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Bone marrow lesions in knee osteoarthritis: regional differences in tibial subchondral bone microstructure and their association with cartilage degeneration

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SUMMARY

Objective: The aim of this study was to investigate how bone microstructure within bone marrow lesions (BMLs) relates to the bone and cartilage across the whole human tibial plateau.
Design: Thirty-two tibial plateaus from patients with osteoarthritis (OA) at total knee arthroplasty and eleven age-matched non-OA controls, were scanned ex vivo by MRI to identify BMLs and by micro CT to quantitate the subchondral (plate and trabecular) bone microstructure. For cartilage evaluation, specimens were processed histologically.
Results: BMLs were detected in 75% of the OA samples (OA-BML), located predominantly in the anterior-medial (AM) region. In contrast to non-OA control and OA-no BML, in OA-BML differences in microstructure were significantly more evident between subregions. In OA-BML, the AM region contained the most prominent structural alterations. Between-group comparisons showed that the AM region of the OA-BML group had significantly higher histological degeneration (OARSI grade) ($P < .0001$, $P < .05$), thicker subchondral plate ($P < .05$, $P < .05$), trabeculae that are more anisotropic ($P < .0001$, $P < .05$), well connected ($P < .05$, $P =$ n.s), and more plate-like ($P < 0.05$, $P < 0.05$), compared to controls and OA-no BML at this site. Compared to controls, OA-no BML had significantly higher OARSI grade ($P < .0001$), and lower trabecular number ($P < .05$).
Conclusion: In established knee OA, both the extent of cartilage damage and microstructural degeneration of the subchondral bone were dependent on the presence of a BML. In OA-no BML, bone microstructural alterations are consistent with a bone attrition phase of the disease. Thus, the use of BMLs as MRI image-based biomarkers appear to inform on the degenerative state within the osteochondral unit.
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Introduction

Knee osteoarthritis (KOA) is a painful and degenerative musculoskeletal condition, characterized by loss of osteochondral integrity. Specifically, destruction of cartilage (loss of cellular integrity and cartilage volume) and pathophysiological changes in the underlying subchondral bone, such as subchondral bone sclerosis, osteophytes, bone marrow lesions (BMLs) and bone cysts, are characteristics of advanced KOA. Although cartilage destruction has received the majority of research attention, there is evidence that changes in the subchondral bone microarchitecture may precede cartilage loss, and are thus important to understanding the pathogenesis and progression of OA[1–6]. Animal models of OA have shown a predictable disease progression in OA, in which initial loss of subchondral bone is followed by sclerotic changes, increased anisotropy and an increase in the plate:rod ratio, prior to cartilage

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degeneration[7]. In human patients, the sequence of KOA subchondral bone changes is less well understood. Several human studies have investigated the microstructure of the proximal tibia in knee OA and found pronounced variations of microstructural parameters in the medial compartment, compared to the lateral, anterior to posterior subregion and also variations dependent on the distance from the articular surface[8–10]. In addition, several knee OA studies, using micro computed tomography (micro CT) to characterize the structure of subchondral bone within the zone of a BML, found strong differences compared to the subchondral bone in the absence of a BML[11–14]. Subchondral bone in BML zones was characterized by a focal sclerotic appearance; thick subchondral plate, increased bone volume percentage, and trabeculae, which were thicker and less separated and more plate-like[11–14]. It has also been reported that BMLs are areas of subchondral bone with altered bone mineralization and active remodelling[12], and that the presence of a BML associates with increased local bone mineral density (BMD)[15,16]. Two clinical studies have indicated that the use of bone remodelling altering therapies (zoledronic acid and strontium ranelate) reduce BML size, with consequent reduction in pain and reduced cartilage volume loss[17,18]. In both studies, better results were recorded in individuals with less structural degeneration, suggesting that early treatment might be more effective. Hence, BMLs have acquired considerable clinical interest, since they appear to inform on clinically important changes in the subchondral bone, and thus might be useful as imaging biomarkers for both disease progression and response to treatment of KOA.

Previously published data observed only small volumes of the subchondral bone corresponding to BMLs[11–14,16], and the relationship between the presence of BMLs and the bone microstructure of the whole tibial plateau has not been described. We hypothesized that the presence of a BML in KOA will affect the structure across the tibial plateau. Thus, the aim of this study was to comprehensively investigate and compare the subchondral bone microarchitecture of the whole tibial plateau in KOA subjects, with and without a tibial BML, and in tibial plateaus without OA, in order to determine the impact of the presence of a BML on subchondral bone structure. We also determined the association between subchondral bone microstructure and loss of cartilage integrity and volume.

