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ALPHA-CTX is associated with subchondral bone turnover and predicts progression of joint space narrowing and osteophytes in osteoarthritis

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Abstract

Objective—To evaluate joint tissue remodeling, with urinary collagen biomarkers, uALPHA CTX and uCTXII, and their association with osteoarthritis (OA) severity, progression, and localized knee bone turnover.

Methods—Participants (N=149) with symptomatic and radiographic knee OA underwent fixed flexion knee radiography at baseline and 3 years, and late-phase bone scintigraphy of both knees at baseline, scored semi-quantitatively for osteophyte (OST) and joint space narrowing (JSN) severity and uptake intensity with scores summed across knees. Urinary concentrations of ALPHA CTX and CTXII were determined by ELISA. Immunohistochemistry of human OA knees was performed to localize the joint tissue origin of the biomarker epitopes.

Results—uALPHA CTX correlated strongly with intensity of bone scintigraphic uptake, and JSN and OST progression (risk ratio=13.2 and 3, respectively). uCTXII was strongly associated with intensity of bone scintigraphic uptake, with JSN and OST severity, and OA progression based on OST. uALPHA CTX localized primarily to high bone turnover areas in subchondral bone; CTXII localized to the bone-cartilage interface, the tidemark, and damaged articular cartilage.

Conclusion—Baseline uALPHA CTX, localized to high turnover areas of subchondral bone, was associated with dynamic bone turnover of knees signified by scintigraphy, and progression of both OST and JSN. uCTXII correlated with JSN and OST severity, and progression of OST. To our knowledge, this represents the first report of serological markers reflecting subchondral bone turnover. These collagen markers may be useful for non-invasive detection and quantification of active subchondral bone turnover and joint remodeling in knee OA.

Osteoarthritis (OA) is the most common form of arthritis and involves multiple components of the joint including synovium, articular cartilage, and bone. The relationship between bone

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and cartilage in OA has been a source of controversy for a long time. Turnover of subchondral bone in OA has been shown to be as much as 20-fold higher than that of normal bone (1). Moreover, bone marrow lesions, considered areas of high turnover detected in the subchondral bone by magnetic resonance imaging (MRI), have been shown to be highly associated with OA and a strong risk factor for OA progression (2). In preclinical settings, studies using anterior cruciate ligament transection (ACLT) in dogs, as well as ACLT and meniscectomy (MNX) in rats, have been instrumental in characterizing the role of subchondral bone changes in OA (3–5). In clinical settings, however, there is a lack of sensitive and non-invasive measures of subchondral bone turnover. The qualification of biomarkers or methods to quantify subchondral bone remodeling may differentiate OA patient phenotypes and provide a means of defining subpopulations that may benefit from interventions focused on the bone-cartilage interface (6). Such investigations would have the added benefit of improving our understanding of OA disease pathogenesis and progression.

Molecules in body fluids (serum, plasma, synovial fluid, and/or urine), which can potentially serve as biochemical markers of joint pathophysiology, include proteins involved in the enzymatic degradation of joint tissues, molecules reflecting the inflammatory component of joint disease, or molecules reflecting proteolysis, synthesis, or turnover of joint tissues (7). Examples include markers of cleavage products by proteases (MMPs, ADAMTS, Cathepsin K), markers of synovial inflammation (PIINP, HA), differentiation and matrix production (PINP, PIINP, Osteocalcin, TRACP), signaling (RANKL, OPG, Dkk1), and matrix destruction (COMP, CTX, CTXII, Col2-1, etc) (8). In addition, imaging modalities, such as radiography and scintigraphy, can be used as surrogate markers of disease progression, although the rate at which these changes occur can be quite slow. However, combining the use of biological markers with imaging markers of disease has been demonstrated to provide independent and thus additive information (9).

