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Pain prediction by serum biomarkers of bone turnover in people with knee osteoarthritis: an observational study of TRAcP5b and cathepsin K in OA

L.N. Nwosu † § [b *], M. Allen † ‡, L. Wyatt † ‡, J.L. Huebner ||, V. Chapman † §, D.A. Walsh † ‡ [a], V.B. Kraus || ¶ [a]

† *Arthritis Research UK Pain Centre, University of Nottingham, UK*
‡ *School of Medicine, University of Nottingham, UK*
§ *School of Life Sciences, University of Nottingham, UK*
|| *Duke Molecular Physiological Institute, USA*
¶ *Division of Rheumatology, Department of Medicine, Duke University, USA*

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SUMMARY

Objectives: To investigate serum biomarkers, tartrate resistant acid phosphatase 5b (TRAcP5b) and cathepsin K (cath-K), indicative of osteoclastic bone resorption, and their relationship to pain and pain change in knee osteoarthritis (OA).
Methods: Sera and clinical data were collected from 129 people (97 with 3-year follow-up) with knee OA from the Prediction of Osteoarthritis Progression (POP) cohort. Knee OA-related outcomes in POP included: WOMAC pain, National Health and Nutrition Examination Survey (NHANES) I (pain, aching and stiffness), subchondral sclerosis, and radiographically determined tibiofemoral and patellofemoral OA. Two putative osteoclast biomarkers were measured in sera: TRAcP5b and cath-K. Medial tibia plateaux were donated at knee arthroplasty for symptomatic OA ($n = 84$) or from 16 post mortem (PM) controls from the Arthritis Research UK (ARUK) Pain Centre joint tissue repository. Osteoclasts were stained for tartrate resistant acid phosphatase (TRAcP) within the subchondral bone of the medial tibia plateaux.
Results: Serum TRAcP5b activity, but not cath-K-immunoreactivity, was associated with density of TRAcP-positive osteoclasts in the subchondral bone of medial tibia plateaux. TRAcP-positive osteoclasts were more abundant in people with symptomatic OA compared to controls. Serum TRAcP5b activity was associated with baseline pain and pain change.
Conclusions: Our observations support a role for subchondral osteoclast activity in the generation of OA pain. Serum TRAcP5b might be a clinically relevant biomarker of disease activity in OA.

Introduction

Pain is the reason for most osteoarthritis (OA)-related medical visits. OA knee pain substantially impacts quality of life and is a key determining factor for loss of joint function[1]. Available drug treatments focus on analgesia, but often do not have sustained benefit and many patients experience unwanted side effects[2].

Although OA affects articular cartilage, it is increasingly recognised as a disease of the whole joint. Changes in subchondral bone are key in the pathogenesis of knee OA, and associated with knee pain[3] and radiographic progression[4]. Bone remodelling and increased pain mediators (cyclooxygenase 2, substance P, TNF-α) in the subchondral bone might occur before overt OA cartilage degeneration[5]. Subchondral bone is densely innervated by sensory nerves[6], and might be a key source of OA pain.

Animal models of OA and imaging studies in man support associations between pain and subchondral structural pathology[7–9]. In particular, increased osteoclast activity indicative of subchondral bone turnover might be associated with OA and pain[7,10]. Osteoclasts are multinucleated giant cells responsible for homeostatic bone resorption that release enzymatic markers, including tartrate

* Address correspondence and reprint requests to: L.N. Nwosu, Arthritis Research UK Pain Centre, Department of Academic Rheumatology, University of Nottingham, City Hospital, Clinical Sciences Building, Hucknall Road, Nottingham NG5 1PB, UK.
E-mail address: lilian.nwosu@newcastle.ac.uk (L.N. Nwosu).
[a] Joint senior authors.
[b] Newcastle University Institute of Cellular Medicine 3rd Floor William Leech Building, Framlington Place Newcastle, NE2 4HH United Kingdom

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resistant acid phosphatase (TRAcP) and cathepsin K (cath-K). TRAcP, originally called type 5 acid phosphatase, can be expressed both by osteoclasts and macrophages[11]; it was identified in human serum and separated electrophoretically into two distinct bands: 5a and 5b. Electrophoretic studies suggest band 5b tartrate resistant acid phosphatase 5b (TRAcP5b) is derived from osteoclasts and 5a from macrophages[12]. Cath-K, a cysteine protease, has been implicated in OA pathogenesis, largely because of its upregulation in areas of cartilage damage and resorbed bone[13,14]. Roles of cath-K in the initial stages of bone resorption have led to it becoming a target for novel therapeutic approaches for diseases such as osteoporosis, where reduced bone resorption can increase bone mineral density and reduce fracture risk[15]. Circulating TRAcP5b activity and cath-K are reduced in clinical trials during bisphosphonate treatment[16,17].