Methods

Magnetic resonance imaging (MRI)

Tibial plateaus and clinical data were obtained from 32 patients, 20 females and 12 males aged 49–79 years, undergoing knee arthroplasty surgery. Each tibial plateau was MR imaged ex vivo in an 8-channel wrist coil (3T MRI Siemens TRIO, Royal Adelaide Hospital, Adelaide), using two clinically relevant sequences; fat suppressed fast spin-echo proton density-weighted (PDFS) and T1 weighted. A BML as presented in Fig. 1, was defined as a zone of altered signal intensity seen on PDFS and T1 weighted sequences in the bone and marrow, located immediately beneath the articular cartilage and visible on at least two consecutive slices[14,19]. The definition and location of BML were by mutual agreement between two investigators (AW and YW) with musculoskeletal MRI expertise. In this study, preoperative MRI was not available. However, previously our group[14] and others[13,20] performed validation studies and confirmed that ex vivo MRI information matches pre-operative imaging findings. Furthermore, to prevent the possibility of false-positive signal change due to surgical trauma, we excluded regions adjacent to the resection surfaces.

Cartilage volume was assessed independently by two researchers (DM and YL) blinded from BML status of the tissue.

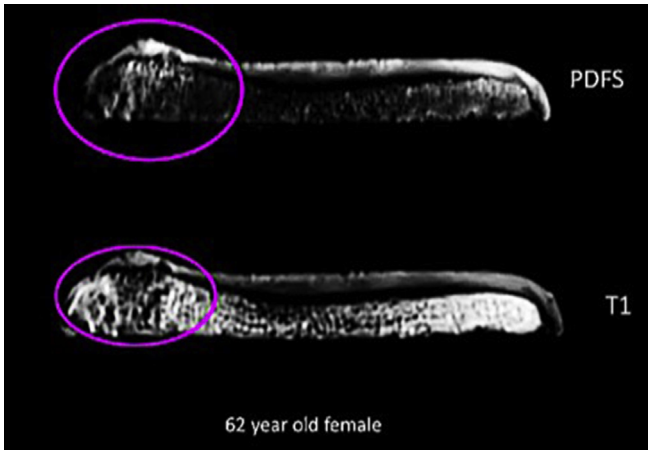

Fig. 1. MRI of the tibial plateau (sagittal view) of a 62-year-old female KOA patient taken ex-vivo (post knee replacement surgery). In PDFS-weighted sequences, BMLs (within pink oval shape) are visualised as an ill-defined area of hyper-intense signal. In T1-weighted sequences, BMLs appear as a hypo-intense signal.

Using the software program OsiriX (Pixmeo-SARL, Switzerland), the external contour around the cartilage boundaries of the medial and lateral compartment were manually marked on the T1-weighted sagittal images and then the automatic volume rendering function was used to calculate the volume in cm^3. The coefficient of variation for the measurement of cartilage volume in the medial and lateral compartments was 2.2%. In addition, using the same software, the external contour of the BML was marked in both sagittal and coronal planes and the automatic volume rendering function was used to calculate the BML volume in cm^3. The coefficient of variation for the measurement of BML volume was 2.4%.

Micro CT

To analyse the microstructure of the subchondral bone, whole tibial plateau samples were scanned by micro CT scanner (Skyscan 1076, Skyscan-Bruker, Kontich, Belgium). After imaging, histopathological assessment of the cartilage degenerative state was performed for AM, PM, AL and PL subregions. The full description of inclusion/exclusion specimen criteria, micro CT method, and histological assessment is available as supplementary material-method.

Statistical analysis

To investigate whether bone and cartilage parameters differ significantly across regions in each group and between groups for each region, linear mixed-effects models were performed, adjusting for clustering on patient and controlling for age, sex and BMI. Assumptions of a linear regression were found to be upheld in each model by inspection of scatter plots and histograms of residuals and predicted values. To investigate whether cartilage volume, OARSI histological scores and BML volume (only in AM region) are associated with bone parameters, linear Generalised Estimating Equation (GEE) models were performed, adjusting for clustering on patient. The outcomes ware cartilage volume and OARSI in each regression with the interaction of region and bone parameter being examined, and a separate model being performed for each group. The critical value for statistical significance was chosen as $P < 0.05$. These analyses were performed using the SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Demographic characteristics of the cohorts

Demographic characteristics and clinical data of the patients are presented in Table I. BMLs or subchondral cysts were not detected in the control group. In the OA group, 24 OA tibial plateaus contained a single BML (OA-BML group) and 8 were without a BML (OA-no BML group). 18 out of 24 (75%) BMLs were detected in the medial compartment and 6 (25%) were detected in the lateral compartment. Since the AM subregion contained the majority of BML detected in the medial compartment [15 out of 18 (83%)] the OA-BML group comprised only samples containing BML in AM and samples with BML detected in other regions were excluded from further analysis.

Intra-group variability of the cartilage volume, cartilage histology and subchondral bone microstructure

Cartilage volume and histological grading

There was no statistically significant interaction between groups and ROIs for the outcome cartilage volume, adjusting for clustering on patient (interaction P value = 0.78). In the control and OA-no BML groups, there was no significant difference in mean cartilage volume measured over the whole medial (M) vs the lateral (L) compartment. In the OA-BML group, the lateral compartment had a 0.33 units greater mean cartilage volume compared to the medial compartment (estimate = 0.33, 95% CI: 0.07, 0.58), although this comparison needs to be treated with caution due to the non-significant interaction.