Mature collagen types I and II are cross-linked triple helical structures that critically contribute to the tensile properties of both bone and articular cartilage, respectively. Collagen type I is the most abundant form of collagen in the human body and the major protein in bone, comprising approximately 90–96% of the entire collagen content of bone. Metabolites of type I collagen (CTXI and N-terminal type I collagen [NTx]) have been positively associated with knee OA progression (10). The CTX epitope, ¹²⁰⁷EKAHGDDR¹²¹⁴, is located in the C-telopeptide $\alpha 1$ chain of collagen type I (11) and exists in two forms--an isomerized and a non-isomerized form. Isomerization of the CTX epitope to the beta form occurs *in situ* with aging (12, 13). The non-isomerized ALPHA CTX is mostly found in newly formed bone. In addition to being an indicator of newly formed bone, urinary ALPHA CTX has been shown to be a sensitive marker for detection and monitoring of skeletal metastases corresponding to sites of high bone turnover (13–15). Type II collagen is the most abundant collagen in articular cartilage representing 10–20% of the wet weight of cartilage (16). The CTXII epitope, ¹²³⁰EKGDPD¹²³⁵, is located on the C-telopeptide fragment of type II collagen. Urinary CTXII has been associated with both OA severity and progression (17–19).

In addition to biochemical markers of disease, there is great interest in the utility of imaging markers as indicators of disease. Dieppe, et. al. were the first to report the association of

increased subchondral bone turnover, detected by bone scintigraphy, with progression of knee OA (20, 21). More recently, bone scintigraphy has been identified as a sensitive indicator of severity of radiographic features and symptoms of knee OA (22). Radiographic features have recently been used to identify distinct phenotypes of osteoarthritis progression including a bone-specific phenotype (23). These studies and others (24) support a role for bone in the pathophysiology of progressive cartilage degradation and OA symptoms.

The goal of this study was to evaluate the association of two markers of matrix destruction, uALPHA CTX, a marker of high turnover and new bone resorption, and uCTXII, a marker of type II collagen degradation, with OA severity and progression in a cohort of subjects with baseline bone scintigraphy and longitudinal radiographic knee evaluation over 3 years.

Patients and Methods

Participants

A total of 149 of the 159 participants from the Prediction of Osteoarthritis Progression (POP) cohort (22) were included in this study; the ten subjects that were excluded had a unilateral total knee replacement at the time of study entry. Participants met the American College of Rheumatology criteria for symptomatic OA of at least one knee (25) and had a Kellgren Lawrence (K/L) grade of 1–3 in at least one knee, thereby meeting the radiographic criteria for knee OA (26). Radiographs as well as bone scans were obtained from all participants at baseline and 3 years later at the follow-up assessment to evaluate disease progression. A total of 87% of participants returned for follow-up; this yielded N=129 without baseline total knee replacement that were available for the knee progressor analyses (Figure 1).

Scintigraphic imaging

Late-phase scintigraphic imaging of the knees, followed by imaging of the whole body, were obtained starting at 2 hours after intravenous administration of ^{99m}Tc -methylene diphosphonate (MDP) as described previously (27). The intensity of the bone scintigraphic radiotracer uptake at each of 16 sites was scored semi-quantitatively by two experienced readers on a scale of 0–3 (0=normal, 1=mild, 2=moderate, 3=intense) and summed for each joint site. Scored sites included knees, shoulders, elbows, wrists, hands, hips, sacroiliac joints, ankles, forefeet, first metatarsophalangeal joints, sternoclavicular joints, acromioclavicular joints, the sternomanubrial joint, the cervical spine, the thoracic spine, and the lumbar spine. Several joints were scored in greater detail than other joint sites, resulting in higher overall possible scores for these sites: these included the knees, for which three regions (medial, lateral, and patellofemoral compartments) were scored resulting in a possible score of 18 for both knees; the ankles, for which four regions (medial talus, lateral talus, medial tibia, and lateral tibia) were scored resulting in a possible total score of 24; and the hands for which the interphalangeal and metacarpophalangeal joints (total score 18) were scored separately from the carpometacarpal joints (total score 6) and the extent of involvement was categorized and scored according to the number of joints involved as described previously (27).

