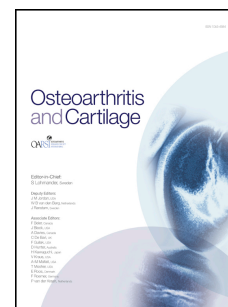


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Pain prediction by serum biomarkers of bone turnover in people with knee osteoarthritis: an observational study of TRAcP5b and cathepsin K in OA

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

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16

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19

20 **ABSTRACT (222)**21 **Objectives**

22 To investigate serum biomarkers, tartrate resistant acid phosphatase 5b (TRAcP5b) and
23 cathepsin K, indicative of osteoclastic bone resorption, and their relationship to pain and pain
24 change in knee osteoarthritis (OA).

25 **Methods**

26 Sera and clinical data were collected from 129 people (97 with 3-year follow-up) with knee
27 OA from the Prediction of Osteoarthritis Progression (POP) cohort. Knee OA-related
28 outcomes in POP included: WOMAC pain, NHANES I (pain, aching and stiffness),
29 subchondral sclerosis, and radiographically determined tibiofemoral and patellofemoral OA.
30 Two putative osteoclast biomarkers were measured in sera: TRAcP5b and cathepsin K.
31 Medial tibia plateaux were donated at knee arthroplasty for symptomatic OA (n=84) or from
32 16 post mortem controls from the Arthritis Research UK (ARUK) Pain Centre joint tissue
33 repository. Osteoclasts were stained for TRAcP within the subchondral bone of the medial
34 tibia plateaux.

35 **Results**

36 Serum TRAcP5b activity, but not cathepsin K-immunoreactivity, was associated with density
37 of TRAcP-positive osteoclasts in the subchondral bone of medial tibia plateaux. TRAcP-
38 positive osteoclasts were more abundant in people with symptomatic OA compared to
39 controls. Serum TRAcP5b activity was associated with baseline pain and pain change.

40 **Conclusions**

41 Our observations support a role for subchondral osteoclast activity in the generation of OA
42 pain. Serum TRAcP5b might be a clinically relevant biomarker of disease activity in OA.

43

44 **Key words:** TRAcP5b, Subchondral bone, Biomarker, Osteoarthritis Pain, Osteoclast

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46 **INTRODUCTION**

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

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

57 Animal models of OA and imaging studies in man support associations between pain and
58 subchondral structural pathology ⁷⁻⁹. In particular, increased osteoclast activity indicative of
59 subchondral bone turnover might be associated with OA and pain ^{7, 10}. Osteoclasts are
60 multinucleated giant cells responsible for homeostatic bone resorption that release enzymatic
61 markers, including tartrate resistant acid phosphatase (TRAcP) and cathepsin K. TRAcP,
62 originally called type 5 acid phosphatase, can be expressed both by osteoclasts and
63 macrophages ¹¹; it was identified in human serum and separated electrophoretically into two
64 distinct bands: 5a and 5b. Electrophoretic studies suggest band 5b (TRAcP5b) is derived from
65 osteoclasts and 5a from macrophages ¹². Cathepsin K, a cysteine protease, has been
66 implicated in OA pathogenesis, largely because of its upregulation in areas of cartilage
67 damage and resorbed bone ^{13, 14}. Roles of cathepsin K in the initial stages of bone resorption
68 have led to it becoming a target for novel therapeutic approaches for diseases such as
69 osteoporosis, where reduced bone resorption can increase bone mineral density and reduce

70 fracture risk ¹⁵. Circulating TRAcP5b activity and cathepsin K are reduced in clinical trials
71 during bisphosphonate treatment ^{16,17}.

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

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

84

85

86 PATIENTS AND METHODS

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

88 **Participants.** 129 participants from the Prediction of Osteoarthritis Progression (POP) cohort
89 ¹⁹ and knee tissue from 100 subjects from the Arthritis Research UK (ARUK) Pain Centre
90 joint tissue repository ²³ were available (Table 1). Included participants met the American
91 College of Rheumatology (ACR) criteria for symptomatic OA ²⁴. Samples from 129 of the
92 POP cohort were available at baseline and from 97 at 3-year follow up. Participants in the
93 POP cohort who had unilateral total knee replacement (TKR) surgery before baseline blood
94 and data collection were excluded, and those who had TKR before follow up were excluded
95 from longitudinal analyses. Cases from the joint tissue repository had knee tissue taken at
96 TKR surgery for symptomatic OA (n = 84), or post mortem (PM) (n = 16) from people who
97 had not sought help for knee pain during the last year of life (asymptomatic control group).
98 Sixteen cases from each of the TKR and PM groups were matched for macroscopic
99 chondropathy scores, age and gender. Macroscopic chondropathy was scored by a single
100 observer as previously described ²⁵, taking account of severity (graded from 0 (normal
101 unbroken surface) to 4 (subchondral bone exposure)) and extent (percentage of area involved
102 by each grade) to calculate a chondropathy score from 0 – 100. Scores for all 4 compartments
103 (medial and lateral tibial plateaux and femoral condyles) were summed to give a total
104 chondropathy score from 0-400. Participants were excluded if they had specific bone disease
105 known to affect bone turnover (e.g. Paget's disease of the bone, osteomalacia), or non-OA
106 diagnoses as a cause of knee pain (e.g. rheumatoid arthritis, acute gout), but not according to
107 medication use (Table 1). Cases with self-reported osteoporosis were also included (Table 1).
108 (Insert table I here)