Bone and cartilage biomarkers have been investigated in OA structural progression[18,19], and some circulating inflammation biomarkers have been associated with OA pain, including C reactive protein (CRP), tumour necrosis factor (TNF)-α, interleukin (IL)-6[20] and interleukin (IL)-1β[21]. One study reports concentrations of N-telopeptide of type I collagen (uNTX-I) being significantly increased in people with OA knee pain (VAS score) independent of radiographic severity[22]. However, validated biomarkers of subchondral osteoclast activity associated with OA pain, or pain progression, have yet to be reported.

We hypothesised that biomarkers which reflect subchondral osteoclast activity, will be associated with OA pain, and might be useful in predicting pain progression in OA. The objectives of this study were to identify and validate serum biomarkers of subchondral osteoclast activity in people with symptomatic knee OA and to evaluate the association of these markers with OA pain, structural severity, and progression.

Patients and methods

Data reports a cross-sectional, case–control, cohort study.

Participants

129 participants from the Prediction of Osteoarthritis Progression (POP) cohort[19] and knee tissue from 100 subjects from the Arthritis Research UK (ARUK) Pain Centre joint tissue repository[23] were available (Table I). Included participants met the American College of Rheumatology (ACR) criteria for symptomatic OA[24]. Samples from 129 of the POP cohort were available at baseline and from 97 at 3-year follow up. Participants in the POP cohort who had unilateral total knee replacement (TKR) surgery before baseline blood and data collection were excluded, and those who had TKR before follow up were excluded from longitudinal analyses. Cases from the joint tissue repository had knee tissue taken at TKR surgery for symptomatic OA ($n = 84$), or post mortem (PM) ($n = 16$) from people who had not sought help for knee pain during the last year of life (asymptomatic control group). Sixteen cases from each of the TKR and PM groups were matched for macroscopic chondropathy scores, age and gender. Macroscopic chondropathy was scored by a single observer as previously described[25], taking account of severity (graded from 0 (normal unbroken surface) to 4 (subchondral bone exposure)) and extent (percentage of area involved by each grade) to calculate a chondropathy score from 0 to 100. Scores for all 4 compartments (medial and lateral tibial plateaux and femoral condyles) were summed to give a total chondropathy score from 0 to 400. Participants were excluded if they had specific bone disease known to affect bone turnover (e.g., Paget's disease of the bone, osteomalacia), or non-OA diagnoses as a cause of knee pain (e.g., rheumatoid arthritis, acute gout), but not according to medication use (Table I). Cases with self-reported osteoporosis were also included (Table I).

Table I
Demographics of patient study groups

	POP cohort		ARUK Pain Centre joint repository	
Number	Baseline; 129	Follow up; 97	TKR; 84*	PM; 16
Age (mean ± SD years)	64 ± 11	67 ± 11	66 ± 10	69 ± 12
Female (%)	72	72	57	56
BMI (mean ± SD kg/m^2)	31.4 ± 6.6	31.6 ± 6.7	31.3 ± 6.8	n/a
Osteoporosis (%)	17	16	0	0
Bisphosphonate use (%)	11	9	0	0

* Matched TKR cases ($n = 16$) were a subgroup of the total TKR cases used.

Imaging

Postero-anterior weight-bearing knee radiographs were obtained as previously described[25–27]. Radiographs of the POP cohort were scored by observers blinded to patient details for Kellgren–Lawrence (K/L) grade (0–4)[28] and individual radiographic features of OA including joint space narrowing (JSN 0–3), osteophytes (OST 0–3), subchondral sclerosis (0 or 1) and patellofemoral OA (0–3) using a standardized atlas[29]. Total scores were summed scores for both knees (right + left) and compartments (tibia − medial, lateral; femur − medial, lateral)[19]. Knee radiographs for cases providing joint tissues at TKR were scored using an atlas of line drawings of medial and lateral JSN and OST[30]. JSN (range 0–6) and OST (range 0–12) scores were summed to provide a total radiographic OA severity score for each knee (range 0–18). Radiographs were not available for PM cases.

