Associations of Symptomatic Knee Osteoarthritis With Histopathologic Features in Subchondral Bone

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Objective. Subchondral bone and the osteochondral junction are thought to contribute to osteoarthritis (OA) knee pain. We undertook this study to identify osteochondral pathologies specifically associated with symptomatic human knee OA.

Methods. Medial tibial plateau samples from 2 groups of subjects (n = 31 per group) were matched for macroscopic chondropathy scores. The symptomatic chondropathy group had undergone total knee replacement for OA knee pain, at which time specimens of the medial tibial plateau were obtained. The asymptomatic chondropathy group included subjects who died of unrelated illness (specimens were obtained at postmortem examination) and who had not previously sought help for knee pain. OA histopathology, immunoreactivity for nerve growth factor (NGF) and CD68 (macrophages), tartrate-resistant acid phosphatase–positive subchondral osteoclasts, and synovitis were compared between groups.

Results. Mankin scores, subchondral bone density, and subchondral CD68-immunoreactive macrophage infiltration were similar between the 2 groups. NGF-like immunoreactivity was found in subchondral mononuclear cells and osteoclasts, as well as in chondrocytes. NGF in osteochondral channels and osteoclast densities in subchondral bone were higher in the symptomatic chondropathy group than in the asymptomatic chondropathy group (P < 0.01 and P = 0.02, respectively), as were synovitis scores (P < 0.01). Osteochondral pathology was not significantly associated with synovitis score. The differences in NGF expression and in osteoclast density remained significant after adjustment for age and synovitis score (P = 0.01 and P = 0.04, respectively). Osteochondral NGF and osteoclast densities, together with synovitis scores, explained ~28% of sample allocation to symptomatic or asymptomatic groups.

Conclusion. Subchondral pathology was associated with symptomatic knee OA, independent of chondropathy and synovitis. Increased NGF expression in osteochondral channels and increased osteoclast density appear to be key features associated with bone pain in knee OA.

INTRODUCTION

Pain is a major source of disability and the reason for hospital visits in patients with knee osteoarthritis (OA). Structural changes including articular cartilage degradation, synovial inflammation, osteophytes, and subchondral osteosclerosis are characteristic of OA, but are not always accompanied by severe pain. Evidence suggests that subchondral bone contributes to knee OA pain (1–7). Subchondral bone marrow lesions (BMLs) detected with magnetic resonance imaging (MRI) in knee OA are strongly associated with pain (1–4,7). Bone attrition, a flattening or depression of the subchondral bone visualized using radiography or MRI, is also associated with the presence of pain (5,6). Microarray analysis of BMLs in OA demonstrated up-regulation of genes implicated in neurogenesis, osteochondral turnover, and inflammation that might contribute to OA pain (8). In animals, OA caused up-regulation of nociceptive markers (calcitonin gene-related peptide and tropomyosin receptor kinase A [TrkA]) in subchondral bone afferents (9).
However, the mechanisms by which subchondral pathology contributes to OA pain are incompletely understood. Synovitis has also been associated with OA pain (1,10–13). Synovial and subchondral pathology can occur together within the same joint, but it is unknown whether these represent discrete painful pathologies that could be separate targets for therapeutic intervention.

Nerve growth factor (NGF) plays a key role in the generation of acute and chronic pain, especially in inflammation (14,15). NGF can bind 2 receptors: the high-affinity TrkA (16) and the low-affinity p75 neurotrophin receptor (17). NGF blockade can be achieved using antibodies or TrkA–IgG fusion proteins that bind NGF and prevent its interaction with TrkA and p75 receptors. Recent clinical trials showed that NGF blockade substantially reduced OA knee pain (18,19). In human OA, NGF is up-regulated in synovium (10) and subchondral bone (20), and increased synovial NGF expression was associated with symptomatic knee OA (10), although the relevance of subchondral NGF expression has not been clarified. Increased density of tartrate-resistant acid phosphatase (TRAP)–positive osteoclasts in subchondral bone has also been shown to be associated with OA and knee symptoms (21,22). Inflammatory CD68–positive macrophages were also detected in subchondral bone marrow compartments in human OA (23).