For outcome OARSI grade a significant statistical interaction was found (interaction P value < .0001). OARSI grade was not different between subregions in the control group, while in both OA-no BML and OA-BML groups differences were found. In the OA-no BML group the AM subregion had 1.6 units greater OARSI grade compared to the PM (estimate = 1.64, 95% CI:0.88, 2.41) and AL (estimate = 1.65, 95% CI: 0.88, 2.43) subregion, and 1.8 units greater compared to the PL (estimate = 1.82, 95% CI: 1.00, 2.63). In OA-BML group, AM region had 2.8 units greater mean OARSI grade compared to the AL subregion (estimate = 2.85, 95% CI: 2.19, 3.50), 2.97 units greater then PL (estimate = 2.97, 95% CI: 2.34, 3.61), and 1.62 units greater than PM (estimate = 1.62, 95% CI: 1.00, 2.23).

Subchondral bone characteristics

A statistically significant interaction was found between groups and ROIs, adjusting for clustering on patient, for the following outcomes: PL.Th, Tb.Pf and DA in controls and OA-no BML, Tb.Sp in control and OA-BML groups, and SMI (all groups). In controls, no significant intragroup variability was detected in mean plate thickness and plate porosity between compartments or subregions. In contrast, in subchondral bone trabeculae, mean SMI and Tb.Sp in control group differed significantly between the ROIs. In OA-no BML, AM had 0.41 units greater Pl.Th compared to AL (estimate = 0.41, 95% CI: 0.23, 0.60), 0.26 units greater then PL (estimate = 0.26, 95% CI:0.07, 0.45), and 0.23 units greater then PM (estimate = 0.23, 95% CI:0.04, 0.42). PL had 0.35 units lower mean DA compared to both AM and PM (both have estimate = −0.35, 95% CI: −0.65, −0.05). The mean SMI value in AM subregion was 0.60 units lower mean SMI compared to AL (estimate = −0.60, 95% CI: −1.01, −0.20) and 0.51 units lower compared to PL (estimate = −0.51, 95% CI: −0.91, −0.11). In OA-BML, prominent structural differences were noted in the AM subregion compared to all other regions, with the largest differences compared to the AL. The AM had 0.68 units higher mean Pl.Th (estimate = 0.68, 95% CI: 0.55, 0.82), 0.93 units higher Tb.Sp (estimate = 0.93, 95% CI:0.58, 1.27), 1.36 units lower mean SMI (estimate = −1.36, 95% CI:−1.66, −1.07) and 12.11 units lower mean Tb.Pf (estimate = −12.11, 95% CI:−14.50, −9.71) compared to the AL region. In this group we also found that BV/TV and DA was higher in AM compared to other regions, but this comparison needs to be treated with caution due to the non-significant interaction. These results show the effect of OA and BMLs on cartilage volume, cartilage histology and subchondral bone microstructure and are presented in Fig. 2 and as Supplementary Table I.

A statistically significant interaction was found between groups and ROIs, adjusting for clustering on patient and controlling for Sex, Age and BMI, for the following outcomes: OARSI, Pl.Th Tb.Pf, SMI and DA for all regions. In the medial compartment, as expected, lower mean cartilage volume was detected in OA-BML compared to the control group. In subchondral bone, the medial compartment of the OA-no BML group had 0.32 units lower mean Pl.Th compared to controls (estimate = −0.32, 95% CI:−0.54, −0.10) and 0.56 compared to OA-BML (estimate = −0.56, 95% CI:−0.74, −0.39), while, OA-BML had 0.24 units greater Pl.Th compared to controls (estimate = 0.24, 95% CI: 0.06, 0.42). In addition, OA-BML had a significantly lower SMI and higher Tb.Sp (both P values <0.05) compared to the medial compartment of controls.

AM and PM subregions in both the OA-no BML and OA-BML groups had significantly greater degeneration of cartilage (higher OARSI grade) compared to controls (P < .0001, <.0001, <.05 and < .0001, respectively). In addition, AM and PM subregions of OA-BML had 1.6 units greater mean OARSI grade (estimate = 1.63, 95% CI: −0.8, 2.4) compared to OA-no BML. In subchondral bone, the AM and PM subregions of the OA-BML group had greater Pl.Th compared to controls and OA-no BML (P < .0001, <.05, respectively). The AM subregions of OA-BML group compared to controls