Scintigraphic imaging of knees and whole body was performed as previously described[19,27]. The radiotracer methylene-diphosphonate labelled with technetium-99m was administered 2 h prior to imaging. Sixteen joint sites were scored semi-quantitatively by 2 experienced observers blinded to patient detail, on a scale of 0–3, where 0 = normal to 3 = intense. The scores were 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.

Pain assessment

In the POP cohort, pain was assessed using the Likert pain scale of the Western Ontario and McMaster Universities Osteoarthritis index (WOMAC-A)[31]. It consists of 5 summed items (pain on walking, stair climbing, nocturnal, rest and weight bearing) scored from 0 = none, 1 = mild, 2 = moderate, 3 = severe and 4 = extreme, to give a total subscore ranging from 0 to 20. Knee symptoms were also ascertained by the National Health and Nutrition Examination Survey (NHANES) I criterion[32] of pain, aching or stiffness on most days of any 1 month in the last year; for subjects answering yes, symptoms were quantified as mild, moderate, or severe, yielding a total score of 0–3 for each knee. Change scores were calculated separately for WOMAC pain and NHANES I pain as follow-up score minus baseline score, summed across both knees, and used to define pain worsening or improvement in participants over 3 years as previously published[19]. Pain scores were not available for ARUK Pain Centre joint tissue repository cases, and sera were not available for PM cases.

Biomarker quantification

TRAcP5b activity and cath-K concentrations were analysed in serum stored at −80°C from participants in the POP cohort and

from TKR patients in the ARUK joint repository group. Experimenter was blinded to patient details. Both biomarkers were measured in undiluted serum by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol. TRAcP5b activity (U/L) was measured using a Bone TRAP® (TRAcP5b) ELISA (immunodiagnostic systems – IDS). Concentrations of cath-K (pg/ml) were measured using a human cath-K ELISA (CUSABIO). Inter-assay coefficient of variation (CVs) for TRAcP5b was 0.89% and cath-K; 9.52%. Twenty-two samples were below the lower limit of detection (LLOD) for TRAcP5b (0.5 U/L). One sample was below the LLOD for cath-K (7.5 pg/ml). A value equal to one half the LLOD was imputed for these samples for the purposes of statistical analyses.

TRAcP positive osteoclast density

Mid-coronal sections (5 μm) of the middle one-third of the medial tibial plateau (an important weight bearing area characteristically affected by OA) were fixed in neutral buffered formalin and then decalcified in 10% EDTA in 10 mM Tris buffer (pH 6.95; at 4°C) prior to embedding in paraffin wax. Sections were stained for TRAcP-positive osteoclasts in two sections per case from the middle one-third of the medial tibial plateau. Samples were deparaffinized in xylene, rehydrated in serial alcohol and distilled water, and recalcified in a solution containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$ in PBS overnight. TRAcP was stained using a commercially available kit (#387A Sigma–Aldrich, UK) following the manufacturer's protocol. The numbers of TRAcP positive osteoclasts within the subchondral bone area were counted manually using a Zeiss Axioscop-50 microscope (Carl Zeiss Ltd, Welwyn Garden City, UK) at 20× magnification to a depth of 400 μm from the calcified cartilage (CC). The scorer was blind to patient details. The number of osteoclasts was divided by the length of the subchondral bone to give an osteoclast density expressed as TRAcP positive cells per mm[33].

Statistical analysis

Data were analysed using Statistical package for the Social Sciences v.22 (SPSS Inc., Chicago, Illinois, USA). Pilot studies were carried out prior to main study for power calculations for sample size. Between group (TKR vs PM, with vs without osteoporosis) comparisons for TRAcP-positive osteoclasts were tested using the Mann–Whitney *U* test. Biomarker data were natural log (Ln) transformed to obtain a normal distribution for use in all analyses. Shapiro–Wilks test confirmed that Ln transformed biomarker data did not significantly diverge from normality. Univariable and multivariable linear regressions were used for all association analyses, including between bone biomarkers and TRAcP-positive osteoclast density, between bone biomarkers and OA outcomes (WOMAC pain, NHANES I pain, subchondral sclerosis, patellofemoral OA, JSN, osteophyte, and KL grade) or total burden of OA at the knee and other joints at baseline based on scintigraphy (cross-sectional study). Univariable and multivariable linear regressions were used to assess associations of baseline TRAcP5b and cath-K with change in pain (WOMAC and NHANES I) over the 3-year follow up in the POP cohort (longitudinal study). A one-factor principal component analysis (PCA) was performed for the joints assessed by bone scintigraphy as previously described[19]. This produced a factor that explained 20% of the variance in the whole body bone scintigraphy data. This factor, reflecting bone formation[34,35] was assessed for association with the osteoclast related biomarkers. All parameter estimates were adjusted for OA risk factors (age, sex, BMI) and, where appropriate, for bisphosphonate use because bisphosphonates are known to inhibit osteoclast activity. In addition to beta coefficients, marginal effects for pain outcomes are presented where statistically significant associations were demonstrated after adjustments. Numerical and graphical data are presented as mean ± 95% confidence interval to denote statistical uncertainty of estimates between groups, whereas mean ± SD is used for descriptive variables. $P < 0.05$ was considered statistically significant.