We hypothesized that structural, cellular, and molecular changes in subchondral bone are associated with symptomatic knee OA. We compared 2 chondropathy groups that had similar macroscopic chondropathy but differing symptom severities. Patients in the symptomatic chondropathy group had previously seen a clinician for knee pain and undergone total knee replacement (TKR) surgery. Subjects in the asymptomatic chondropathy group had not sought care for knee pain and died of unrelated illness. We hypothesized that NGF expression by cells within subchondral bone was associated with symptomatic OA.

PATIENTS AND METHODS

Samples. The study included 31 consecutive samples from symptomatic chondropathy patients who had donated tibial plateau at TKR surgery for OA and 31 samples from asymptomatic chondropathy subjects obtained at autopsy. All symptomatic chondropathy patients undergoing TKR had reported severe knee pain. No asymptomatic chondropathy subjects had sought medical attention for knee pain during the last year, and thus this group was highly likely to have experienced less pain than the symptomatic chondropathy patients. Asymptomatic chondropathy samples were selected from 782 consecutive postmortem donors by matching them to each symptomatic chondropathy sample using both the macroscopic chondropathy score and the percentage of joint surface with grade 4 chondropathy lesion (subchondral bone exposure), with a maximum difference of ±5 for each measure between the matched cases.

Informed consent was obtained from TKR patients and the next of kin consented for postmortem subjects. Protocols were approved by Nottingham 1 Research Ethics Committee (05/Q2403/24) and Derby Research Ethics Committee 1 (11/H0405/2). Symptomatic chondropathy samples were from patients fulfilling the American College of Rheumatology classification criteria for OA (24) at the time of TKR.

Macroscopic chondropathy score and osteophytes. Following tissue harvesting, articular surfaces of the medial tibial plateau were evaluated by a single assessor (RH) for the extent and severity of loss of surface integrity (25). Articular surface defects were graded using the following scale: 0 (normal, smooth unbroken surface), 1 (swelling and softening), 2 (superficial fibrillation), 3 (deep fibrillation), or 4 (subchondral bone exposure). The proportion of articular surface area corresponding to each grade was used to calculate a score using the following formula (25): macroscopic chondropathy score (0–100) = (grade 1 × 0.14) + (grade 2 × 0.34) + (grade 3 × 0.65) + grade 4. Osteophytes were recorded as present or absent on direct visualization of postmortem samples.

Radiographic OA severity score. Radiographic OA severity scores were derived using preoperative posteroanterior knee radiographs as previously described (25). An atlas of line drawings of the knee joint was used to grade medial and lateral joint space narrowing and osteophytes (26). The scores for tibiofemoral joint space narrowing (range 0–6) and osteophytes (range 0–12) were summed to provide a total radiographic OA severity score (range 0–18) (25).

Sample processing. Midcoronal sections of the middle 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, 4°C), prior to wax embedding. Synovial tissues were fixed in formalin and wax-embedded without decalcification.

Histology and grading. Tibial plateau sections (5 μm) were stained with hematoxylin and eosin or Safranin O–fast green. OA articular cartilage changes were graded using the Mankin scale (27): cartilage surface integrity (0–6, where 0 = normal and 6 = complete disorganization), tidemark integrity (0–1, where 0 = intact and 1 = crossed by vessels), chondrocyte morphology (0–3, where 0 = normal and 3 = hypocellular), and proteoglycan loss (0–4, where 0 = normal, no loss of Safranin-O stain and 4 = complete loss of stain). Subchondral bone marrow replacement by fibrovascular tissue was assessed as either present or absent. Subchondral osteosclerosis was histologically assessed using trabecular bone volume/total volume (BV/TV) and subchondral plate area (μm²/μm), which were quantified using computer-assisted image analysis (Zeiss). Osteochondral channel densities were assessed for subchondral bone, calcified cartilage, and noncalcified cartilage, separately in each region. Channels passing through one region
into another were counted as being in the region occupied by the larger part of the channel. Synovial inflammation was assessed using a synovitis histologic score (0–3, where 0 = no synovitis and 3 = severe synovitis) developed by Haywood et al (28).