109 **Imaging.** Postero-anterior weight-bearing knee radiographs were obtained as previously
110 described²⁵⁻²⁷. Radiographs of the POP cohort were scored by observers blinded to patient
111 details for Kellgren-Lawrence (K/L) grade (0-4)²⁸ and individual radiographic features of
112 OA including joint space narrowing (JSN 0-3), osteophytes (OST 0-3), subchondral sclerosis
113 (0 or 1) and patellofemoral OA (0-3) using a standardized atlas²⁹. Total scores were summed
114 scores for both knees (right + left) and compartments (tibia – medial, lateral; femur – medial,
115 lateral)¹⁹. Knee radiographs for cases providing joint tissues at TKR were scored using an
116 atlas of line drawings of medial and lateral JSN and OST³⁰. JSN (range 0–6) and OST (range
117 0–12) scores were summed to provide a total radiographic OA severity score for each knee
118 (range 0–18). Radiographs were not available for post mortem cases.

119 Scintigraphic imaging of knees and whole body was performed as previously described^{19,27}.
120 The radiotracer methylene-diphosphonate labelled with technetium-99m was administered 2
121 hours prior to imaging. Sixteen joint sites were scored semiquantitatively by 2 experienced
122 observers blinded to patient detail, on a scale of 0–3, where 0 = normal to 3 = intense. The
123 scores were summed for each joint site. Scored sites included knees, shoulders, elbows,
124 wrists, hands, hips, sacroiliac joints, ankles, forefeet, first metatarsophalangeal joints,
125 sternoclavicular joints, acromioclavicular joints, the sternomanubrial joint, the cervical spine,
126 the thoracic spine, and the lumbar spine.

127 **Pain assessment.** In the POP cohort, pain was assessed using the Likert pain scale of the
128 Western Ontario and McMaster Universities Osteoarthritis index (WOMAC-A)³¹. It consists
129 of 5 summed items (pain on walking, stair climbing, nocturnal, rest and weight bearing)
130 scored from 0 = none, 1 = mild, 2 = moderate, 3 = severe and 4 = extreme, to give a total
131 subscore ranging from 0-20. Knee symptoms were also ascertained by the National Health
132 and Nutrition Examination Survey (NHANES) I criterion³² of pain, aching or stiffness on
133 most days of any one month in the last year; for subjects answering yes, symptoms were

134 quantified as mild, moderate, or severe, yielding a total score of 0-3 for each knee. Change
135 scores were calculated separately for WOMAC pain and NHANES I pain as follow-up score
136 minus baseline score, summed across both knees, and used to define pain worsening or
137 improvement in participants over 3 years as previously published¹⁹. Pain scores were not
138 available for ARUK Pain Centre joint tissue repository cases, and sera were not available for
139 PM cases.

140 **Biomarker quantification.** TRAcP5b activity and cathepsin K concentrations were analysed
141 in serum stored at -80°C from participants in the POP cohort and from TKR patients in the
142 ARUK joint repository group. Experimenter was blinded to patient details. Both biomarkers
143 were measured in undiluted serum by enzyme-linked immunosorbent assay (ELISA)
144 according to the manufacturer's protocol. TRAcP5b activity (U/L) was measured using a
145 Bone TRAP® (TRAcP5b) ELISA (immunodiagnostic systems – IDS). Concentrations of
146 cathepsin K (pg/ml) were measured using a human cathepsin K (cath-K) ELISA
147 (CUSABIO). Inter-assay coefficient of variation (CVs) for TRAcP5b was 0.89% and
148 cathepsin K; 9.52%. Twenty-two samples were below the lower limit of detection (LLOD)
149 for TRAcP5b (0.5U/L). One sample was below the LLOD for cathepsin K (7.5pg/ml). A
150 value equal to one half the LLOD was imputed for these samples for the purposes of
151 statistical analyses.

152 **TRAcP positive osteoclast density.** Mid-coronal sections (5µm) of the middle one-third of
153 the medial tibial plateau (an important weight bearing area characteristically affected by OA)
154 were fixed in neutral buffered formalin and then decalcified in 10% EDTA in 10mM Tris
155 buffer (pH 6.95; at 4°C) prior to embedding in paraffin wax. Sections were stained for
156 TRAcP-positive osteoclasts in two sections per case from the middle one-third of the medial
157 tibial plateau. Samples were deparaffinized in xylene, rehydrated in serial alcohol and
158 distilled water, and recalcified in a solution containing 1mM CaCl₂ and 1mM MgCl₂ in PBS

159 overnight. TRAcP was stained using a commercially available kit (#387A Sigma-Aldrich,
160 UK) following the manufacturer's protocol. The numbers of TRAcP positive osteoclasts
161 within the subchondral bone area were counted manually using a Zeiss Axioscop-50
162 microscope (Carl Zeiss Ltd, Welwyn Garden City, UK) at 20x magnification to a depth of
163 400 μ m from the calcified cartilage. The scorer was blind to patient details. The number of
164 osteoclasts was divided by the length of the subchondral bone to give an osteoclast density
165 expressed as TRAcP positive cells per mm³³.