Results

Demographics of participants

The 129 participants from the POP cohort with symptomatic knee OA at baseline comprised 72% females with an overall mean ± SD age of 64 ± 11 years and mean ± SD BMI of 31.4 ± 6.6 kg/m^2 (Table I). A 3-year follow up of 97 participants from the POP cohort included 72% females with an overall mean ± SD age of 67 ± 11 years and mean ± SD BMI of 31.6 ± 6.7 kg/m^2. The mean ± SD baseline concentrations of TRAcP5b and cath-K for these participants were 0.8 ± 0.4 U/L and 170.7 ± 110.7 pg/ml, respectively. Mean (SD) baseline WOMAC and NHANES I pain scores were 5.2 ± 3.2 and 2.9 ± 1.2, respectively. Pain change, defined as follow-up minus baseline score in the POP participants, was a mean (95% CI) of 0.13 (−0.85 to 1.1) and −0.3 (−0.6 to 0.0008) for WOMAC and NHANES I pain, respectively.

ARUK Pain Centre joint repository knee tissue and sera, were obtained at TKR from 84 people (57% female) who had symptomatic knee OA, with an overall mean ± SD age of 66 ± 10 years and mean ± SD BMI of 31.3 ± 6.8 kg/m^2 (Table I). Knee tissues were obtained at PM from 16 subjects (56% female) who did not seek help for knee pain in the last year of their life (mean ± SD age 69 ± 12 years). The mean ± SD baseline concentrations of TRAcP5b and cath-K in the TKR subjects were 3.35 ± 1.48 U/L and 9.54 ± 18.1 pg/ml, respectively.

Associations between osteoclast density in OA subchondral bone, serum osteoclast biomarkers, and symptomatic knee OA

To investigate whether the biomarkers TRAcP5b and cath-K are serum markers of subchondral osteoclast activity, we assessed their associations with TRAcP-positive osteoclast density in OA subchondral bone from patient samples ($n = 68$) obtained at TKR for knee OA. TRAcP-positive osteoclasts were identified in OA subchondral bone samples at a mean (95% CI) density of 1.5 (0.95–2) mm^{-1}. TRAcP5b and cath-K were detectable in the serum of the TKR group by immunoassay. Serum TRAcP5b was associated with density of TRAcP-positive osteoclasts, independent of age, sex, and BMI. In contrast, serum cath-K was not statistically significantly associated with TRAcP-positive osteoclast density (Table II). In the POP cohort, as expected neither TRAcP5b nor cath-K was statistically significantly associated with new bone formation, as assessed by namely knee or total body bone scintigraphy scores (Supplementary Table 1).

Asymptomatic (PM) and symptomatic (TKR) chondropathy groups ($n = 16$), matched for macroscopic chondropathy scores

Table II
Relationship of serum biomarkers to TRAcP positive osteoclast density

	TRAcP5b		Cath-K	
	β (95% CI)	*P*	β (95% CI)	*P*
TRAcP osteoclast density	**0.74 (0.04–1.44)**	**0.04**	0.13 (−0.26 to 0.52)	0.50
TRAcP osteoclast density†	**0.74 (0.01–1.47)**	**0.047**	0.12 (−0.29 to 0.53)	0.57

†Adjusted for baseline age, sex, BMI.
Bold highlights represent statistical significant data