**Immunohistochemistry.** Sections underwent antigen retrieval (10 mM citrate buffer, 90°C, 20 minutes) and were blocked with 5% bovine serum albumin containing goat serum, followed by incubation with rabbit monoclonal antibody to NGF (EP1320Y; Abcam), and biotinylated goat anti-rabbit IgG secondary antibody (BA1000; Vector). CD68 immunoreactivity was visualized after citrate buffer antigen retrieval (1 mg/ml pepsin in 0.5M acetic acid, 37°C, 2 hours) and incubation with mouse monoclonal anti-human CD68 (MA5-13324; ThermoFisher) and biotinylated horse anti-mouse IgG secondary antibody (BA2001; Vector). NGF and CD68 immunoreactivities were visualized using avidin–biotin–peroxidase complex (Vector) with nickel-enhanced diaminobenzidine development (29). Sections were counterstained with hematoxylin so that different regions were more apparent.

NGF expression was measured as the proportion of osteochondral channels in each sample that displayed NGF-immunoreactive cells. Subchondral tissues within 400 μm of the cement line in the osteochondral junction were classified as bone marrow or fibrovascular tissues. NGF-like immunoreactivity was graded in each subchondral tissue type as 0 (none), 1 (focal/sparse distribution), or 2 (high density); it was graded in chondrocytes as 0 (<5% of cells), 1 (5–20% of cells), or 2 (>20% of cells) (20). CD68-immunoreactive macrophages were graded in subchondral tissues as 0 (none), 1 (focal/sparse distribution), or 2 (high density) (20).

**TRAP staining.** Differentiated osteoclasts were identified by TRAP staining, using a commercially available kit (#386A; Sigma-Aldrich) according to the manufacturer’s protocol. TRAP-positive osteoclasts were counted within 400 μm of the cement line in the osteochondral junction and divided by the length of the subchondral bone to give an osteoclast density expressed as TRAP-positive/mm (22). One dark-purplish or reddish cell with ≥3 nuclei was recorded as 1 osteoclast.

### Table 1. Patient and sample characteristics*

|                         | Symptomatic chondropathy (n = 31 knees) | Asymptomatic chondropathy (n = 31 knees) | Postmortem repository (n = 782 knees) |
|-------------------------|----------------------------------------|-----------------------------------------|--------------------------------------|
| Macroscopic chondropathy score, 0–100 | 74 (56–80) | 76 (56–81)† | 33 (24–51) |
| % of joint surface area with grade 4 chondropathy | 30 (0–48)‡ | 30 (0–50)† | 0 (0–0) |
| Sex, % male | 51.6 | 61.3 | 54.5 |
| Age, years | 67 (55–73)‡ | 74 (66–84)§ | 69 (60–80) |
| Total radiographic OA severity score, 0–18¶ | 13 (10.5–13.5) | – | – |
| Tibiofemoral JSN score, 0–6# | 5 (5–5.8) | – | – |
| MT JSN score, 0–3 | 3 (3) | – | – |
| Osteophyte score, 0–12** | 8 (5.5–8) | – | – |
| MT osteophyte score, 0–3 | 2 (2–2) | – | – |
| Samples with osteophytes, no. (%)†† | – | 16 (53.3)† | 113 (15.3) |
| MFC osteophytes | – | 18 (62.1)† | 111 (15.0) |
| LFC osteophytes | – | 15 (50.0)† | 87 (11.7) |
| MT osteophytes | – | 13 (43.3)† | 82 (11.1) |
| LT osteophytes | – | 10 (50.0)† | 41 (11.4) |

* Except where indicated otherwise, values are the median (interquartile range). OA = osteoarthritis; MT = medial tibial plateau; MFC = medial femoral condyle; LFC = lateral femoral condyle; LT = lateral tibial plateau.
† P < 0.01 versus the postmortem repository.
‡ P < 0.01 versus asymptomatic chondropathy.
§ P = 0.03 versus the postmortem repository.
¶ Summation of tibiofemoral joint space narrowing (JSN) and osteophyte scores.
# Summation of medial and lateral tibiofemoral JSN scores.
** Summation of medial and lateral tibial and femoral osteophyte scores.
†† Not all samples could be assessed for osteophyte presence; percentages are based on the number of assessments available.
Image analysis. All histologic scoring and quantification was performed using a Zeiss Axioskop 50 microscope, by a single observer (KA) who was blinded with regard to details of the diagnostic group.