Table I
Demographic characteristics of the study groups

	Control (n = 11)	OA-no BML (n = 8)	OA-BML (n = 15)	P value
Age (years)	70.8 ± 16.3	69.5 ± 4.0	66 ± 15	[a]0.9, [b]0.9, [c]0.9
Male n (%)	7 (63.6%)	3 (37.5%)	6 (40%)	[a]0.5, [b]0.5, [c]0.9
Female n (%)	4 (36.4%)	5 (62.5%)	9 (60%)	[a]0.5, [b]0.5, [c]0.9
BMI	24.9 ± 2.7	33.1 ± 5.7	35.2.1 ± 6.1	**[a]<.005,[b]<.0001,** [c]0.2
K&L grade	NA	2 (1–4)	3 (2–4)	0.7
Medial OA n (%)	NA	2 (25%)	12 (80%)	**<.005**
Lateral OA n (%)	NA	2 (25%)	1 (6.6%)	0.2
Patellofemoral OA n (%)	NA	2 (25%)	1 (6.6%)	0.2
Unknown OA n (%)	NA	2 (25%)	1 (6.6%)	0.2
Varus	NA	2 (25%)	12 (86.6%)	**<.005**
Valgus	NA	1 (12.5%)	1 (6.6)	0.6
Neutral	NA	5 (62.5%)	1 (6.6%)	**<.005**

[*]BML seen only in anterior medial subregion of tibial plateau**, BMI**: body mass index, **K&L grade:** Kellgren and Lawrence grade.[a]Control vs OA-no BML, [b]Control vs OA-BML, [c]OA-no BML vs OA-BML; The critical value for statistical significance was chosen as P < 0.05.

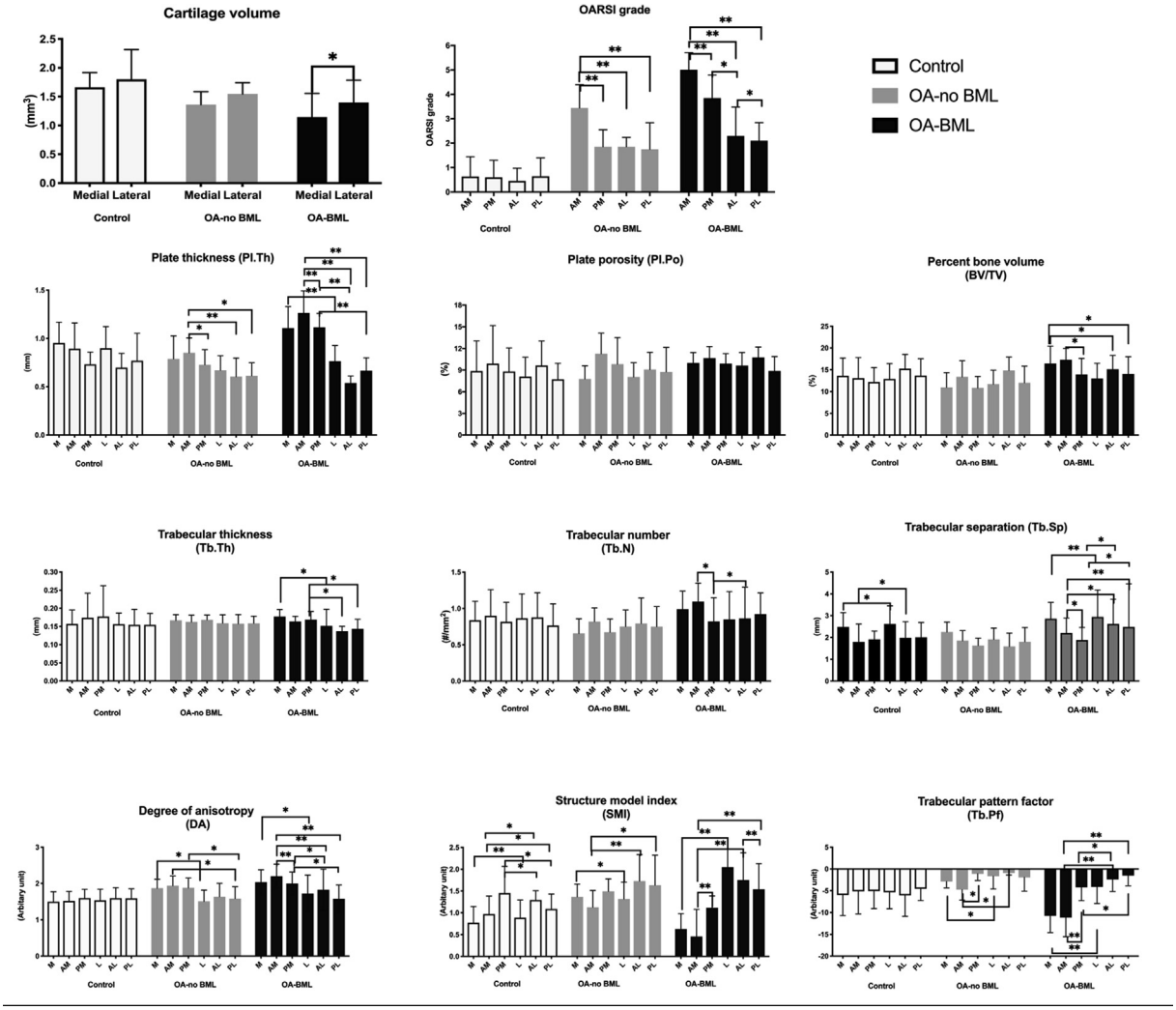

Fig. 2. Intragroup variability. Linear mixed-effects model results of bone and cartilage parameters. Significant difference, as mean ± standard deviation, Comparison *P* value $^{*}P < 0.05$, $^{**}P < 0.0001$. Cartilage volume, BV/TV, Tb.Th and Tb.N is with non-significant interaction.