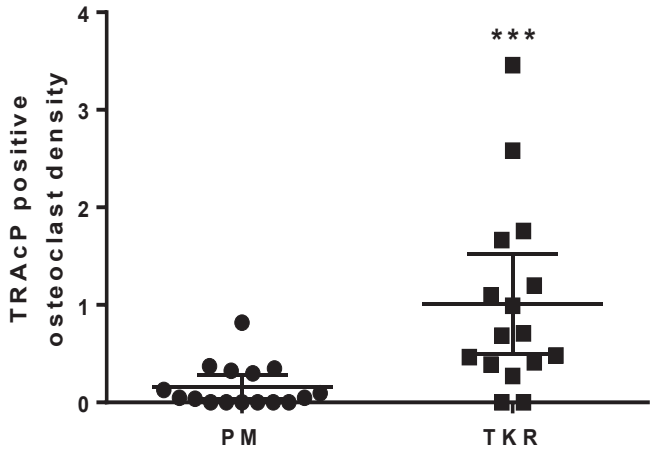

Fig. 1. TRAcP positive osteoclasts were statistically significantly higher in people with symptomatic OA (TKR) compared to PM controls who also presented with chondropathy but did not seek help for knee pain. Data indicate mean ± S.E.M. for $n = 16$ per group. Differences between groups were analysed using a Mann–Whitney U testTKR.

mean (95% CI); (200 (186–215) and 209 (196–221), respectively ($P = 0.38$)) were assessed for TRAcP-positive osteoclasts. TRAcP-positive osteoclasts in subchondral bone were significantly more abundant in people with symptomatic knee OA (mean density 1.0 (0.50–1.5) mm^{-1}) compared to the asymptomatic PM controls (0.16 (0.04–0.28) mm^{-1}), $P = 0.001$ (Figs. 1 and 2(A) and (B)).

Association of bone biomarkers with OA pain and structural severity

In a cross-sectional, baseline serum TRAcP5b in the POP cohort ($n = 129$) was associated with WOMAC pain score ($\beta = 1.24$, 95% CI 0.21–2.26; $P = 0.02$) (Table III) and subchondral sclerosis ($\beta = 0.35$, 95% CI 0.07–0.63; $P = 0.02$) (Table IV), even after adjusting for age, sex, and BMI. This association persisted after adjusting for bisphosphonate use. Based on marginal effect sizes, the mean baseline TRAcP5b levels would need to be 2.3-fold–2.8-fold higher to predict a 1 unit higher baseline WOMAC pain score. Baseline serum TRAcP5b activity was not significantly different in participants who reported osteoporosis compared to those who did not ($P = 0.47$). Baseline serum TRAcP5b was also associated with NHANES I pain score (Table III), and baseline serum cath-K in the POP cohort was associated with radiographic severity of patellofemoral OA (Table IV), but statistical significance was lost after adjusting for age, sex, and BMI.

Table III
Relationship of serum biomarkers of osteoclast activity to OA pain

	TRAcP5b		Cath-K	
	β (95% CI)	P	β (95% CI)	P
WOMAC pain	**1.64 (0.58–2.71)**	**0.003**	−0.05 (−0.87 to 0.78)	0.91
WOMAC pain†	**1.24 (0.21–2.26)**	**0.02**	−0.21 (−0.99 to 0.57)	0.60
WOMAC pain†¶	**1.28 (0.24–2.32)**	**0.02**	−0.21 (−0.99 to 0.57)	0.60
NHANES I pain	**0.45 (0.06–0.84)**	**0.02**	0.15 (−0.14 to 0.45)	0.31
NHANES I pain†	0.26 (−0.10 to 0.62)	0.16	0.10 (−0.17 to 0.37)	0.48
NHANES I pain†¶	0.27 (−0.10 to 0.63)	0.15	0.10 (−0.17 to 0.37)	0.48

†Adjusted for age, sex, BMI and ¶ for bisphosphonates. WOMAC pain marginal effect sizes (fold increase in TRAcP5b associated with 1 unit higher WOMAC pain score); 2.3, †2.8 and ¶2.7.
Bold highlights represent statistical significant data.

Table IV
Relationship of serum biomarkers of osteoclast activity to structural OA features

