who were lost to followup had significantly higher mean baseline WOMAC pain scores compared to those who completed the study (p = 0.01). Otherwise, there were no significant differences in the demographic characteristics between those who completed the study and those who were lost to followup.

The average annual change (± SD) in tibial cartilage volume was 91 (± 116) mm^3/yr in the medial and 114 (± 128) mm^3/yr in lateral compartments. These compartmental differences in cartilage loss were not statistically significant (p = 0.10). There were no significant differences in the median levels of the individual biomarkers between those who completed the study and those who were lost to followup (Table 1).

Relationship between markers of bone metabolism and annual change in knee cartilage volume. The relationship between individual markers and annual change in cartilage volume was examined (Table 2). When markers of formation were considered, there was a trend for higher levels of PINP to be associated with a reduced rate of medial cartilage loss ($p = 0.05$). This relationship was significant after adjustment for age, sex, BMI, baseline cartilage volume, and tibial plateau area ($p = 0.02$). A similar association was seen with osteocalcin using multiple regression analyses ($p = 0.01$).

Markers of resorption (CTX-I and NTX-I) were also inversely associated with a reduced rate of medial cartilage loss (Table 2). These relationships persisted after adjustment for potential confounders ($p = 0.02$, $p = 0.02$, respectively). The negative linear regression coefficients indicate that for every unit increase in CTX-I and NTX-I, there was a reduced rate of medial cartilage loss. No significant associations were obtained between any individual marker and rate of cartilage loss in the lateral compartment.

Similar results were obtained when men and women were analyzed separately, and the direction of effect in all cases was the same (Table 3). The difference in rate of cartilage loss between men and women was not statistically significant for any of the bone biomarkers ($p = 0.18$ to $p = 0.92$). When individual and total WOMAC dimensions were included in the multiple linear regression equations, similar results were obtained (data not shown). When the subject receiving bisphosphonate therapy was excluded from the analysis, similar results were obtained.

While there were significant associations between the formation marker PINP and resorption markers (CTX-I, NTX-I, ICTP; $r = 0.33$–0.63, $p < 0.001$), and the formation marker osteocalcin and resorption markers (CTX-I and NTX-I; $p < 0.05$), the strength of these was weak ($r = 0.05$–0.34). However, the relationship between markers of formation, PINP and osteocalcin, was stronger ($r = 0.73$, $p < 0.001$).

Relationship between markers of bone resorption and annual change in knee cartilage volume in subgroups with high or low levels of bone formation (PINP or osteocalcin). As bone formation and bone resorption are tightly coupled, we investigated whether cartilage loss was associated with bone formation to resorption ratios, and also used subgroup analysis. No significant associations were obtained between any marker ratios of formation and resorption and rate of cartilage loss in either the medial or lateral compartment (Table 4). Similarly, no significant associations were obtained when high and low ratio subgroups were considered separately. However, ratios do not differentiate between where both markers are both high or both low. To more fully examine the interrelated effect of formation and resorption, the population was divided according to whether the level of markers of formation (PINP or osteocalcin) was high (≥ mean for the population) or low (< mean for the population). The relationship between a marker of resorption (CTX-I, NTX-I, ICTP) and rate of cartilage loss was then determined (Table 5).

When the subgroup with high formation marker PINP was considered, there was a significant association between

Table 2. The relationship between serum bone markers and annual change in tibial cartilage volume (mm^3/yr). Formation and resorption biomarkers were found to have a skewed distribution. Values based on natural log-transformed data.