had trabeculae that were more plate-like (lower SMI), (estimate = −0.56, 95% CI:−0.97, −0.14), more anisotropic (higher DA) (estimate = 0.75, 95% CI: 0.46, 1.05) and well connected (lower Tb.Pf) (estimate = −6.02, 95% CI:−9.24, −2.79). When the AM subregion of OA-BML group were compared to OA-no BML, higher DA (estimate = 0.36, 95% CI:0.07, 0.66) and lower SMI (estimate = −0.68, 95% CI:−1.08, −0.29) and Tb.Pf (estimate = −7.44, 95% CI:−10.65, −4.22) were found. The PM subregion of OA-no BML had a significantly higher SMI (estimate = 0.49, 95% CI:0.02, 0.96) and higher Tb.Pf (estimate = 5.29, 95% CI:1.45, 9.14) compared to controls. Higher SMI (estimate = 0.35, 95%CI: −0.01, 0.72) and Tb.Pf (estimate = 4.20, 95% CI:1.11, 7.29) was found in OA-no BML compared to OA-BML. The statistical significance of these data is presented in Fig. 3 and in Supplementary Table II. Data for the lateral, anterior lateral and posterior lateral compartments are reported as Supplementary Tables III, IV and V.

Association between subchondral bone microstructural parameters, cartilage volume and OARSI histological grade

In the control group, there was a statistically significant interaction between plate thickness and medial compartment for the outcome cartilage volume (interaction *P* value = 0.02). For every mm increase in Pl.Th, there was a mean cartilage volume decrease of 0.8 mm (estimate = −0.80, 95% CI:−1.11, −0.49). In the OA-no BML group, a statistically significant interaction was found between bone parameters BV/TV, Tb.N, TbS and Tb.Pf and the medial compartment for the outcome cartilage volume ($P < .05$). For every increase of one unit in BV/TV, Tb.N and Tb.S, the mean cartilage volume decreased by 0.05, 0.73 and 0.39, respectively, ($P < .05$, <.05, <.0001, respectively). In the OA-BML group, statistically significant interaction was not found. These results are presented in Table II.

For the AM subregion of the control group, statistically significant interactions were found between ROI and bone parameters; BV/TV, Tb.N, Tb.Sp, (interaction $P < .0001$), DA and Pl.Th (interaction $P < .05$) for the outcome OARSI grade. The OARSI grade increased for every unit of increase in trabecular number and DA, and the OARSI grade decreased for every unit of increase in trabecular separation and plate thickness. For the PM subregion in the control group, statistically significant interactions were found between ROI and bone parameters; SMI and Tb.Sp (interaction $P < .05$ and <0.0001 respectively). For every unit increase in SMI, OARSI grade increased by 0.85 units and for every unit increase in Tb.Sp, OARSI grade decreased by 1.25 units. For the AM subregion in