	TRAcP5b		Cath-K	
	β (95% CI)	P	β (95% CI)	P
Subchondral sclerosis	**0.32 (0.04–0.59)**	**0.03**	−0.01 (−0.22 to 0.20)	0.92
Subchondral sclerosis†	**0.35 (0.07–0.63)**	**0.02**	−0.01 (−0.23 to 0.20)	0.91
Subchondral sclerosis†¶	**0.35 (0.07–0.64)**	**0.02**	−0.01 (−0.23 to 0.20)	0.91
Osteophyte	0.49 (−1.07 to 2.06)	0.53	0.80 (−0.36 to 1.96)	0.18
Osteophyte†	0.40 (−1.19 to 1.20)	0.62	0.68 (−0.49 to 1.86)	0.25
Osteophytes†¶	0.24 (−1.37 to 1.84)	0.77	0.67 (−0.50 to 1.84)	0.26
Joint space narrowing	0.25 (−0.33 to 0.83)	0.40	0.28 (−0.16 to 0.71)	0.21
Joint space narrowing†	0.19 (−0.37 to 0.75)	0.49	0.16 (−0.25 to 0.57)	0.44
Joint space narrowing†¶	0.28 (−0.43 to 0.69)	0.65	0.15 (−0.26 to 0.56)	0.46
Patellofemoral OA	−0.56 (−2.21 to 1.10)	0.51	**1.26 (0.04–2.47)**	**0.04**
Patellofemoral OA†	−0.46 (−2.12 to 1.20)	0.59	1.11 (−0.11 to 2.32)	0.07
Patellofemoral OA†¶	−0.56 (−2.25 to 1.13)	0.51	1.11 (−0.11 to 2.32)	0.07
KL grade	0.17 (−0.42 to 0.75)	0.58	0.32 (−0.11 to 0.75)	0.15
KL grade†	0.10 (−0.46 to 0.66)	0.72	0.21 (−0.20 to 0.63)	0.32
KL grade†¶	0.06 (−0.51 to 0.63)	0.83	0.21 (−0.21 to 0.62)	0.33

†Adjusted for age, sex, BMI and ¶ for bisphosphonates.
Bold highlights represent statistical significant data.

Association of baseline TRAcP5b with OA pain change

To evaluate the predictive capability of TRAcP5b and cath-K for change in OA pain (WOMAC and NHANES I), we assessed the

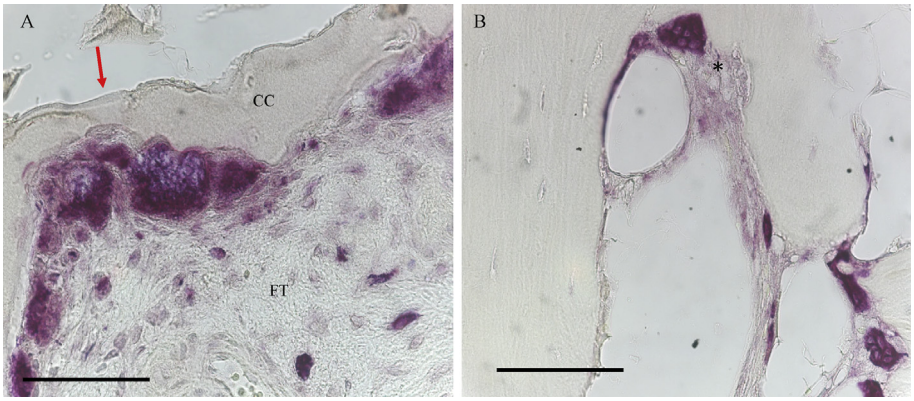

Fig. 2. TRAcP positive osteoclasts in the subchondral bone of OA patients at TKR. TRAcP positive osteoclasts stained in sections from the medial tibial plateau show severely eroded cartilage (red arrow – A). TRAcP staining showed active multinucleated osteoclasts (purple) within bone marrow spaces (B) and in areas of fibrovascular replacement (A). TRAcP positive osteoclasts on the edge of the bone signify sites of bone resorption and a resorption cavity (asterisk) as evidence of bone remodelling. FT – fibrovascular tissue. Scale bars = 100 μm.

associations of bone biomarkers at baseline with change in pain scores during a 3-year follow up ($n = 97$). Baseline TRAcP5b was associated with pain change as evaluated by the NHANES I pain questionnaire ($\beta = 0.69$, 95% CI 0.19–1.20; $P = 0.008$) after adjustment for age, sex, BMI, and baseline NHANES I pain, but not with WOMAC pain ($\beta = 0.71$, 95% CI −0.90 to 2.33; $P = 0.38$) (Table V). Associations between baseline serum TRAcP5b and change in pain (NHANES I) remained statistically significant after adjusting for bisphosphonate use (Table V). Based on marginal effect sizes, the mean baseline levels of TRAcP5b would need to be 5.3-fold–11-fold higher to predict an additional 1 unit increase in NHANES I pain score between baseline and follow up. Baseline cath-K was not associated with pain change (either WOMAC or NHANES I) (Table V).

Based upon our regression findings, although the magnitude of the association between TRAcP5b and WOMAC pain was similar to NHANES I, there were no statistically significant relationships.