Radiographic imaging

Posteroanterior fixed-flexion knee radiographs were obtained using the SynaFlexer lower limb positioning frame as described previously (22). The radiographs were scored by two graders (GEM and VBK) for K/L grade and for individual radiographic features of OA, including joint space narrowing (JSN) and osteophytes (OST). Each compartment of the knee was scored semi-quantitatively for JSN (medial and lateral) and OST (medial and lateral tibia and femur) on a scale of 0–3 (0=normal, 1=mild, 2=moderate, 3=severe) according to the Osteoarthritis Research Society International (OARSI) standardized atlas (25), resulting in a possible range of scores of 0–6 for total JSN and 0–12 for total OST. OA severity was determined for JSN separate from OST by summing scores across both knees. Films were blinded to clinical status and both baseline and follow-up radiographs for an individual participant were scored together to determine progression. Progression of JSN separate from OST was calculated as the sum of the change in scores (over 3 years from baseline to follow-up) for both knees. Radiographs of the participants who underwent an interval joint replacement could not be scored for radiographic features of OA and were therefore classified as categorical progressors (Figure 1).

Quantification of ALPHA CTX and CTXII in Urine

Non-fasting second morning void urine samples were collected from all participants and stored at -80°C until analyzed. The concentrations of urinary ALPHA CTX fragments and CTXII were measured by ELISA following the manufacturer's recommendations (Nordic Biosciences, Herlev, Denmark and IDS, Boldon, UK, respectively). The uALPHA CTX assay is a sandwich ELISA which uses highly specific monoclonal antibodies raised against α -EKAHDDGR (α CTX), demonstrating <1% cross reactivity with the β -form (11). The uCTXII assay is a competitive ELISA which uses a monoclonal antibody raised against EKGPD and is specific to the type II collagen C-telopeptide fragment (28). All values were normalized to urinary creatinine levels measured by ELISA (Quidel San Diego, CA) and reported as $\mu\text{g}/\text{mmol}$.

Immunolocalization

Full-depth human OA joint sections were cut from paraffin embedded tissue biopsies isolated from 5 different knee replacement arthroplasties retrieved from the Department of Orthopedics at Frederikshavn Hospital in Denmark, as a courtesy of Dr. Ole Simonsen. For immunodetection of ALPHA CTX and CTXII (29), we used the monoclonal antibodies from the immunoassay kits following the same procedure using standard peroxidase substrate (EnVision, DAKO, Denmark). Sections were deparaffinized, blocked for endogenous peroxidase with H_2O_2 for 30 minutes in 99% ethanol and then hydrated. Sections were prepared by demasking in a sodium citrate buffer (pH 6.0, Sigma-Aldrich, Denmark) by incubation overnight in a 60°C waterbath. After antigen retrieval, the sections were blocked in 0.5% casein in TBS (Sigma-Aldrich, Denmark) for 20 minutes at room temperature. Sections were then incubated for 2 hours at RT with the primary antibodies ($10\mu\text{g}/\text{mL}$), followed by a 30-min incubation with horseradish peroxidase (HRP) conjugated anti-mouse polymer (EnVision, DAKO, Denmark). Development was performed using diaminobenzidine-tetrahydrochloride (DAB+) (DAKO, Denmark). Finally, the sections

were counter-stained with Mayer's acidic hematoxylin, dehydrated, and mounted in Pertex. Mouse IgG was used as a negative control. Digital pictures were obtained using a computer assisted microscope and only modified by enhancing the contrast and light; no background was removed or altered.

Statistical Analyses

All biomarker data were log transformed to obtain a normal distribution prior to analyses. The lower limit of detection for ALPHA CTX was 0.08 ng/ml and there were 16 samples for which levels fell below this threshold. For the purposes of statistical analyses, a value of 0.04 ng/ml, or ½ the lower limit of detection, was used for these 16 samples. Pearson correlations were used to evaluate the association of biomarkers with both radiographic (JSN and OST) and scintigraphic features of OA, and Pearson partial correlations were assessed to account for effects of age, gender, BMI, use of hormone replacement therapy, selective estrogen receptor modulators (SERMS), calcitonin, and bisphosphonates.