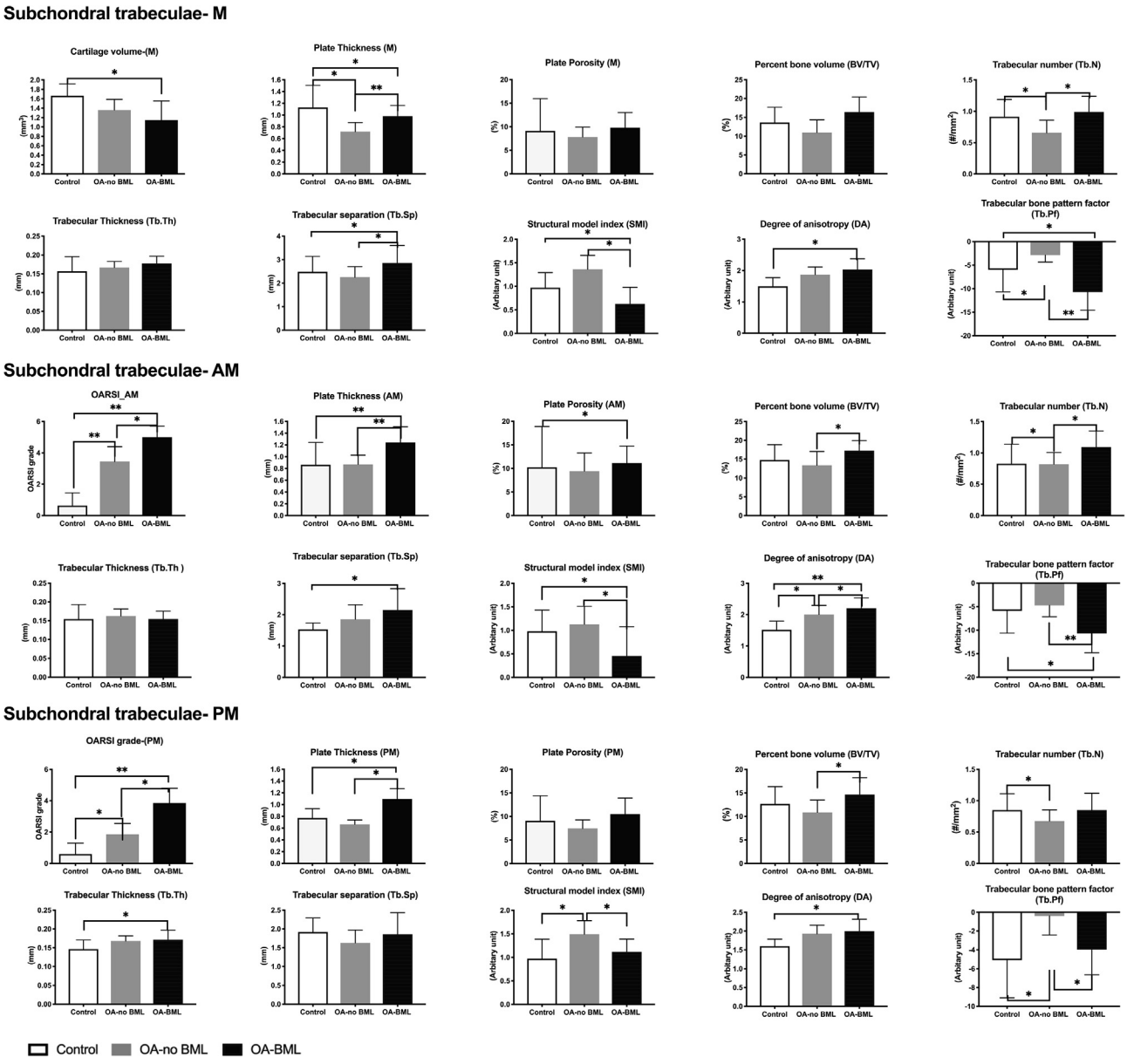

Fig. 3. Differences between groups. Linear mixed-effects model results of bone and cartilage parameter in medial compartment, anterior medial and posterior medial subregions. Significant difference, as mean ± standard deviation, Comparison *P* value $^{*}P < 0.05$, $^{**}P < 0.0001$.

the OA-no BML group, statistically significant interactions were noted between ROI and Tb.Sp for the outcome OARSI grade (interaction $P < .0001$). For every unit increase in Tb.Sp, the OARSI grade decreased by 2.1 units (comparison $P < .0001$). Also, in the OA-no BML group, we found significant interactions between ROI and bone parameters BV/TV ($P < .0001$), SMI ($P < .05$), Tb.N ($P < .05$), DA ($P < .05$), plate thickness ($P < .05$), but there were no significant post-hoc comparisons for AM and PM ROIs.

For the AM subregion in OA-BML group, statistically significant interactions were found between ROI and bone parameters; SMI, DA and Pl.Th for the outcome OARSI grade ($P < .05$ for all). For every unit increase in SMI, the OARSI grade decreased by 0.48 units (estimate = −0.48, 95% CI: −0.82, −0.14), $P < .05$). There was no significant post-hoc comparison for DA and Pl.Th. These results are presented in Table III.

Discussion

To our knowledge, this is the first comprehensive study of the subchondral bone microstructure (subchondral plate and trabeculae) across the whole OA tibial plateau in association with presence or absence of BMLs. In this study, there were a number of major findings relating to the subchondral bone microarchitecture of the tibial plateau in KOA. Firstly, and in contrast to non-OA (control) subjects and in OA subjects with no BML, in OA subjects with tibial BMLs, differences in microstructure were significantly more evident between subregions. Secondly, in KOA subjects, microstructural differences in the subchondral plate and trabeculae were dependent on the presence or absence of a BML in the tibial plateau, which also related to the extent of cartilage degradation. These observations suggest that BMLs identified by MRI clinical

Table II
Linear generalized estimating equation model results of medial compartment cartilage volume vs interaction of bone parameters in groups, adjusting for clustering on patient