Discussion

In the context of knee OA, increased density of TRAcP-positive osteoclasts was associated with knee symptoms. Serum concentrations of TRAcP5b, which we show to be a marker of subchondral osteoclast numbers, was statistically significantly associated with OA pain and pain change. These data provide important new evidence that subchondral bone remodelling contributes to OA. Moreover, serum TRAcP5b may have potential as a biomarker to assist in the selection of patients who could benefit from treatments targeting bone resorption in OA.

Subchondral bone changes are an integral part of the OA pathology. Bone remodelling at joint margins leads to osteophyte formation, and subchondral uptake of a radiotracer (methylene-diphosphonate labelled with technetium-99m) detected by scintigraphy, reflecting bone formation, has previously been associated with both radiographic OA disease progression and with knee pain[27,36,37]. Bone remodelling requires osteoclast activity. We tested whether osteoclast enzymes released during bone resorption, cath-K and TRAcP5b[38,39], could serve as markers of subchondral osteoclast activity. Our data, linking osteoclast activity, as reflected by serum TRAcP5b, with OA pain provide a clear biological mechanism that could explain the reported analgesic benefit of anti-resorptives such as bisphosphonates in human[40,41] and rodent OA[7]. We also observed at baseline, an association of serum TRAcP5b with subchondral bone sclerosis as well as with WOMAC pain scores, further suggesting a link between subchondral bone remodelling and pain generation in OA. Other cartilage and bone biomarker studies have reported on associations with structure and structural progression in OA[42], but not with OA pain progression. In the current study, we report strong associations between baseline serum TRAcP5b and subsequent change in symptoms measured by NHANESI.

Increased numbers of TRAcP-positive osteoclasts in subchondral bone have been reported in human[33] and rodent[43] OA, and preclinical and imaging studies report possible involvement of osteoclasts in osteoarthritic pain[7,10]. In the current study, we show that in samples matched for chondropathy, osteoclast density was higher in people who sought treatment for knee pain (TKR) compared to those who did not (PM), indicating that osteoclast densities might contribute to OA symptoms independent of OA structural severity. In addition, by altering joint shape and loading, osteoclast-mediated subchondral bone remodelling might contribute to further cartilage damage.

Osteoclasts are derived from monocytes, which originate within the bone marrow. Activated osteoclasts release both cath-K and TRAcP5b during the course of bone resorption, although only serum TRAcP5b, and not cath-K, was associated with subchondral osteoclast numbers in the current study. The statistically significant association between TRAcP5b serum levels and osteoclast numbers suggest that a high proportion of circulating TRAcP5b might originate from subchondral bone during OA disease activity, whereas circulating cath-K may be derived from additional sources (e.g., chondrocytes)[33,44]. Further work would require investigating serum concentrations of cath-K with chondrocytes.

TRAcP5b has two enzymatic roles after its release from osteoclasts. It acts as a phosphatase at acidic pH, and also as a generator of reactive oxygen species (ROS) at neutral pH. ROS may participate in the breakdown of endocytosed bone matrix products in resorbing osteoclasts[45] and be involved in pain generation in OA[46]. In the current study, we report for the first time, statistically significant associations of serum TRAcP5b with WOMAC pain scores in OA. Other studies have shown inflammatory biomarkers, CRP, TNF-α, IL-6[20] and IL-1β[21] associated with OA pain. Anti-cytokine treatments have been tested in clinical trials for OA pain, but lack of clinically important improvements over placebo might indicate that these molecules mediate OA pain only alongside other factors, or in subgroups of patients[47,48].

High concentrations of serum TRAcP5b have been detected in diseases characterized by increased osteoclastic activity such as Paget's disease, haemodialysis, primary hyperparathyroidism[49] and malignancies involving bone resorption, for example breast cancer with bone metastases[39]. In the current study, patients with other bone diseases were excluded and parameter estimates adjusting for bisphosphonates did not alter statistically significant associations observed between serum TRAcP5b, structural pathology, pain, and pain change in OA. Histological examination of the subchondral bone did not reveal malignant infiltration in any case in our current study, but we do not disregard the possibility of systemic effects of malignancy. Furthermore, concentrations of serum TRAcP5b were not different in participants with or without osteoporosis suggesting that relationships of TRAcP5b activity to symptomatic knee OA were independent of the presence of osteoporosis. Serum TRAcP5b concentrations were reported to be decreased following administration of the bisphosphonate alendronate in postmenopausal women with osteoporosis[16]. From studies that show analgesic effects of bisphosphonates, and with findings from the current study, we suggest that bisphosphonates might reduce pain in OA by reducing osteoclast activity.