A one-factor principal components analysis (PCA) was used to determine the association of biomarkers with the total body burden of OA outside the knee as assessed by bone scintigraphy. PCA was performed using the following late-phase bone scan components: shoulder, thoracic spine, cervical spine, lumbar spine, carpometacarpal joint, sacroiliac joint, hip, sternoclavicular joint, sternomanubrial joint, acromioclavicular joint, ankle joint, forefeet, and first metatarsophalangeal joint; this single factor explained 20% of the variance in the bone scan data. Elbow, hand, wrist, and interphalangeal joints were excluded due to missing values for 19 participants; however, the carpometacarpal joint was retained despite missing values due to the known strong impact on biomarkers (30). Pearson partial correlations were used to determine the associations of the bone scan scores with each of the biomarkers, controlling for age, gender, BMI, and use of hormone replacement therapy, selective estrogen receptor modulators (SERMS), calcitonin, and bisphosphonates.

The non-parametric, Kruskal-Wallis test was used to assess differences in the distribution of age, gender, and BMI between OA progression subgroups. Poisson logistic regression was used to evaluate the relationships between baseline biomarker (uALPHA CTX, uCTXII) concentrations and continuous OA progression scores, described above. Log risk ratio estimates for progression were exponentiated and presented as risk ratios (RR) for progression such that each step increase on a log scale of the biomarker leads to an increased risk for progression corresponding to the RR. To account for participants that underwent knee replacement surgery, logistic regression was used to evaluate the relationships between baseline biomarker (uALPHA CTX, uCTXII) concentrations and categorical OA progression defined as any increase in osteophyte score, joint space narrowing score, or total knee replacement. SAS (version 9.3, SAS Institute, Cary NC) and SPSS were used for all analyses, and a p-value < 0.05 was considered statistically significant.

Results

Characteristics of the participants

The study sample consisted of a total of 149 participants with symptomatic knee OA at baseline including 111 (74%) women, with overall mean age of 63 ± 12 years and mean BMI of 31 ± 3 kg/m². The mean $\pm$ SD levels of both ALPHA CTX and CTXII measured in baseline urine samples, corrected by creatinine, were 145.3 ± 362 ng/mmol and 711.9 ± 2861 ng/mmol, respectively. uALPHA CTX and uCTXII were significantly associated ($r=0.95$, $p<0.0001$; 95% CI: 0.93–0.97).

Bone scans were used to assess activity of bone turnover of the knee and other joint sites including the spine, hip, shoulder, acromioclavicular, sternoclavicular, sternomanubrial, elbow, wrist, hand, carpometacarpal, sacroiliac, ankle, forefoot, and first metatarsophalangeal joint. uALPHA CTX was significantly associated with bone scan scores of the medial knee ($r=0.197$, $p=0.017$) and the ankle ($r=0.266$, $p=0.001$). uCTXII was significantly correlated with bone scan scores for the medial knee ($r=0.324$; $p<0.0001$), the lateral knee ($r=0.202$; $p=0.015$), the lumbar spine ($r=0.311$; $p=0.0001$), the elbow ($r=0.216$, $p=0.03$), wrist ($r=0.224$, $p=0.02$), and ankle ($r=0.167$, $p=0.04$). In a subset analysis of subjects with bilateral symptomatic knee OA at baseline ($n=125$), the association of uALPHA CTX with medial knee bone scan score was somewhat greater ($r=0.245$, $p=0.0057$) than the association in the whole cohort; however, the association of uCTXII with medial knee bone scan score in the symptomatic subset was not strengthened ($r=0.25$, $p=0.003$).