Region	Linear Regression Coefficient (95% CI)*	p	Multiple Linear Regression Coefficient (95% CI)**	p
Medial compartment				
Markers of formation				
PINP, μg/l[a]	–53.2 (–107.2, 0.74)	0.05	–59.7 (–109.7, –9.7)	0.02
Osteocalcin, ng/ml[b]	–35.4 (–79.4, 8.7)	0.11	–53.5 (–95.1, –12.0)	0.01
Markers of resorption				
CTX-I, ng/ml[c]	–51.2 (–88.2, –14.2)	0.007	–44.2 (–80.1, –8.2)	0.02
NTX-I, nM BCE[d]	–70.8 (–133.7, –7.9)	0.03	–68.4 (–127.2, –9.6)	0.02
ICTP, ng/ml[b]	–46.8 (–121.2, 27.6)	0.22	–31.2 (–103.2, 40.9)	0.39
Lateral compartment				
Markers of formation				
PINP, μg/l	–10.4 (–70.7, 49.9)	0.73	–19.6 (–72.6, 33.4)	0.47
Osteocalcin, ng/ml	–21.9 (–70.6, 26.8)	0.37	–21.4 (–66.4, 23.6)	0.35
Markers of resorption				
CTX-I, ng/ml	–6.5 (–48.9, 35.9)	0.76	–4.1 (–42.3, 34.1)	0.83
NTX-I, nM BCE	3.3 (–68.0, 74.7)	0.93	–17.2 (–80.1, 45.7)	0.59
ICTP, ng/ml	9.5 (–73.0, 92.1)	0.82	6.9 (–67.2, 80.9)	0.85

* Change in cartilage volume mm^3 per unit increase in respective biomarker. ** Change in cartilage volume mm^3 per unit increase in respective biomarker including age, sex, BMI, baseline cartilage volume, and baseline tibial plateau area in the regression equation. Biomarker data available at baseline for 122 subjects[a], 124 subjects[b], 123 subjects[c], 120 subjects[d], respectively. Of 15 subjects lost to followup, 9 had biomarker data at baseline. For definitions see Table 1.

Table 3. The relationship between serum marker ratios and annual change in tibial cartilage volume (mm^3/yr). Formation and resorption biomarkers were found to be skewed. Ratios based on natural log-transformed data.

	Linear Regression Coefficient* (95% CI)	p	Multiple Linear Regression Coefficient** (95% CI)	p
Medial compartment				
Osteocalcin:CTX-I	23.4 (–19.2, 66.1)	0.28	0.01 (–42.4, 42.4)	0.99
Osteocalcin:ICTP	–14.9 (–54.6, 24.7)	0.46	–36.5 (–75.8, 2.8)	0.07
Osteocalcin:NTX-I	–1.6 (–43.1, 39.8)	0.94	–13.6 (–53.1, 26.0)	0.50
PINP:CTX-I	37.2 (–11.4, 85.9)	0.13	17.9 (–29.9, 65.6)	0.46
PINP:ICTP	–28.6 (–82.1, 24.9)	0.29	–46.3 (–97.5, 4.9)	0.08
PINP:NTX-I	–3.0 (–55.7, 49.8)	0.91	–9.9 (–59.4, 39.6)	0.69
Lateral compartment				
Osteocalcin:CTX-I	–13.2 (–60.7, 34.2)	0.58	–19.2 (–62.3, 24.0)	0.38
Osteocalcin:ICTP	–18.7 (–62.3, 24.9)	0.40	–17.6 (–58.1, 22.9)	0.39
Osteocalcin:NTX-I	–22.1 (–67.8, 23.6)	0.34	–10.0 (–51.4, 31.4)	0.63
PINP:CTX-I	5.6 (–49.0, 60.2)	0.84	–5.9 (–54.9, 43.1)	0.81
PINP:ICTP	–14.9 (–74.0, 44.1)	0.62	–24.1 (–77.2, 28.9)	0.37
PINP:NTX-I	–20.5 (78.6, 37.6)	0.49	–12.3 (–63.5, 38.8)	0.63

* Change in cartilage volume mm^3 per unit increase in respective biomarker ratio. ** Change in cartilage volume mm^3 per unit increase in respective biomarker ratio including age, sex, BMI, baseline cartilage volume, and baseline tibial plateau area in the regression equation. For definitions see Table 1.

Table 4. The relationship between serum bone markers and annual change in medial tibial cartilage volume (mm^3/yr) in subgroups of subjects with low (< mean) and high (≥ mean) levels of serum bone formation markers. Biomarkers were found to have a skewed distribution. Values based on natural log-transformed data.