Statistical analysis. Analyses were performed with JMP 10 software (SAS Institute). The significance of differences between groups was assessed by Mann-Whitney U test or chi-square test. Logistic regression was performed to adjust for age and synovitis scores and to calculate McFadden’s pseudo $R^2$. The $R^2$ for each linear regression model was recorded for each of the individual histologic measures (NGF alone, osteoclasts alone, or synovitis score alone) and also for the linear regression model where all measures were included together (NGF, osteoclasts, and synovitis). Associations were assessed by Spearman’s rank correlation. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated. $P$ values less than 0.05 were considered significant.

RESULTS

Patient details. Subject demographics and sample details of samples selected for this study and of source repository cases are shown in Table 1. Per study protocol, the selected asymptomatic chondropathy group had similar macroscopic chondropathy scores and proportion of joint surface area displaying grade 4 chondropathy to the symptomatic group. However, the samples from the asymptomatic chondropathy group showed more severe OA changes than were observed overall in the samples from the postmortem repository from which they were selected. The asymptomatic chondropathy group had a higher median age than the symptomatic chondropathy group. There were no cases in which medications for osteoporosis were used in either group.

Histologic characteristics. Histologic characteristics of the study groups are shown in Figure 1 and Table 2. Osteochondral channels containing inflammatory cells and blood vessels were observed in subchondral bone plate, calcified cartilage, and noncalcified cartilage (Figures 1A and B). Mankin score, proportion of cases with fibrovascular marrow replacement, histologic BV/TV, subchondral plate area, and osteochondral channel densities were similar between the symptomatic and asymptomatic chondropathy groups. How-

**Figure 1.** Histopathologic features in subchondral bone. A–E, Nerve growth factor (NGF) immunoreactivity. Sample from a subject in the symptomatic chondropathy group exhibits an NGF-positive osteochondral channel (A) (arrowheads), while a sample from a subject in the asymptomatic chondropathy group exhibits an NGF-negative osteochondral channel (B) (arrowhead). NGF-immunoreactive cells (brown) were found in osteochondral channels (A), fibrovascular tissue (C), and bone marrow (D). Multinucleated osteoclasts were immunoreactive for NGF (E). F and G, Analysis of macrophage infiltration. CD68-immunoreactive macrophages were mainly observed in bone marrow (F) and fibrovascular tissue (G). H, Tartrate-resistant acid phosphatase staining showed multinucleated osteoclasts (purple). Bars = 50 μm.
ever, synovitis scores were higher in the symptomatic group than in the asymptomatic group, and this difference remained significant after adjustment for age (adjusted OR \(\text{OR adj} \) 2.75 [95% CI 1.35–6.20]; \(P = 0.01\)).

In samples of medial tibial plateau, NGF immunoreactivity was detected in chondrocytes, subchondral mononuclear cells, and multinucleate osteoclast-like cells adherent to bone (Figure 1). NGF-immunoreactive cells were found in osteochondral channels and in subchondral fibrovascular tissue and bone marrow. CD68-immunoreactive macrophages were observed mainly in subchondral bone marrow and fibrovascular tissues. A higher proportion of osteochondral channels contained NGF-immunoreactive cells in the symptomatic group than in the asymptomatic chondropathy group (Figure 2). This difference remained significant after adjustment for age and synovitis histologic score (OR \(\text{adj} \) 1.05 [95% CI 1.01–1.10]; \(P = 0.04\)). The percentage of NGF-positive osteochondral channels was significantly correlated with the number of TRAP-positive osteoclasts (\(r_s = 0.34, P = 0.01\)).