Strong association of uALPHA CTX and uCTX-II with total knee bone turnover

Both with and without the contribution of the patellofemoral joint, tibiofemoral knee bone scan scores, were significantly associated with uALPHA CTX ($r=0.18$, $p=0.03$) and uCTXII ($r=0.36$, $p<0.0001$). There was no significant association of the markers with the total OA factor score for joints outside the knee. After controlling for age, gender, BMI, hormone, calcitonin and bisphosphonate use, uALPHA CTX remained significantly associated with the total knee bone scan score ($r=0.188$; $p=0.041$), and uCTXII was significantly related to tibiofemoral knee bone scan scores, with ($r=0.291$; $p=0.0014$) and without ($r=0.248$; $p=0.007$) the patellofemoral joint (Table 1).

Strong association of uCTXII but not ALPHA CTX with radiographic OA severity

Using the radiographic information, independent of age, gender, BMI, and use of hormones, calcitonin and bisphosphonates, uCTXII was significantly associated with total JSN ($r=0.278$, $p=0.0007$) and total OST ($r=0.336$, $p<0.0001$; Table 2). Regionally, uCTXII was significantly associated with osteophytes in both the medial ($r=0.288$; $p=0.0004$) and lateral knee compartments ($r=0.320$; $p<0.0001$); uCTXII was also significantly associated with JSN in the medial ($r=0.201$, $p=0.015$), but not in the lateral compartment ($r=0.112$, $p=0.18$). When controlling for age, gender, BMI, use of hormones, bisphosphonates, calcitonin, and total OST or JSN separately, uCTXII was independently associated with total OST ($\beta=0.020$, $p=0.008$) but not JSN (Model $r^2=0.19$). uALPHA CTX was not associated with either JSN or OST.

Strong association of ALPHA CTX and uCTXII with radiographic knee OA progression

Progression status of the participants was defined as the change in OST or JSN scores over three years, summed across both knees. Of the 129 participants for which data were available, 35 (27.1%) were classified as non-progressors, 60 (46.5%) were classified as OST progressors, 17 (13.2%) were classified as OST and JSN progressors, and 17 (13.2%) as total knee replacement (TKR) progressors, having undergone surgery for OA during the 3-year follow-up interval (Table 3) with the understanding that TKR is performed for many reasons and therefore is not a straightforward indicator of OA progression. There were no significant differences in gender, age or BMI between groups. Accounting for those with any progression by including those who underwent interval knee replacement surgery, there were 94 (73%) progressors and 35 (27%) non-progressors. As compared to non-progressors, progressors had higher baseline concentrations of uCTXII ($p=0.004$), but not ALPHA CTX ($p=0.69$).

To evaluate the predictive capability of these biomarkers for radiographic knee OA progression, we evaluated the associations of baseline uALPHA CTX and uCTXII and change in JSN and OST scores. Interestingly, higher baseline ALPHA CTX (log) predicted progression of JSN and OST. Each step on a log scale of uALPHA CTX leads to a 13.2 and 3-fold change in the risk ratio (RR) of having increased progression of JSN and OST, respectively. These associations persisted upon adjusting for age, gender, BMI, hormone, calcitonin and bisphosphonate use: $\beta=0.88$, $p=0.04$ for uALPHA CTX (log) association with JSN, and $\beta=0.40$, $p=0.01$ for uALPHA CTX (log) association with OST. Hormone use was independently associated with JSN ($\beta=1.82$, $p=0.03$), while BMI was independently associated with progression of osteophytes ($\beta=0.03$, $p=0.005$).

uCTXII was related to progression of OST, with each step on a log scale of uCTXII leading to a 6-fold change in the risk ratio (RR), $p<0.0001$; this association persisted upon adjusting for age, gender, BMI, hormone, calcitonin and bisphosphonate use ($RR=5.4$, $p<0.0001$). BMI was independently associated with progression of osteophytes ($\beta=0.02$, $p=0.02$). There were no significant associations of uCTXII and progression of JSN ($\beta=0.82$, $p=0.07$).