166 **Statistical analysis.** Data were analysed using Statistical package for the Social Sciences
167 v.22 (SPSS Inc., Chicago, Illinois, USA). Pilot studies were carried out prior to main study
168 for power calculations for sample size. Between group (TKR vs. PM, with vs. without
169 osteoporosis) comparisons for TRAcP-positive osteoclasts were tested using the Mann-
170 Whitney U test. Biomarker data were natural log (Ln) transformed to obtain a normal
171 distribution for use in all analyses. Shapiro-Wilks test confirmed that Ln transformed
172 biomarker data did not significantly diverge from normality. Univariable and multivariable
173 linear regressions were used for all association analyses, including between bone biomarkers
174 and TRAcP-positive osteoclast density, between bone biomarkers and OA outcomes
175 (WOMAC pain, NHANES I pain, subchondral sclerosis, patellofemoral OA, JSN,
176 osteophyte, and KL grade) or total burden of OA at the knee and other joints at baseline
177 based on scintigraphy (cross-sectional study). Univariable and multivariable linear
178 regressions were used to assess associations of baseline TRAcP5b and cathepsin K with
179 change in pain (WOMAC and NHANES I) over the 3-year follow up in the POP cohort
180 (longitudinal study). A one-factor principal component analysis (PCA) was performed for the
181 joints assessed by bone scintigraphy as previously described¹⁹. This produced a factor that
182 explained 20% of the variance in the whole body bone scintigraphy data. This factor,
183 reflecting bone formation^{34, 35} was assessed for association with the osteoclast related

184 biomarkers. All parameter estimates were adjusted for OA risk factors (age, sex, BMI) and,
185 where appropriate, for bisphosphonate use because bisphosphonates are known to inhibit
186 osteoclast activity. In addition to beta coefficients, marginal effects for pain outcomes are
187 presented where statistically significant associations were demonstrated after adjustments.
188 Numerical and graphical data are presented as mean \pm 95% confidence interval to denote
189 statistical uncertainty of estimates between groups, whereas mean \pm SD is used for
190 descriptive variables. $P < 0.05$ was considered statistically significant.

191

192

193

194 RESULTS

195 Demographics of participants

196 The 129 participants from the POP cohort with symptomatic knee OA at baseline comprised
197 72% females with an overall mean \pm SD age of 64 ± 11 years and mean \pm SD BMI of $31.4 \pm$
198 6.6 kg/m^2 (Table 1). A 3-year follow up of 97 participants from the POP cohort included 72%
199 females with an overall mean \pm SD age of 67 ± 11 years and mean \pm SD BMI of 31.6 ± 6.7
200 kg/m^2 . The mean \pm SD baseline concentrations of TRAcP5b and cathepsin K for these
201 participants were $0.8 \pm 0.4\text{U/L}$ and $170.7 \pm 110.7\text{pg/ml}$, respectively. Mean (SD) baseline
202 WOMAC and NHANES I pain scores were 5.2 ± 3.2 and 2.9 ± 1.2 , respectively. Pain
203 change, defined as follow-up minus baseline score in the POP participants, was a mean (95%
204 CI) of $0.13 (-0.85-1.1)$ and $-0.3 (-0.6-0.0008)$ for WOMAC and NHANES I pain,
205 respectively.

206 ARUK Pain Centre joint repository knee tissue and sera, were obtained at TKR from 84
207 people (57% female) who had symptomatic knee OA, with an overall mean \pm SD age of $66 \pm$
208 10 years and mean \pm SD BMI of $31.3 \pm 6.8 \text{ kg/m}^2$ (Table 1). Knee tissues were obtained at
209 PM from 16 subjects (56% female) who did not seek help for knee pain in the last year of
210 their life (mean \pm SD age 69 ± 12 years). The mean \pm SD baseline concentrations of
211 TRAcP5b and cathepsin K in the TKR subjects were $3.35 \pm 1.48\text{U/L}$ and $9.54 \pm 18.1 \text{ pg/ml}$,
212 respectively.

213 Associations between osteoclast density in OA subchondral bone, serum osteoclast 214 biomarkers, and symptomatic knee OA

215 To investigate whether the biomarkers TRAcP5b and cathepsin K are serum markers of
216 subchondral osteoclast activity, we assessed their associations with TRAcP-positive
217 osteoclast density in OA subchondral bone from patient samples (n=68) obtained at TKR for

218 knee OA. TRAcP-positive osteoclasts were identified in OA subchondral bone samples at a
219 mean (95% CI) density of 1.5 (0.95 – 2) mm⁻¹. TRAcP5b and cathepsin K were detectable in
220 the serum of the TKR group by immunoassay. Serum TRAcP5b was associated with density
221 of TRAcP-positive osteoclasts, independent of age, sex, and BMI. In contrast, serum
222 cathepsin K was not statistically significantly associated with TRAcP-positive osteoclast
223 density (Table 2). In the POP cohort, as expected neither TRAcP5b nor cathepsin K was
224 statistically significantly associated with new bone formation, as assessed by namely knee or
225 total body bone scintigraphy scores (supplementary Table 1).