The association between NGF expression in osteochondral channels and symptomatic chondropathy remained significant after adjustment for osteoclast density (OR \(\text{adj} \) 1.05 [95% CI 1.01–1.09]; \(P < 0.01\)), but the significant association between osteoclast density and symptomatic chondropathy did not persist after adjustment for NGF expression in osteochondral channels (OR \(\text{adj} \) 1.10 [95% CI 0.96–1.32]; \(P = 0.20\)). Synovitis scores were not significantly associated with either NGF-immunoreactive osteochondral channels \((r = 0.07, P = 0.62)\) or subchondral TRAP-positive osteoclasts \((r = 0.11, P = 0.44)\).

McFadden’s pseudo \(R^2\) values for the symptomatic chondropathy group versus the asymptomatic chondropathy group were as follows: synovitis score 0.17, NGF expression in osteochondral channels 0.13, and subchondral osteoclast

### Table 2. Osteochondral histology and synovitis scores*

|                          | Symptomatic chondropathy \(n = 31\) knees | Asymptomatic chondropathy \(n = 31\) knees |
|--------------------------|----------------------------------------|------------------------------------------|
| Total Mankin score, 0–14†| 9 (7–11)                               | 8 (7–11)                                 |
| Cartilage surface integrity, 0–6| 4 (3–6)                           | 4 (3–6)                                 |
| Chondrocyte appearance, 0–3| 2 (2–3)                               | 2 (2–2)                                 |
| Tidemark integrity, 0–1| 1 (0–1)                                | 0 (0–1)                                 |
| Proteoglycan loss, 0–4| 2 (2–3)                                | 2 (2–3)                                 |
| Subchondral bone marrow replacement, no. (%)| 11 (35)                          | 14 (45)                                 |
| Histologic BW/TV‡     | 50.0 (42.0–61.3)                        | 57.3 (39.0–63.0)                        |
| Subchondral plate area, \(\mu m^2/\mu m^2\) | 608.3 (460.0–810.6) | 651.5 (431.7–1050.0) |
| Total osteochondral channel density, \(mm^3\)| 5.4 (3.7–6.4)            | 4.9 (3.5–7.4)                           |
| Subchondral bone, \(mm\)| 4.8 (3.3–6.1)                        | 4.7 (3.4–7.2)                           |
| Calcified cartilage, \(mm\)| 0.24 (0.09–0.57) | 0.25 (0.0–0.46)                         |
| Noncalcified cartilage, \(mm\)| 0 (0–0)                        | 0 (0–0)                                 |
| Synovitis histologic score, 0–3| 3 (2.75–3)                         | 1 (1–2.5)                               |

* Except where indicated otherwise, values are the median (interquartile range).
† Summation of cartilage surface integrity, chondrocyte appearance, tidemark integrity, and proteoglycan loss.
‡ Trabecular bone volume per total volume (BW/TV).
§ Summation of osteochondral channel densities in subchondral bone, calcified cartilage, and noncalcified cartilage.
¶ \(P < 0.01\) versus asymptomatic chondropathy.
density 0.05. The pseudo R² value for the combination of all 3 histopathologic features was 0.28.

DISCUSSION

In this study, we demonstrated that components of subchondral pathology are associated with symptomatic chondropathy in people undergoing knee arthroplasty for painful OA. We showed that NGF expression in osteochondral channels and subchondral TRAP-positive osteoclast density are individually associated with symptomatic chondropathy. We confirmed previous findings (10) that suggested symptomatic OA is associated with synovitis and showed that associations with subchondral pathology are not dependent on the severity of chondropathy or synovitis. OA can affect all tissues in the joint, and our data support the notion that different joint tissue compartments make discrete contributions to pain in OA.

We found that the proportion of osteochondral channels positive for NGF immunoreactivity was a sensitive measure to distinguish between symptomatic and asymptomatic groups, supporting the hypothesis that osteochondral NGF plays a role in the generation of OA pain. This association appears to be beyond any effect of synovitis or cartilage damage on joint pain. The number of osteochondral channels penetrating noncalcified cartilage is increased in OA (20), but our findings suggest that this alone may not be sufficient to explain OA pain. We showed that NGF immunoreactivity in osteochondral channels was correlated with tidemark integrity, suggesting that expression of sensitizing factors such as NGF mediates the effects of osteochondral channels on OA pain.