To validate previous findings elucidating the origin of ALPHA CTX [12] and CTXII [27], immunolocalization studies were conducted on human OA full-depth knee OA joint tissue sections. ALPHA CTX was found in the subchondral bone and the eroded surface of the cartilage (Figure 2A). CTXII was localized in the deep zone cartilage above the cartilage-bone interface, extending upwards into the rim of the cartilage (e.g. chondrophyte) (Figure 2C). No non-specific immunoreactivity was observed with the IgG control.

Discussion

Changes in subchondral bone are intrinsic to the diagnosis of OA. As visualized on radiographs, bone changes, such as osteophytes, are a cumulative reflection of disease progression. In addition, subchondral bone turnover has been suggested to be an important factor in disease progression (31–33), however, it is much more difficult to investigate owing to its dynamic nature. Examinations of periarticular bone in the knees and hips of patients with OA have confirmed that the subchondral bone is abnormal in OA joints, with

sclerosis of the subchondral plate and altered trabecular structure (34, 35), exemplified by the thinning and fenestration of the vertical trabeculae and under-mineralized bone consequent to the high remodeling rates. Bone scintigraphy was the first technique used to demonstrate increased subchondral bone turnover in OA (20). These pivotal studies by Dieppe and colleagues strongly suggested the importance of subchondral bone in the pathogenesis of OA and in progression of disease pathology (21, 36–40).

This study provided the opportunity to explore the utility of two collagen biomarkers in a cohort evaluated for dynamic bone turnover and radiographic knee OA progression. Urinary ALPHA CTX has been described as a marker of newly formed bone indicative of high turnover areas specific for newly synthesized bone (13–15, 24) and is promising as a marker for detecting changes attributable to high localized bone turnover states or diseases. Previously, increased bone resorption (based on NTXI and CTXI) was associated with increased radiographic OA progression (10). In our cohort, uALPHA CTX was strongly associated with the dynamic assessment of bone turnover in the knee but no other joints, suggesting that uALPHA CTX may be able to detect the turnover of knee joints over the background of bone metabolism, possibly indicative of an enhanced repair response at this location. This interpretation is compatible with the available literature showing that uptake on bone scintigraphy reflects the surface area of new bone formation as opposed to bone resorption (41, 42). There were no significant associations of uALPHA CTX with the static assessment, baseline radiographic features of OA, suggesting that uALPHA CTX is associated with the dynamic biology of the highly remodeled subchondral bone. After adjusting for all parameters, baseline levels of uALPHA CTX were associated with the progression of both JSN and OST, suggesting that increased localized bone turnover is a risk factor for knee OA progression and supports a role for bone in the pathophysiology of progressive cartilage degradation. These biochemical data are compatible with other studies demonstrating that localized bone turnover is an indicator of risk for knee OA progression based on computed tomography and bone trabecular integrity markers (43, 44).

Further insights into ALPHA CTX can be gleaned from studies related to the interpretation of 99mTc-MDP bone scintigraphy in metastatic bone disease. When a tumor invades bone, in addition to causing bone destruction, it also causes reactive bone formation or repair. Late-phase uptake on images generated with 99mTc-MDP, which binds hydroxapatite crystals of bone, has been shown to preferentially represent new bone formation; the high degree of labeling at bone forming surfaces is believed to be due to the large surface area provided by the newly formed mineral crystals, prior to complete calcification of osteoid that inhibits radiotracer penetration into bone (41, 42, 45).

Since uptake on bone scintigraphy reflects the surface area of new bone formation as opposed to bone resorption (41, 42), it might be expected that uALPHA CTX, a marker of new bone formation, would correlate better with bone scintigraphy than uCTXII, a marker of type II collagen degradation. However, uALPHA CTX can be derived from the whole skeleton whereas uCTXII is derived specifically from joint tissues and therefore less subject to confound by systemic biomarker contributions. Furthermore, joint catabolism and anabolism are coupled; it is therefore logical that uCTXII, a degradative marker, could correlate with bone scintigraphy, a joint anabolic marker.