226 Asymptomatic (PM) and symptomatic (TKR) chondropathy groups (n = 16), matched for
227 macroscopic chondropathy scores mean (95% CI); (200 (186 – 215) and 209 (196 – 221),
228 respectively (p = 0.38)) were assessed for TRAcP-positive osteoclasts. TRAcP-positive
229 osteoclasts in subchondral bone were significantly more abundant in people with
230 symptomatic knee OA (mean density 1.0 (0.50 – 1.5) mm⁻¹) compared to the asymptomatic
231 PM controls (0.16 (0.04 – 0.28) mm⁻¹), p = 0.001 (Figure 1 and Figure 2A & B).
232 (Insert table II here) and (Figure 1 and 2)

233 **Association of bone biomarkers with OA pain and structural severity.**

234 In a cross-sectional, baseline serum TRAcP5b in the POP cohort (n=129) was associated with
235 WOMAC pain score ($\beta = 1.24$, 95%CI 0.21 - 2.26; p = 0.02) (Table 3) and subchondral
236 sclerosis ($\beta = 0.35$, 95%CI 0.07 - 0.63; p = 0.02) (Table 4), even after adjusting for age, sex,
237 and BMI. This association persisted after adjusting for bisphosphonate use. Based on
238 marginal effect sizes, the mean baseline TRAcP5b levels would need to be 2.3-fold to 2.8-
239 fold higher to predict a 1 unit higher baseline WOMAC pain score. Baseline serum TRAcP5b
240 activity was not significantly different in participants who reported osteoporosis compared to
241 those who did not (p = 0.47). Baseline serum TRAcP5b was also associated with NHANES I

242 pain score (Table 3), and baseline serum cathepsin K in the POP cohort was associated with
243 radiographic severity of patellofemoral OA (Table 4), but statistical significance was lost
244 after adjusting for age, sex, and BMI.

245 (Insert table III and IV here)

246 **Association of baseline TRAcP5b with OA pain change**

247 To evaluate the predictive capability of TRAcP5b and cathepsin K for change in OA pain
248 (WOMAC and NHANES I), we assessed the associations of bone biomarkers at baseline with
249 change in pain scores during a 3-year follow up (n = 97). Baseline TRAcP5b was associated
250 with pain change as evaluated by the NHANES I pain questionnaire ($\beta = 0.69$, 95%CI 0.19 –
251 1.20; $p = 0.008$) after adjustment for age, sex, BMI, and baseline NHANES I pain, but not
252 with WOMAC pain ($\beta = 0.71$, 95%CI -0.90 – 2.33; $p = 0.38$) (Table 5). Associations between
253 baseline serum TRAcP5b and change in pain (NHANES I) remained statistically significant
254 after adjusting for bisphosphonate use (Table 5). Based on marginal effect sizes, the mean
255 baseline levels of TRAcP5b would need to be 5.3-fold to 11-fold higher to predict an
256 additional 1unit increase in NHANES I pain score between baseline and follow up. Baseline
257 cathepsin K was not associated with pain change (either WOMAC or NHANES I) (Table 5).

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

261 (Insert table V here)

262

263 **DISCUSSION**

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

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

286 Increased numbers of TRAcP-positive osteoclasts in subchondral bone have been reported in
287 human³³ and rodent⁴³ OA, and preclinical and imaging studies report possible involvement
288 of osteoclasts in osteoarthritic pain^{7, 10}. In the current study, we show that in samples
289 matched for chondropathy, osteoclast density was higher in people who sought treatment for
290 knee pain (TKR) compared to those who did not (PM), indicating that osteoclast densities
291 might contribute to OA symptoms independent of OA structural severity. In addition, by
292 altering joint shape and loading, osteoclast-mediated subchondral bone remodelling might
293 contribute to further cartilage damage.

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

303 TRAcP5b has two enzymatic roles after its release from osteoclasts. It acts as a phosphatase
304 at acidic pH, and also as a generator of reactive oxygen species (ROS) at neutral pH. ROS
305 may participate in the breakdown of endocytosed bone matrix products in resorbing
306 osteoclasts⁴⁵ and be involved in pain generation in OA⁴⁶. In the current study, we report for
307 the first time, statistically significant associations of serum TRAcP5b with WOMAC pain
308 scores in OA. Other studies have shown inflammatory biomarkers, C-reactive protein (CRP),
309 tumour necrosis factor (TNF)- α , interleukin (IL)-6²⁰ and interleukin (IL)-1 β ²¹ associated
310 with OA pain. Anti-cytokine treatments have been tested in clinical trials for OA pain, but

311 lack of clinically important improvements over placebo might indicate that these molecules
312 mediate OA pain only alongside other factors, or in subgroups of patients^{47,48}.

313 High concentrations of serum TRAcP5b have been detected in diseases characterized by
314 increased osteoclastic activity such as Paget's disease, haemodialysis, primary
315 hyperparathyroidism⁴⁹ and malignancies involving bone resorption, for example breast
316 cancer with bone metastases³⁹. In the current study, patients with other bone diseases were
317 excluded and parameter estimates adjusting for bisphosphonates did not alter statistically
318 significant associations observed between serum TRAcP5b, structural pathology, pain, and
319 pain change in OA. Histological examination of the subchondral bone did not reveal
320 malignant infiltration in any case in our current study, but we do not disregard the possibility
321 of systemic effects of malignancy. Furthermore, concentrations of serum TRAcP5b were not
322 different in participants with or without osteoporosis suggesting that relationships of
323 TRAcP5b activity to symptomatic knee OA were independent of the presence of
324 osteoporosis. Serum TRAcP5b concentrations were reported to be decreased following
325 administration of the bisphosphonate alendronate in postmenopausal women with
326 osteoporosis¹⁶. From studies that show analgesic effects of bisphosphonates, and with
327 findings from the current study, we suggest that bisphosphonates might reduce pain in OA by
328 reducing osteoclast activity.