NGF may directly activate sensory neurons that express TrkA and modulate the expression of TrkA or p75 receptor (30). Anti-NGF antibodies can reduce OA pain (18,19), indicating the importance of NGF in pain generation, although their anatomic site of action remains uncertain. NGF has been localized to human synovium, where it was associated with OA pain (10). OA chondrocytes may also express NGF (10), although we were unable to demonstrate association of chondrocyte-derived NGF with symptomatic chondropathy. Increased NGF-immunoreactive cells in osteochondral channels could contribute to OA pain by increasing colocalized sensory nerve activity. NGF-immunoreactive cells were colocalized with sensory nerve fibers within osteochondral channels in human subchondral bone (20). Indeed, most sensory neurons innervating the subchondral bone in rat knee joints were found to be TrkA-immunoreactive (31), and TrkA expression in subchondral bone afferents was further increased during monoiodoacetate-induced OA in rats (9).

Our findings showed that osteoclast density in subchondral bone was associated with symptomatic knee OA, and the differences between the 2 groups remained significant after adjustment for age and synovitis histologic score. Osteoclasts might increase pain either by directly changing the subchondral biochemical milieu or by altering subchondral bone structure. Osteoclasts release protons that generate a local acidosis, potent activators of nociceptors that can increase pain signaling (32). Our findings also indicate that osteoclasts are a source of NGF, which could then sensitize primary afferents in the subchondral bone.

Classification of cases as symptomatic or asymptomatic was significantly predicted by NGF immunoreactivity but not by subchondral trabecular bone density. Our current results therefore extend findings from a previous study (22), which demonstrated the potential role of increased osteoclast density in subchondral bone in the generation of OA pain. High serum concentrations of TRAP-5b, an indicator of osteoclast number, were shown to be associated with subchondral osteoclast density, OA pain, and worse pain prognosis (22). We now show that the association of osteoclast density with symptomatic OA is not explained by associations with chondropathy, synovitis, or age, suggesting a direct effect of osteoclasts on OA pain. Increased subchondral osteoclast number was also associated with pain behavior in rats, and
reducing the number of osteoclasts led to decreases in weight-bearing pain (33,34).

Studies of osteoclast inhibitors such as bisphosphonates, denosumab, and strontium ranelate show reductions in joint pain in people with knee OA (35,36). Zoledronic acid, a bisphosphonate, reduced knee pain and BML size in OA patients (36), although findings from a meta-analysis of randomized controlled trials did not support analgesic effects of bisphosphonates in knee OA (37). Our data suggest that OA knee pain has multiple sources, and targeting osteoclasts will have clinically important benefits only in cases in which osteoclast activity is the predominant driver of pain.

We observed associations between NGF and osteoclast densities in subchondral bone. Multinucleated osteoclasts were immunoreactive for NGF, and NGF expression in osteochondral channels was significantly correlated with the number of TRAP-positive osteoclasts detected. NGF expression in osteochondral channels was associated with symptomatic knee OA after adjustment for osteoclast density, but the association between osteoclast density and symptoms did not persist after adjustment for NGF. Our data support the notion that NGF is a more important factor than osteoclast density in subchondral bone with regard to the generation of OA pain. Furthermore, NGF can act as an autocrine or paracrine factor regulating osteoclast activity and bone remodeling. NGF and TrkA are expressed by osteoclasts, and the addition of NGF to monocyte cultures induces the formation of TRAP-positive multinucleated cells (38). An anti-NGF antibody reduced subchondral osteoclast numbers in a rat model of OA pain (39).