Levels of urinary CTXII are specific to type II collagen and are indicative of both calcified and non-calcified MMP-induced cartilage degradation. Urinary CTXII was associated with medial compartment knee bone turnover and lumbar spine bone turnover. Interestingly, not only were levels of uCTXII independently associated with the knee by bone scintigraphy, but uCTXII was independently associated with OA severity, as determined by OST and not JSN.

Given that this marker is specific for type II collagen, the expectation would have been an association with joint space narrowing, which is indicative of the loss of cartilage, and not changes in bone metabolism, however, uCTXII often appears to be associated with structural changes in bone(30), as well as with biomarkers of bone metabolism (46) which have led to varying explanations for this phenomenon. Lohmander, et al has suggested that uCTXII originates from mineralized tissue involving osteophytes, as well as the cartilage-bone interface (47). In fact, CTXII has been localized to the cartilage-bone interface in OA (29). In addition, type II collagen is expressed during the development of osteophytes (48); this is supported by the data in the present study showing the immunolocalization of the CTXII epitope in the calcified cartilage and osteophytes of human cartilage.

After adjusting for all parameters, uCTXII was associated only with static (baseline) OST severity (after controlling for JSN severity) and only with progression of OST, not JSN. Other studies have shown that CTXII is in fact a marker of OST burden and not JSN (upon adjusting OST for JSN, and JSN for OST)(30). In contrast, after adjustment, ALPHA CTX was not associated with static (baseline) OST or JSN but was associated with OST and JSN progression. Localized high bone turnover of the subchondral bone is strongly associated with change in joint space narrowing (43, 44). It is therefore to be expected that the bone turnover marker, ALPHA CTX, would be associated with progression of JSN. CTXII, a degradative marker, is likely associated with bone scintigraphy due to the coupling of joint tissue catabolic and anabolic responses; it therefore could be correlated to, but not directly reflecting, the bone formation process represented by bone scintigraphy. Rather it seems to reflect bone remodeling associated with chondrocyte/osteocyte formation and progression.

In summary, the data demonstrate that ALPHA CTX is indicative of dynamic bone turnover involved in attempted repair, as measured by bone scintigraphy and that this parameter predicts subsequent radiographic joint progression. This is consistent with the known ability for positive bone scintigraphy to predict radiographic progression (20). ALPHA CTX is not associated with static concurrent radiographic disease because some of it may be quiescent from a disease activity or repair perspective. ALPHA CTX was strongly correlated with, and localized to the subchondral bone, suggesting that ALPHA CTX may be a sensitive diagnostic and prognostic marker for the subchondral bone remodeling associated with OA and OA progression. The fact that levels of these two markers are correlated is consistent with a coupling of joint anabolism (reflected in ALPHA CTX) and joint catabolism (reflected in CTXII).

In this study, both uCTXII and uALPHA CTX were associated with OA progression and correlated with important disease parameters. Consequently, it may be speculated that a

possible structural treatment for OA may attenuate both ALPHA CTX and CTXII, reflecting two different disease parameters. Interestingly, some anti-resorptive treatments, such as bisphosphonates, have been shown to inhibit ALPHA CTX and BETA CTX to the same extent (49), whereas other interventions, such as calcitonin, seems to have a more pronounced effect on BETA alone, as well as decrease urinary levels of CTXII (49, 50). Taken together, these results are consistent with an interpretation that high turnover of the subchondral bone (reflected in ALPHA CTX) and the cartilage-bone interface (reflected in CTXII) is a mediator of progressive joint disease including cartilage degradation and attempted bone repair.

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Figure 1.

Flow chart depicting the enrollment of study participants and the numbers of participants who were included in the analyses of osteoarthritis (OA) progression over a three year period. Categorical progressors were participants who underwent a joint replacement during the followup period and therefore could not be scored for radiographic features of OA. Participants scored for progression were eligible for an increase in osteophyte score or joint space narrowing scores of which 77 were progressors based on an increase in osteophyte score and/or joint space narrowing score.