329 OA has traditionally been viewed as a disorder of the tibiofemoral joint (TFJ), but the
330 patellofemoral joint (PFJ) is one of the most commonly affected compartments in OA and
331 also an important source of pain in OA⁵⁰. The association observed between serum cathepsin
332 K and patellofemoral but not tibiofemoral OA suggests that different biomarkers might
333 reflect OA disease activity in different joint compartments of the knee. Patellofemoral OA
334 with cartilage loss of the patella and trochlea groove is reported in about half of patients
335 diagnosed with knee OA⁵¹.

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

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

357 Our findings identify serum TRAcP5b as a marker of subchondral osteoclast activity and
358 suggest its potential utility as a biomarker for OA pain and pain change. TRAcP5b deserves
359 further investigation as a biomarker of bone remodelling to aid in identifying people for

360 whom osteoclast activity contributes to OA pain, and who might be particularly responsive to
361 analgesic and disease modification potential of anti-resorptive agents.

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371 **AUTHOR CONTRIBUTIONS**

372 All authors were involved in drafting the article or revising it critically for important
373 intellectual content, and all authors approved the final version to be published. Drs. Walsh
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375 integrity of the data and the accuracy of the data analysis.

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

377 Acquisition of data; LNN, MA, LW

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

379 **Competing interests**

380 The authors have no competing interests.

381

382 **References**

- 383 1. Thomas, E., G. Peat, L. Harris, R. Wilkie, and P.R. Croft, The prevalence of pain and
384 pain interference in a general population of older adults: cross-sectional findings from
385 the North Staffordshire Osteoarthritis Project (NorStOP). *Pain* 2004; 110(1):361-368.
- 386 2. Creamer, P., Osteoarthritis pain and its treatment. *Current Opinion in Rheumatology*
387 2000; 12(5):450-455.
- 388 3. Felson, D.T., C.E. Chaisson, C.L. Hill, S.M. Totterman, M.E. Gale, K.M. Skinner, et
389 al., The association of bone marrow lesions with pain in knee osteoarthritis. *Ann*
390 *Intern Med* 2001; 134(7):541-9.
- 391 4. Tanamas, S.K., A.E. Wluka, J.-P. Pelletier, J.M. Pelletier, F. Abram, P.A. Berry, et
392 al., Bone marrow lesions in people with knee osteoarthritis predict progression of
393 disease and joint replacement: a longitudinal study. *Rheumatology* 2010.
- 394 5. Ogino, S., T. Sasho, K. Nakagawa, M. Suzuki, S. Yamaguchi, M. Higashi, et al.,
395 Detection of pain-related molecules in the subchondral bone of osteoarthritic knees.
396 *Clinical Rheumatology* 2009; 28(12):1395-1402.
- 397 6. Salaffi, F., A. Ciapetti, and M. Carotti, The sources of pain in osteoarthritis: a
398 pathophysiological review. *Reumatismo* 2014; 66(1):57-71.
- 399 7. Strassle, B.W., L. Mark, L. Leventhal, M.J. Piesla, X. Jian Li, J.D. Kennedy, et al.,
400 Inhibition of osteoclasts prevents cartilage loss and pain in a rat model of
401 degenerative joint disease. *Osteoarthritis and Cartilage* 2010; 18(10):1319-1328.
- 402 8. Hunter, D.J., A. Guermazi, F. Roemer, Y. Zhang, and T. Neogi, Structural correlates
403 of pain in joints with osteoarthritis. *Osteoarthritis and Cartilage* 2013; 21(9):1170-
404 1178.
- 405 9. Barr, A.J., T.M. Campbell, D. Hopkinson, S.R. Kingsbury, M.A. Bowes, and P.G.
406 Conaghan, A systematic review of the relationship between subchondral bone

- 407 features, pain and structural pathology in peripheral joint osteoarthritis. *Arthritis*
408 *Research & Therapy* 2015; 17(1):1-36.
- 409 10. Sagar, D.R., S. Ashraf, L. Xu, J.J. Burston, M.R. Menhinick, C.L. Poulter, et al.,
410 Osteoprotegerin reduces the development of pain behaviour and joint pathology in a
411 model of osteoarthritis. *Annals of the Rheumatic Diseases* 2014; 73(8):1558-1565.
- 412 11. Yaziji, H., A.J. Janckila, S.C. Lear, A.W. Martin, and L.T. Yam,
413 Immunohistochemical detection of tartrate-resistant acid phosphatase in non-
414 hematopoietic human tissues. *Am J Clin Pathol* 1995; 104(4):397-402.
- 415 12. Halleen, J.M., Tartrate-resistant acid phosphatase 5B is a specific and sensitive
416 marker of bone resorption. *Anticancer Res* 2003; 23(2A):1027-9.
- 417 13. Morko, J.P., M. Söderström, A.-M.K. Säämänen, H.J. Salminen, and E.I. Vuorio, Up
418 regulation of cathepsin K expression in articular chondrocytes in a transgenic mouse
419 model for osteoarthritis. *Annals of the Rheumatic Diseases* 2004; 63(6):649-655.
- 420 14. Morko, J., R. Kiviranta, M.T.K. Mulari, K.K. Ivaska, H.K. Väänänen, E. Vuorio, et
421 al., Overexpression of cathepsin K accelerates the resorption cycle and osteoblast
422 differentiation in vitro. *Bone* 2009; 44(4):717-728.
- 423 15. Bone, H.G., D.W. Dempster, J.A. Eisman, S.L. Greenspan, M.R. McClung, T.
424 Nakamura, et al., Odanacatib for the treatment of postmenopausal osteoporosis:
425 development history and design and participant characteristics of LOFT, the Long-
426 Term Odanacatib Fracture Trial. *Osteoporosis International* 2015; 26(2):699-712.
- 427 16. Nenonen, A., S. Cheng, K.K. Ivaska, S.L. Alatalo, T. Lehtimäki, H. Schmidt-Gayk, et
428 al., Serum TRACP 5b is a useful marker for monitoring alendronate treatment:
429 comparison with other markers of bone turnover. *J Bone Miner Res* 2005;
430 20(10):1804-12.