	Low Bone Formation Subgroup (< mean)*				High Bone Formation Subgroup (≥ mean)*			
	Linear Regression Coefficient (95% CI)†	p	Multiple Linear Regression Coefficient (95% CI)††	p	Linear Regression Coefficient (95% CI)†	p	Multiple Linear Regression Coefficient (95% CI)††	p
PINP subgroup								
CTX-I	–27.3 (–84.4, 29.8)	0.34	–26.4 (–90.8, 38.1)	0.42	–100.2 (–167, –33.9)	0.004	–77.0 (–143, –10.7)	0.02
NTX-I	12.1 (–77.4, 101.6)	0.79	8.2 (–82.7, 99.0)	0.86	–163.4 (–260.7, –66.1)	0.001	–155.0 (–244.0, –66.0)	0.001
ICTP	–109.8 (–210.0, –9.5)	0.03	–80.7 (–187.3, 26.0)	0.14	36.6 (–88.8, 161.9)	0.56	26.6 (–94.3, 147.6)	0.66
Osteocalcin subgroup								
CTX-I	–14.8 (–68.3, 38.6)	0.58	–16.6 (–77.4, 44.1)	0.58	–86.2 (–149.7, –22.7)	0.009	–72.6 (–134.4, –10.8)	0.02
NTX-I	2.2 (–88.6, 92.9)	0.96	5.8 (–87.4, 99.0)	0.90	–126.5 (–216.2, –36.8)	0.007	–121.4 (–200.2, –42.7)	0.003
ICTP	–38.5 (–121.5, 44.4)	0.36	–22.1 (–107.9, 63.8)	0.61	–56.8 (–215.5, 101.8)	0.48	–45.4 (–203.5, 112.8)	0.57

* n = 61 in low and high PINP subgroups, n = 62 in low and high osteocalcin subgroups. † Change in cartilage volume mm^3 per unit increase in respective bone resorption biomarker. †† Change in cartilage volume mm^3 per unit increase in respective bone resorption biomarker including age, sex, BMI, baseline cartilage volume, and baseline tibial plateau area in the regression equation. For definitions see Table 1.

increasing resorption markers CTX-I and NTX-I and reduced rate of medial cartilage loss (p = 0.02, p = 0.001, respectively). Similarly, in the high formation marker osteocalcin subgroup, increasing resorption markers CTX-I and NTX-I were significantly associated with a reduced rate of medial cartilage loss (p = 0.02, p = 0.003, respectively). In contrast, in the low formation marker subgroups (PINP and osteocalcin), no significant associations were obtained between any of the markers of resorption (CTX-I, NTX-I, ICTP) and rate of cartilage loss. No significant associations were obtained with the marker of resorption ICTP.

If the population was subgrouped based on high or low resorption markers (CTX-I, NTX-I, ICTP), instead of high or low formation markers, similar results were obtained. In the high resorption marker CTX-I subgroup, increasing formation markers PINP (regression coefficient ß = –94, 95% CI –160, –29; p = 0.005) and osteocalcin (ß = –61, 95% CI –120, –2; p = 0.045) were significantly associated with a reduced rate of medial cartilage loss. Similarly, in the high resorption marker NTX-I subgroup, increasing formation markers PINP (ß = –72, 95% CI –140, –4; p = 0.04) and osteocalcin (ß = –83, 95% CI –148, –19; p = 0.01) were significantly associated with a reduced rate of medial cartilage loss.

In contrast, in the low CTX-I subgroup, the relationship between PINP (ß = –12, 95% CI –130, 106; p = 0.84) and osteocalcin (ß = –56, 95% CI –141, 28; p = 0.19) and rate of cartilage loss was not significant. Again, the relationship between PINP (ß = –61, 95% CI –150, 28; p = 0.18) and

Table 5. Studies of markers of bone metabolism at baseline in progression of human knee osteoarthritis (OA).