Figure 2.

Immunolocalization of alpha-C-telopeptide of type I collagen (ALPHA-CTX) and C-telopeptide of type II collagen (CTX-II) in human OA cartilage and bone from patients with severe erosions of the cartilage. **A** and **C**, Staining for ALPHA-CTX (**A**) and CTX-II (**C**) in sections from a severely eroded area with an eroded surface (ES), thickening of the subchondral bone (SB) and bone invasion fronts (IF) through the calcified cartilage (CC). **B** and **D**, Staining for ALPHA-CTX (**B**) and CTX-II (**D**) in sections from an area from the rim of the cartilage where an osteophyte (OP) is forming (i.e., chondrophyte) seen by the diffuse structure of the tissue. No background immunostaining was observed with the IgG control (results not shown). Bars = 200 μ m for panels A and C, 500 μ m for panels B and D.

Table 1

Pearson correlations of biomarkers with burden of OA, as assessed by late-phase bone scintigraphy

	log(uALPHA CTX), r(p)	log(uCTXII), r(p)
Tibiofemoral Knee	0.182 (0.027)	0.359 (<0.0001)
	0.102 (0.272) [#]	0.248 (0.007)[#]
Total Knee	0.250 (0.002)	0.368 (<0.0001)
	0.188 (0.041)[#]	0.291 (0.0014)[#]
Total OA Factor (No Knee)	−0.080 (0.376)	0.164 (0.068)
	−0.002 (0.980)[#]	0.159 (0.085)[#]

[#] Adjusted for age, gender, BMI, and use of hormone replacement therapy, selective estrogen receptor modulators, calcitonin, and bisphosphonates.

Table 2

Pearson correlations of biomarkers with severity of radiographic features of osteoarthritis.

	log(uALPHA CTX), r(p)	log(uCTXII), r(p)
Total JSN	0.082 (0.32)	0.278 (0.0007)
	0.017 (0.84) [#]	0.220 (0.009) [#]
Total OST	0.080 (0.333)	0.336 (<0.0001)
	-0.0014 (0.986) [#]	0.309 (0.0002) [#]
	log(uALPHA CTX), β(p)	log(uCTXII), β(p)
Total JSN	NA	0.019 (0.318)±
	NA	0.011 (0.578) *
Total OST	NA	0.022 (0.0028)±
	NA	0.020 (0.0076) *

NA= Not assessed due to lack of significance in unadjusted model.

[#] Adjusted for age, gender, BMI, and use of hormone replacement therapy, selective estrogen receptor modulators, calcitonin, and bisphosphonates.

± Adjusted for JSN and OST only.

* Adjusted for age, gender, BMI, and use of hormone replacement therapy, selective estrogen receptor modulators, calcitonin, bisphosphonates, JSN, and OST.

Table 3

Demographics and baseline biomarker concentrations of the POP cohort subgroups with progression data.

	NP	OST	OST/JSN	TKR
Subjects (n)	35	60	16	17
Gender (%F)	77%	72%	71%	65%
Age (years) *	61 ± 12	64 ± 11	64 ± 14	66 ± 13
BMI (kg/m ²) *	30 ± 5	32 ± 7	31 ± 8	30 ± 6
uALPHA CTX (ng/mmol) [^]	80 (48,164)	81 (53,137)	139 (71, 231)	91 (38, 208)
uCTXII (ng/mmol) [^]	271 (163, 356)	320 (249, 556)	427 (310, 616)	471 (257, 812)

NP=Non-progressor, OST = progressor by osteophyte, OST/JSN = progressor by osteophyte and joint space narrowing, TKR = progressor by total knee replacement.

* Values for Age and BMI are mean ± standard deviation.

[^] Values for uALPHA CTX and uCTXII are median (25th, 75th Interquartile range).