- 431 17. Muñoz-Torres, M., R. Reyes-García, P. Mezquita-Raya, D. Fernández-García, G.
432 Alonso, J.d. Dios Luna, et al., Serum cathepsin K as a marker of bone metabolism in
433 postmenopausal women treated with alendronate. *Maturitas* 2009; 64(3):188-192.
- 434 18. Valdes, A.M., I. Meulenbelt, E. Chassaing, N.K. Arden, S. Bierma-Zeinstra, D. Hart,
435 et al., Large scale meta-analysis of urinary C-terminal telopeptide, serum cartilage
436 oligomeric protein and matrix metalloprotease degraded type II collagen and their role
437 in prevalence, incidence and progression of osteoarthritis. *Osteoarthritis Cartilage*
438 2014; 22(5):683-9.
- 439 19. Huebner, J.L., A.C. Bay-Jensen, K.M. Huffman, Y. He, D.J. Leeming, G.E.
440 McDaniel, et al., ALPHA-CTX is associated with subchondral bone turnover and
441 predicts progression of joint space narrowing and osteophytes in osteoarthritis.
442 *Arthritis & Rheumatology* 2014:n/a-n/a.
- 443 20. Stannus, O.P., G. Jones, L. Blizzard, F.M. Cicuttini, and C. Ding, Associations
444 between serum levels of inflammatory markers and change in knee pain over 5 years
445 in older adults: a prospective cohort study. *Ann Rheum Dis* 2013; 72(4):535-40.
- 446 21. Attur, M., I. Belitskaya-Lévy, C. Oh, S. Krasnokutsky, J. Greenberg, J. Samuels, et
447 al., Increased interleukin-1 β gene expression in peripheral blood leukocytes is
448 associated with increased pain and predicts risk for progression of symptomatic knee
449 osteoarthritis. *Arthritis & Rheumatism* 2011; 63(7):1908-1917.
- 450 22. Ishijima, M., T. Watari, K. Naito, H. Kaneko, I. Futami, K. Yoshimura-Ishida, et al.,
451 Relationships between biomarkers of cartilage, bone, synovial metabolism and knee
452 pain provide insights into the origins of pain in early knee osteoarthritis. *Arthritis*
453 *Research & Therapy* 2011; 13(1):R22-R22.

- 454 23. Stoppiello, L.A., P.I. Mapp, D. Wilson, R. Hill, B.E. Scammell, and D.A. Walsh,
455 Structural associations of symptomatic knee osteoarthritis. *Arthritis & Rheumatology*
456 2014; 66(11):3018-3027.
- 457 24. Altman, R., E. Asch, D. Bloch, G. Bole, D. Borenstein, K. Brandt, et al.,
458 Development of criteria for the classification and reporting of osteoarthritis.
459 Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria
460 Committee of the American Rheumatism Association. *Arthritis Rheum* 1986;
461 29(8):1039-49.
- 462 25. Walsh, D.A., A. Yousef, D.F. McWilliams, R. Hill, E. Hargin, and D. Wilson,
463 Evaluation of a Photographic Chondropathy Score (PCS) for pathological samples in
464 a study of inflammation in tibiofemoral osteoarthritis. *Osteoarthritis and Cartilage*
465 2008; 17(3):304-312.
- 466 26. McDaniel, G., J.B. Renner, R. Sloane, and V.B. Kraus, Association of knee and ankle
467 osteoarthritis with physical performance. *Osteoarthritis and Cartilage* 2011;
468 19(6):634-638.
- 469 27. Kraus, V.B., G. McDaniel, T.W. Worrell, S. Feng, T.P. Vail, G. Varju, et al.,
470 Association of bone scintigraphic abnormalities with knee malalignment and pain.
471 *Ann Rheum Dis* 2009; 68(11):1673-9.
- 472 28. Kellgren, J.H. and J.S. Lawrence, Radiological Assessment of Osteo-Arthrosis.
473 *Annals of the Rheumatic Diseases* 1957; 16(4):494-502.
- 474 29. Altman, R.D. and G.E. Gold, Atlas of individual radiographic features in
475 osteoarthritis, revised. *Osteoarthritis Cartilage* 2007; 15 Suppl A:A1-56.
- 476 30. Nagaosa, Y., M. Mateus, B. Hassan, P. Lanyon, and M. Doherty, Development of a
477 logically devised line drawing atlas for grading of knee osteoarthritis. *Annals of the*
478 *Rheumatic Diseases* 2000; 59(8):587-595.