Ours is the first study to evaluate associations between symptomatic OA and pathologic changes in discrete tissue compartments of the human knee. Patients with more severe chondropathy have been shown to be more likely to display synovitis and subchondral bone changes (40). However, in the current study, subchondral changes were not significantly associated with synovitis grade, and each compartment might contribute discretely to OA pain. Our findings support a heterogeneous model of OA pain, resulting from multiple mechanisms in different peripheral tissues. The balance between pain mechanisms varies by person. Latent class analysis indicated that synovitis was a defining characteristic for one subgroup of patients with OA (10). Our findings here suggest that subchondral pathology can define a subgroup of patients with symptomatic chondropathy, only partially overlapping with those whose OA pain is driven by synovitis. MRI evidence of cartilage defects (41), bone marrow lesions (7), and synovitis (12) can also discretely predict OA pain. We extended these findings to identify NGF-immunoreactive osteochondral channels and subchondral osteoclast densities as key pathologic features that make discrete contributions to OA symptoms.

Our results showed that 28% of allocation to symptomatic and asymptomatic chondropathy groups can be explained by the combination of synovitis score, NGF expression in osteochondral channels, and subchondral osteoclast density. Synovitis score and NGF expression in osteochondral channels, separately, contributed to group allocation at similar rates (17% and 13%, respectively), and both may be important targets for future OA treatments.

This study has several potential limitations. Some subjects in our asymptomatic chondropathy group could have experienced knee pain, but relatives may have been unaware of these symptoms. However, all patients undergoing TKR reported severe knee pain, and it is highly likely that people who did not undergo surgery had less pain overall than those who did. The symptomatic and asymptomatic chondropathy groups differed in age, although significant associations with subchondral pathology and synovitis persisted after adjusting our analyses for age. Samples were obtained from the midcoronal section of the medial tibial plateau, a key weight-bearing area, but findings might differ for other joint regions such as femoral condyles. Symptomatic chondropathy patients had late-stage OA and were undergoing arthroplasty, and different pain mechanisms might be important in cases with less severe structural change.

Osteoclast activity itself was not examined in this study, but cells with ≥3 nuclei were counted as 1 osteoclast to estimate active osteoclasts, as resorption activity has been shown under some circumstances to correlate with the number of nuclei present (42). However, osteoclast numbers do not necessarily correlate with osteoclast activity (i.e., bisphosphonate treatment) (43). More direct measures, such as biomarkers of collagen breakdown, might further clarify whether associations between symptoms and osteoclast number reflect mediation by osteoclast activity.

Our models did not explain all of the variance in classification to symptomatic and asymptomatic groups. Some variation might be attributable to case ascertainment (e.g., subjects in the asymptomatic group might have experienced some knee pain). Factors not explored here, such as other histopathologic changes, cytokines/molecules, psychological factors, biomechanical loading, and obesity, are also likely to contribute to OA pain. BMLs are associated with knee OA pain, and they have also been found to be associated with cartilage surface integrity and subchondral bone marrow replacement by fibrovascular tissue (8), both of which were similar in the symptomatic and asymptomatic chondropathy groups in our study. However, MRI scans were not available for subjects in our study, and further investigation is needed to clarify the association of BMLs with NGF expression in osteochondral channels and with TRAP-positive osteoclast densities. Case-matching asymptomatic chondropathy samples (from a total postmortem group of 782 samples) to symptomatic chondropathy samples allowed us to identify histopathologic factors that contribute to OA symptoms, but further research is needed to determine their importance relative to contributions from chondropathy itself.

In conclusion, we have identified histopathologic features of subchondral bone that are associated with symptomatic chondropathy. NGF expression in osteochondral channels was associated with symptomatic knee OA independently.
of any effects of chondropathy, synovitis, or subchondral TRAP-positive osteoclast densities. Increased NGF expression appears to be a key feature associated with subchondral bone pain in knee OA and could contribute to the previously observed association between osteoclasts and OA pain. Our findings support a heterogeneous model of OA pain, with discrete contributions from different compartments in the joint. Different treatment regimens could improve pain due to synovitis or subchondral pathology, necessitating the development of biomarkers to help target therapies to those who will most benefit. Other treatments targeting molecular pathways that are shared between tissue compartments will have a greater potential for efficacy in unselected OA populations.

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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. Dr. Aso had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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