Table V
Relationship of baseline serum biomarkers to change in OA pain

	TRAcP5b		Cath-K	
	β (95% CI)	P	β (95% CI)	P
WOMAC pain change	−1.44 (−3.23 to 0.34)	0.11	0.33 (−1.03 to 1.67)	0.63
WOMAC pain change†¥	0.71 (−0.90 to 2.33)	0.38	0.29 (−0.83 to 1.40)	0.61
WOMAC pain change†¥¶	0.72 (−0.90 to 2.35)	0.38	0.30 (−0.85 to 1.45)	0.61
NHANES I pain change	0.46 (−0.08 to 1.0)	0.10	−0.09 (−0.50 to 0.33)	0.69
NHANES I pain change†¥	**0.69 (0.19–1.20)**	**0.008**	0.02 (−0.36 to 0.41)	0.91
NHANES I pain change†¥¶	**0.67 (0.16–1.18)**	**0.01**	0.07 (−0.33 to 0.47)	0.73

†Adjusted for baseline age, sex, BMI, and ¥ for baseline pain score (e.g., change in WOMAC pain adjusted for baseline WOMAC pain), and ¶ for bisphosphonates. Change scores are follow up scores minus baseline scores. Mean ± SD baseline WOMAC and NHANES I pain = 5.2 ± 3.2 and 2.9 ± 1.2 respectively. Mean ± SD follow-up WOMAC and NHANES I pain = 5.4 ± 4 and 2.6 ± 1.5 respectively. NHANES I pain marginal effect sizes (fold increase in TRAcP5b associated with 1 unit greater NHANES I pain score increase between baseline and follow up); 11.0, †¥5.3, †¥¶5.6. Bold highlights represent statistical significant data.

OA has traditionally been viewed as a disorder of the tibiofemoral joint (TFJ), but the patellofemoral joint (PFJ) is one of the most commonly affected compartments in OA and also an important source of pain in OA[50]. The association observed between serum cath-K and patellofemoral but not tibiofemoral OA suggests that different biomarkers might reflect OA disease activity in different joint compartments of the knee. Patellofemoral OA with cartilage loss of the patella and trochlea groove is reported in about half of patients diagnosed with knee OA[51].

Both TRAcP5b and cath-K are released by osteoclasts and are involved in bone resorption during bone turnover. Neither serum TRAcP5b nor cath-K was associated in the current study with bone scintigraphy scores; this underscores the specificity of these markers for bone resorption rather than bone formation. In another study, alpha-C-telopeptide of type I collagen [α-CTX], a marker of degradation of newly formed bone, was associated with bone scintigraphy[19]. Serum biomarkers of osteoclast activity, such as TRAcP5b, reflect the specific domain of bone resorption and thereby provide distinct and complementary information to that provided by other bone turnover markers[34,35].

Our study is necessarily subject to a number of limitations. There were no knee tissues available from the participants of the POP cohort so we could not directly correlate TRAcP osteoclasts to TRAcP5b serum concentrations, pain or subchondral sclerosis in this cohort. Likewise, there were no serum samples available for the asymptomatic chondropathy group (PM) so circulating TRAcP5b could not be quantified. We also assumed that people in the PM group had experienced less pain than the patients in the symptomatic chondropathy group (TKR), since to the best of our knowledge, they had not sought medical attention for knee pain during their last year of life. In the current study, we investigated the association of TRAcP-positive osteoclasts from tibia samples to serum TRAcP5b. Osteoclast activity in the femoral condyles might further contribute to serum TRAcP5b[52]. In addition, lack of statistically significant association for most of the analyses with cath-K, and between cath-K and TRAcP5b might be due to limitations in the sensitivity of the cath-K assay used.

Our findings identify serum TRAcP5b as a marker of subchondral osteoclast activity and suggest its potential utility as a biomarker for OA pain and pain change. TRAcP5b deserves further investigation as a biomarker of bone remodelling to aid in identifying people for whom osteoclast activity contributes to OA pain, and who might be particularly responsive to analgesic and disease modification potential of anti-resorptive agents.

Author contributions

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Drs. Walsh and Kraus had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design; LNN, VC, DAW, VBK.

Acquisition of data; LNN, MA, LW.

Analysis and interpretation of data; LNN, MA, JLH, VC, DAW, VBK.

Competing interests

The authors have no competing interests.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2017.01.002.

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