- 479 31. Bellamy, N., W.W. Buchanan, C.H. Goldsmith, J. Campbell, and L.W. Stitt,
480 Validation study of WOMAC: a health status instrument for measuring clinically
481 important patient relevant outcomes to antirheumatic drug therapy in patients with
482 osteoarthritis of the hip or knee. *J Rheumatol* 1988; 15(12):1833-40.
- 483 32. Davis, M.A., W.H. Ettinger, and J.M. Neuhaus, Obesity and osteoarthritis of the knee:
484 evidence from the National Health and Nutrition Examination Survey (NHANES I).
485 *Semin Arthritis Rheum* 1990; 20(3 Suppl 1):34-41.
- 486 33. Prieto-Potin, I., R. Largo, J. Roman-Blas, G. Herrero-Beaumont, and D. Walsh,
487 Characterization of multinucleated giant cells in synovium and subchondral bone in
488 knee osteoarthritis and rheumatoid arthritis. *BMC Musculoskeletal Disorders* 2015;
489 16(1):226.
- 490 34. Malmud, L.S. and N.D. Charkes, Bone scanning: principles, technique and
491 interpretation. *Clin Orthop Relat Res* 1975(107):112-22.
- 492 35. Roelofs, A.J., C.A. Stewart, S. Sun, K.M. Blazewska, B.A. Kashemirov, C.E.
493 McKenna, et al., Influence of bone affinity on the skeletal distribution of fluorescently
494 labeled bisphosphonates in vivo. *J Bone Miner Res* 2012; 27(4):835-47.
- 495 36. Dieppe, P., J. Cushnaghan, P. Young, and J. Kirwan, Prediction of the progression of
496 joint space narrowing in osteoarthritis of the knee by bone scintigraphy. *Ann Rheum*
497 *Dis* 1993; 52(8):557-63.
- 498 37. McCrae, F., J. Shouls, P. Dieppe, and I. Watt, Scintigraphic assessment of
499 osteoarthritis of the knee joint. *Ann Rheum Dis* 1992; 51(8):938-42.
- 500 38. Costa, A.G., N.E. Cusano, B.C. Silva, S. Cremers, and J.P. Bilezikian, Cathepsin K:
501 its skeletal actions and role as a therapeutic target in osteoporosis. *Nat Rev Rheumatol*
502 2011; 7(8):447-456.

- 503 39. Sarvari, B.K., D. Sankara Mahadev, S. Rupa, and S.A. Mastan, Detection of Bone
504 Metastases in Breast Cancer (BC) Patients by Serum Tartrate-Resistant Acid
505 Phosphatase 5b (TRACP 5b), a Bone Resorption Marker and Serum Alkaline
506 Phosphatase (ALP), a Bone Formation Marker, in Lieu of Whole Body Skeletal
507 Scintigraphy with Technetium^{99m} MDP. *Indian J Clin Biochem* 2015; 30(1):66-71.
- 508 40. Laslett, L.L., D.A. Dore, S.J. Quinn, P. Boon, E. Ryan, T.M. Winzenberg, et al.,
509 Zoledronic acid reduces knee pain and bone marrow lesions over 1 year: a
510 randomised controlled trial. *Ann Rheum Dis* 2012; 71(8):1322-8.
- 511 41. Varena, M., F. Zucchi, S. Failoni, A. Becciolini, and M. Berruto, Intravenous
512 neridronate in the treatment of acute painful knee osteoarthritis: a randomized
513 controlled study. *Rheumatology* 2015; 54(10):1826-1832.
- 514 42. Bay-Jensen, A.C., D. Reker, C.F. Kjelgaard-Petersen, A. Mobasheri, M.A. Karsdal,
515 C. Ladel, et al., Osteoarthritis year in review 2015: soluble biomarkers and the BIPED
516 criteria. *Osteoarthritis and Cartilage* 2016; 24(1):9-20.
- 517 43. Botter, S.M., G.J. van Osch, S. Clockaerts, J.H. Waarsing, H. Weinans, and J.P. van
518 Leeuwen, Osteoarthritis induction leads to early and temporal subchondral plate
519 porosity in the tibial plateau of mice: an in vivo microfocal computed tomography
520 study. *Arthritis Rheum* 2011; 63(9):2690-9.
- 521 44. Zhang, Y., J. Li, J. Zhu, G. Zhou, W.J. Zhang, Y. Cao, et al., Enhanced cartilage
522 formation by inhibiting cathepsin K expression in chondrocytes expanded in vitro.
523 *Biomaterials* 2012; 33(30):7394-404.
- 524 45. Vääräniemi, J., J.M. Halleen, K. Kaarlonen, H. Ylipahkala, S.L. Alatalo, G.
525 Andersson, et al., Intracellular Machinery for Matrix Degradation in Bone-Resorbing
526 Osteoclasts. *Journal of Bone and Mineral Research* 2004; 19(9):1432-1440.