Study	Population	Biomarker Measured	Imaging	Outcome/Assessment of Progression	Main Findings
Bettica[8]	71 subjects, progressive knee OA 36 subjects, nonprogressive knee OA	Urinary NTX and CTX	Radiograph	Change of ≥ 1 osteophytes or JSN from grade 1 at baseline over 4 yrs	Baseline NTX and CTX urinary excretion were significantly higher in subjects with progressive compared to nonprogressive knee OA
Bruyere[10]	212 subjects, knee OA	Serum osteocalcin	Radiograph	Change in mean and minimal joint space width over 3 yrs	Baseline serum osteocalcin was not correlated with changes in mean or minimal joint space width
Bruyere[11]	62 subjects, knee OA	Serum osteocalcin and CTX-I	MRI	Changes in cartilage volume and thickness over 1 yr	Baseline serum osteocalcin and CTX-I were not correlated with changes in cartilage volume and thickness

For definitions see Table 1.

osteocalcin (ß = –59, 95% CI –119, 2; p = 0.06) was not significant in the low NTX-I subgroup. In the low ICTP subgroup, increasing PINP (ß = –92, 95% CI –172, –12; p = 0.03) and osteocalcin (ß = –81, 95% CI –154, –7; p = 0.03) were significantly associated with a reduced rate of cartilage loss, while no significant association was found between PINP (ß = –30, 95% CI –99, 39; p = 0.38) and osteocalcin in the high ICTP subgroup.

DISCUSSION

In this well characterized population of subjects with symptomatic knee OA[18,23-25], higher individual markers of bone formation (PINP and osteocalcin) and bone resorption (NTX-I and CTX-I) were significantly associated with a reduced rate of medial cartilage loss. There was no significant association between ratios of formation and resorption and cartilage loss. However, when subjects with high (≥ mean) bone formation markers (PINP and osteocalcin) were examined, increasing markers of bone resorption (NTX-I and CTX-I) were significantly associated with a reduced rate of cartilage loss. These results suggest that by considering markers of bone formation and resorption together, it is possible to identify subgroups within the OA population who have reduced rates of cartilage loss.

There are conflicting data on the relationship between markers of bone formation and the progression of OA. Our study showed that higher levels of formation markers (PINP and osteocalcin) were associated with reduced cartilage loss. Our results are consistent with previous studies in which low levels of osteocalcin have been associated with incident knee OA[26], and a study showing a relationship with elevated cartilage loss in healthy men[27]. In contrast, Bruyere, *et al* showed no significant relationship between baseline osteocalcin and radiographic progression of knee OA measured by joint space width after 3 years[10], or changes in cartilage volume and thickness over 1 year as assessed by MRI[11], in 62 patients with knee OA. These discrepancies in results may be due to differences in study methodology. The first study[10] used a radiographic assessment of progression, while our study used a more sensitive measure of cartilage loss assessed by MRI. Although the second study[11] used MRI to assess cartilage loss, that study may have been unable to show an effect due to a shorter followup duration and smaller sample size.

Conflicting data also exist regarding the relationship between markers of bone resorption (CTX-I, NTX-I) and OA progression. Increased resorption, assessed by urinary NTX and CTX[8] but not the serum marker of resorption CTX-I[11], has been associated with progression of knee OA. In contrast, our study showed that higher serum levels of NTX-I and CTX-I were associated with a reduced rate of cartilage loss. This result differs from that reported by Bettica, *et al*[8]. However, compared to results of our study, that previous study used a less sensitive measure of disease progression based on osteophyte and joint space narrowing grades. The discrepancies with the previous MRI study[11], in which no significant association between bone resorption marker CTX-I and changes in cartilage volume or thickness was identified, may be due to the previous study's shorter duration of followup and limited power due to a smaller sample size. We did not observe any significant relationship between ICTP and disease progression. The reason various biomarkers of resorption produced different results may be explained if the proteolytic enzymes involved are considered. NTX-I is cleaved by cathepsin K alone, while CTX-I is cleaved by both cathepsin K and matrix metalloprotease (MMP) activity, and ICTP only by MMP activity[28]. Thus these data suggest that the enzymes and mechanisms involved in the cleavage of these bone resorption biomarkers may play different roles in affecting cartilage volume loss and disease progression.