Predictor	Comparison	Group	Estimate (95% CI)	Comparison *P* value	Interaction *P* value
Pl.Th	**Pl.Th, ROI = M**	**Controls**	**−0.80 (−1.11, −0.49)**	**<.0001**	**0.0239**
	Pl.Th, ROI = M	OA-no BML	1.70 (0.54, 2.86)	0.0040	0.1148
	Pl.Th, ROI = M	OA-BML	−0.42 (−0.92, 0.09)	0.1071	0.2111
Pl.Po	Pl.Po, ROI = M	Controls	0.02 (0.00, 0.03)	0.0179	0.3119
	Pl.Po, ROI = M	OA-no BML	−0.03 (−0.08, 0.02)	0.2704	0.1519
	Pl.Po, ROI = M	OA-BML	0.04 (−0.00, 0.09)	0.0711	0.4452
BV/TV	BV/TV, ROI = M	Controls	0.04 (−0.00, 0.08)	0.0613	0.8487.
	BV/TV, ROI = M	**OA-no BML**	**−0.05 (−0.09, −0.01)**	**0.0192**	**0.0474**
	BV/TV, ROI = M	OA-BML	−0.06 (−0.12, −0.00)	0.0375	0.3711
Tb.N	Tb.N, ROI = M	Controls	−0.04 (−0.58, 0.51)	0.8989	0.7141
	Tb.N, ROI = M	**OA-no BML**	**−0.73 (−1.13, −0.33)**	**0.0004**	**0.0203**
	Tb.N, ROI = M	OA-BML	0.65 (0.06, 1.24)	0.0309	0.2728
Tb.Th	Tb.Th, ROI = M	Controls	−2.81 (−7.72, 2.10)	0.2616	0.4689
	Tb.Th, ROI = M	OA-no BML	5.40 (−4.25, 15.06)	0.2727	0.4056
	Tb.Th, ROI = M	OA-BML	−2.79 (−10.46, 4.88)	0.4757	0.7717
TbSp	Tb.Sp, ROI = M	Controls	0.07 (−0.22, 0.36)	0.6494	0.2853
	Tb.Sp, ROI = M	**OA-no BML**	**0.39 (0.21, 0.56)**	**<.0001**	**0.0461**
	Tb.Sp, ROI = M	OA-BML	−0.15 (−0.38, 0.07)	0.1834	0.6250
SMI	SMI, ROI = M	Controls	−0.11 (−0.85, 0.64)	0.7783	0.6352
	SMI, ROI = M	OA-no BML	0.08 (−0.45, 0.60)	0.7776	0.6208
	SMI, ROI = M	OA-BML	0.22 (−0.31, 0.76)	0.4139	0.5395
DA	DA, ROI = M	Controls	−0.36 (−1.13, 0.41)	0.3560	0.3658
	DA, ROI = M	OA-no BML	−0.17 (−0.94, 0.60)	0.6645	0.1947
	DA, ROI = M	OA-BML	0.43 (−0.17, 1.02)	0.1576	0.2266
Tb.Pf	Tb.Pf, ROI = M	Controls	−0.00 (−0.04, 0.04)	0.9052	0.3203
	Tb.Pf, ROI = M	OA-no BML	0.06 (−0.03, 0.14)	0.1764	**0.0297**
	Tb.Pf, ROI = M	OA-BML	0.04 (0.00, 0.07)	0.0309	0.1740

The critical value for statistical significance was chosen as $P < 0.05$.

imaging might serve as a tool to assess OA severity at the tissue level.

Structural differences in the subchondral bone plate and trabeculae in KOA subjects

The present study demonstrates that subregional differences in the control group and OA-no BML were minimal. In comparison, in the OA-BML group, substantial differences were found in bone microstructure between regions.

Because the analysis was confined to those samples which contained a BML in the AM, the most substantial microstructural differences were found in the AM subregion of OA-BML. Trabecular bone of the AM region showed more prominent sclerosis, higher bone volume, and more numerous and plate-like trabeculae compared to PM. Comparing the AM subregion to AL and PL, significant differences were found in all parameters describing the microstructure of the subchondral plate and trabeculae.

Recently, Roberts *et al.* described the distribution of forces across the tibia that result in changes of subchondral bone microstructure, and found that regional variation in bone microstructure within tibial compartments is significantly associated with dynamic and static indices of knee joint loading[21,22]. With respect to BMLs, it has been found that high peak knee adduction moment and high knee adduction moment impulse, as well as static alignment were significantly related to the presence of BMLs[23]. These findings support the notion that excessive and biomechanically unfavourable loading contributes to the occurrence of BMLs. In addition, these findings could also explain regional and subregional variation in bone microstructure of the OA BML group. The conclusion was that BML areas represent “hot spots” in the tibial plateau where changes in the bone microstructure might be the result of an acute localised tissue response as well as a pathophysiological interaction between the bone and cartilage[1,24].

When we compared microstructural parameters between groups, we found substantially thinner subchondral plate in the medial compartment of OA-no BML, compared to both control and OA-BML. In contrast, the subchondral plate of OA-BML was thicker in the AM and PM subregions compared to the same subregions of both control and OA-no BML groups. We also found a greater porosity of the subchondral plate in OA-BML compared to controls, likely reflecting altered vascularity and/or bone resorption within the subchondral plate. The increased porosity could also affect the permeability of the osteochondral interface and play a direct role in the pathogenesis of OA. Higher porosity of the subchondral plate was observed to co-localise with the point of mechanical load during ambulation in a rat knee model of post-traumatic OA[25].