- 527 46. Moon, S.J., Y.J. Woo, J.H. Jeong, M.K. Park, H.J. Oh, J.S. Park, et al., Rebamipide
528 attenuates pain severity and cartilage degeneration in a rat model of osteoarthritis by
529 downregulating oxidative damage and catabolic activity in chondrocytes.
530 *Osteoarthritis Cartilage* 2012; 20(11):1426-38.
- 531 47. Cohen, S., S. Proudman, A. Kivitz, F. Burch, J. Donohue, D. Burstein, et al., A
532 randomized, double-blind study of AMG 108 (a fully human monoclonal antibody to
533 IL-1R1) in patients with osteoarthritis of the knee. *Arthritis Research & Therapy*
534 2011; 13(4):1-12.
- 535 48. Auw Yang, K.G., N.J. Raijmakers, E.R. van Arkel, J.J. Caron, P.C. Rijk, W.J.
536 Willems, et al., Autologous interleukin-1 receptor antagonist improves function and
537 symptoms in osteoarthritis when compared to placebo in a prospective randomized
538 controlled trial. *Osteoarthritis Cartilage* 2008; 16(4):498-505.
- 539 49. Scarnecchia, L., S. Minisola, M.T. Pacitti, V. Carnevale, E. Romagnoli, R. Rosso, et
540 al., Clinical usefulness of serum tartrate-resistant acid phosphatase activity
541 determination to evaluate bone turnover. *Scand J Clin Lab Invest* 1991; 51(6):517-24.
- 542 50. Kornaat, P.R., J.L. Bloem, R.Y. Ceulemans, N. Riyazi, F.R. Rosendaal, R.G.
543 Nelissen, et al., Osteoarthritis of the knee: association between clinical features and
544 MR imaging findings. *Radiology* 2006; 239(3):811-7.
- 545 51. Kim, Y.-M. and Y.-B. Joo, Patellofemoral Osteoarthritis. *Knee Surgery & Related*
546 *Research* 2012; 24(4):193-200.
- 547 52. Hudelmaier, M., C. Glaser, J. Hohe, K.H. Englmeier, M. Reiser, R. Putz, et al., Age-
548 related changes in the morphology and deformational behavior of knee joint cartilage.
549 *Arthritis Rheum* 2001; 44(11):2556-61.

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553 Figure 1

554 TRAcP positive osteoclasts were statistically significantly higher in people with symptomatic
555 OA (TKR) compared to PM controls who also presented with chondropathy but did not seek
556 help for knee pain. Data indicate mean \pm SEM for n = 16 per group. Differences between
557 groups were analysed using a Mann Whitney-U test. TKR – total knee replacement, PM –
558 post mortem.

559 Figure 2

560 TRAcP positive osteoclasts in the subchondral bone of OA patients at TKR.

561 TRAcP positive osteoclasts stained in sections from the medial tibial plateau show severely
562 eroded cartilage (red arrow - A). TRAcP staining showed active multinucleated osteoclasts
563 (purple) within bone marrow spaces (B) and in areas of fibrovascular replacement (A).
564 TRAcP positive osteoclasts on the edge of the bone signify sites of bone resorption and a
565 resorption cavity (asterisk) as evidence of bone remodelling. CC – calcified cartilage, FT –
566 fibrovascular tissue. Scale bars = 100 μ m.

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569 Table I

570 Demographics of patient study groups

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

571 ^a Matched TKR cases (n=16) were a subgroup of the total TKR cases used. TKR; total knee
 572 replacement, PM; post mortem.

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576 Table II

577 Relationship of serum biomarkers to TRAcP positive osteoclast density

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

578 [†]Adjusted for baseline age, sex, BMI.

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581 Table III

582 Relationship of serum biomarkers of osteoclast activity to OA pain

	TRAcP5b		Cathepsin K	
	β (95% CI)	P	β (95% CI)	P
WOMAC pain	1.64 (0.58 to 2.71)	0.003	-0.05 (-0.87 to 0.78)	0.91
WOMAC pain [†]	1.24 (0.21 to 2.26)	0.02	-0.21 (-0.99 to 0.57)	0.60
WOMAC pain ^{†¶}	1.28 (0.24 to 2.32)	0.02	-0.21 (-0.99 to 0.57)	0.60
NHANES I pain	0.45 (0.06 to 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

583 [†]Adjusted for age, sex, BMI and [¶] for bisphosphonates. WOMAC pain marginal effect sizes
584 (fold increase in TRAcP5b associated with 1 unit higher WOMAC pain score); 2.3, [†]2.8 and
585 [¶]2.7.

586

587 Table IV

588 Relationship of serum biomarkers of osteoclast activity to structural OA features

	TRAcP5b		Cathepsin K	
	β (95% CI)	P	β (95% CI)	P
Subchondral sclerosis	0.32 (0.04 to 0.59)	0.03	-0.01 (-0.22 to 0.20)	0.92
Subchondral sclerosis [†]	0.35 (0.07 to 0.63)	0.02	-0.01 (-0.23 to 0.20)	0.91
Subchondral sclerosis ^{†¶}	0.35 (0.07 to 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 to 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

589 [†]Adjusted for age, sex, BMI and [¶] for bisphosphonates.

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593 Table V

594 Relationship of baseline serum biomarkers to change in OA pain

	TRAcP5b		Cathepsin 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 to 1.20)	0.008	0.02 (-0.36 to 0.41)	0.91
NHANES I pain change†‡§	0.67 (0.16 to 1.18)	0.01	0.07 (-0.33 to 0.47)	0.73

595 †Adjusted for baseline age, sex, BMI, and ‡ for baseline pain score (e.g. change in WOMAC
596 pain adjusted for baseline WOMAC pain), and § for bisphosphonates. Change scores are
597 follow up scores minus baseline scores. Mean \pm SD baseline WOMAC and NHANES I pain
598 = 5.2 ± 3.2 and 2.9 ± 1.2 respectively. Mean \pm SD follow-up WOMAC and NHANES I pain
599 = 5.4 ± 4 and 2.6 ± 1.5 respectively. NHANES I pain marginal effect sizes (fold increase in
600 TRAcP5b associated with 1 unit greater NHANES I pain score increase between baseline and
601 follow up); 11.0, †‡5.3, †‡§5.6

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