The concept of considering catabolic and anabolic processes together has been used to examine the relationship between bone formation and resorption in osteoporosis[13]. This is important as it is recognized that bone formation and bone resorption are tightly coupled. Combining cartilage

synthesis and degradation markers in an uncoupling index strengthened the ability to identify risk of rapid progression[15], and use of a combination of markers was more sensitive in revealing radiographic features than using markers individually[17]. However, no previous study has examined the interrelated effect of bone formation and resorption markers in an OA population.

We did not observe an association between bone formation and resorption ratios and cartilage loss. However, a potential limitation of using ratios is that subjects with high bone formation and high bone resorption, or low formation and low resorption, will have the same ratio. That is, the ratio cannot discriminate subjects who are either high or low for both bone formation and resorption markers. To address this, we took a different approach and examined the relationship between resorption markers (CTX-I, NTX-I, ICTP) and cartilage loss in subgroups with high and low formation markers (PINP and osteocalcin). In the high, but not the low formation subgroups, increasing resorption markers (CTX-I, NTX-I) were significantly associated with a reduced rate of cartilage loss. Thus these results suggest that combining markers of bone formation and resorption has the potential to identify different subgroups among the OA population.

The mechanism by which increasing markers of bone resorption are associated with a reduced rate of cartilage loss only in the high formation subgroup remains unclear. OA is a dynamic process, and many extraneous factors contribute to the disease pathogenesis. Our results suggest that cartilage loss is less likely to occur when increased bone metabolism is present. Structural changes in the subchondral bone such as sclerosis, trabecular remodeling, and osteophyte formation are associated with trabecular microfractures and stiffening of the subchondral bone plate. These changes may alter the biomechanical properties of the bone, leading to damage to the overlying cartilage[29]. Individuals with a more active metabolic response may be able to maintain a more favorable subchondral biomechanical environment, optimizing the foundation of the overlying cartilage. When disease was in a phase of increased activity with increased cartilage loss, a relationship between bone metabolism and cartilage loss was not seen. It may be that a point is reached whereby factors other than bone metabolism become more important, and facilitate progressive cartilage loss. As the role of the bony response is overwhelmed, the relationship between cartilage loss and bone metabolism is weakened.

Our study has a number of potential limitations. Although we studied a well characterized population, we were unable to account for the possible involvement of other joints. One further limitation was the use of nonfasted serum. Recent publications have shown that bone resorption and formation markers may show higher variability in serum obtained from fasting compared to nonfasting individuals[30]. The use of nonfasting serum may result in non-differential misclassification, which reduces the power of the study to identify significant relationships. Despite this, the levels of bone markers in our study were comparable to those in similar populations[8,31,32]. Moreover, we were able to identify statistically significant and consistent relationships using independent formation and resorption markers. Indeed, other researchers have also been able to show that bone markers measured from nonfasting samples predict fracture and bone loss[33,34]. Thus it is likely that sampling of bone markers after fasting would strengthen the association with cartilage loss. Although measurement of the serum markers was subject to strict quality assurance, the data for CTX-I should be viewed as less reliable. Although the levels were detectable, 36% of values were within a range that we had less confidence in, that is, they were below the LLOQ. Excluding those subjects with levels below the LLOQ may lead to a biased population. However, similar results were obtained when those subjects with values below the LLOQ were excluded from the analysis. Moreover, the results obtained for CTX-I were consistent with those obtained for another independent bone resorption marker, NTX-I. These results are biologically plausible, as the same proteolytic enzyme, cathepsin K, is involved in the cleavage of both these resorption markers[28].

Overall, our results suggest that higher bone remodeling, that is, higher serum levels of bone formation and resorption, is associated with reduced cartilage loss. As most of the drugs used to prevent or arrest osteoporosis decrease bone turnover, these findings indicate that they may have a detrimental effect on articular cartilage in patients with OA of the knee. Thus further research will be required to clarify the effect of anticatabolic drugs on cartilage integrity. Considering markers of formation and resorption together, it is possible to identify subgroups within the OA population who have reduced rates of cartilage loss, which may assist in identification of therapeutic targets in OA. Our results will need to be confirmed in a larger independent study, and further work will be required to identify the optimal combination of bone turnover markers and to examine these relationships in further subgroups. These results highlight the heterogeneity of knee OA, and in the longer term, may lead to novel ways to target this disease.

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