Trabecular bone of the medial compartment in the OA-no BML group was characterized by lower bone volume and trabecular number and higher SMI compared to both controls and OA-BML. In contrast, trabecular bone of the AM subregion did not differ significantly between OA-no BML and controls. In the PM subregion, OA-no BML had higher mean values for SMI and Tb.Pf compared to controls, indicating persistence of a rod-like structure with low connectivity. Furthermore, we found that trabecular bone in the AM subregion of OA-no BML, compared to the same region of OA-BML, was characterized predominantly by lower bone volume and fewer trabeculae that are more rod-like (indicated by greater SMI). The PM subregion of OA-no BML had lower mean bone volume, more rod-like trabeculae that were more isolated or less connected (indicated by larger Tb.Pf), compared to PM of OA-BML. A thinner subchondral plate and the reduced number of trabeculae in OA suggest a process of bone attrition at some prior stage in the OA disease progression. In addition, it has been suggested recently that rod-like structures (indicated by greater SMI) might have a protective effect on cartilage during impact loading[26,27]. However, in a separate study it has been noted that SMI alone may not be the optimal parameter to make assessment of rod-like and/or plate-like structure[28]. Further clarification and definition of the microstructural parameters representing rod-like and/or plate-like structure is needed.

Previously, similar findings (to OA-no BML) were only reported in animal models, such as in the Duncan Hartley guinea pig model of OA[29]. Recently, Chen *et. al.* described a novel finding in

Table III
Linear GEE model results of AM and PM OARSI score vs interaction of bone parameters and ROI of the groups, adjusting for clustering on patient

Predictor	Comparison	Group	Estimate (95% CI)	Comparison *P* value	Interaction *P* value
Pl.Th	Pl.Th, ROI = AM	Controls	−0.81 (−1.59, −0.03)	0.0419	0.0015
	Pl.Th, ROI = PM	Controls	0.00 (−2.02, 2.02)	1.0000	–
	Pl.Th, ROI = AM	OA-no BML	1.35 (−2.02, 4.72)	0.4313	0.0187
	Pl.Th, ROI = PM	OA-no BML	0.87 (−2.89, 4.63)	0.6508	–
	Pl.Th, ROI = AM	OA-BML	−0.24 (−1.86, 1.38)	0.7702	0.0026
	Pl.Th, ROI = PM	OA-BML	−1.78 (−4.00, 0.44)	0.1164	–
Pl.Po	Pl.Po, ROI = AM	Controls	−0.06 (−0.20, 0.08)	0.4284	0.6743
	Pl.Po, ROI = PM	Controls	−0.10 (−0.21, 0.01)	0.0860	–
	Pl.Po, ROI = AM	OA-no BML	−0.05 (−0.49, 0.39)	0.8348	0.0027
	Pl.Po, ROI = PM	OA-no BML	−0.40 (−0.57, −0.22)	<.0001	–
	Pl.Po, ROI = AM	OA-BML	0.01 (−0.12, 0.14)	0.8686	0.7575
	Pl.Po, ROI = PM	OA-BML	0.04 (−0.04, 0.12)	0.3484	–
BV/TV	BV/TV, ROI = AM	Controls	−0.12 (−0.19, −0.04)	0.0025	**<.0001**
	BV/TV, ROI = PM	Controls	−0.07 (−0.15, 0.00)	0.0591	–
	BV/TV, ROI = AM	OA-no BML	−0.00 (−0.27, 0.26)	0.9834	**<.0001**
	BV/TV, ROI = PM	OA-no BML	−0.11 (−0.24, 0.02)	0.0855	–
	BV/TV, ROI = AM	OA-BML	−0.02 (−0.17, 0.12)	0.7477	0.0761
	BV/TV, ROI = PM	OA-BML	−0.07 (−0.18, 0.04)	0.2343	–
Tb.N	Tb.N, ROI = AM	Controls	1.66 (1.04, 2.27)	**<.0001**	**<.0001**
	Tb.N, ROI = PM	Controls	−0.15 (−1.84, 1.54)	0.8638	–
	Tb.N, ROI = AM	OA-no BML	−2.64 (−6.02, 0.74)	0.1254	0.0003
	Tb.N, ROI = PM	OA-no BML	−2.26 (−4.64, 0.12)	0.0622	–
	Tb.N, ROI = AM	OA-BML	0.15 (−0.92, 1.22)	0.7860	0.0858
	Tb.N, ROI = PM	OA-BML	0.01 (−0.91, 0.93)	0.9807	–
Tb.Th	Tb.Th, ROI = AM	Controls	−8.50 (−16.14, −0.85)	0.0295	0.1794
	Tb.Th, ROI = PM	Controls	−8.88 (−18.99, 1.23)	0.0853	–
	Tb.Th, ROI = AM	OA-no BML	4.66 (2.63, 6.69)	0.6001	0.4222
	Tb.Th, ROI = PM	OA-no BML	3.95 (−12.11, 20.02)	0.6297	–
	Tb.Th, ROI = AM	OA-BML	−6.84 (−20.94, 7.26)	0.3420	0.4166
	Tb.Th, ROI = PM	OA-BML	−1.52 (−3.52, 5.23)	0.1461	–
Tb.Sp	Tb.Sp, ROI = AM	Controls	−2.39 (−4.10, −0.68)	0.0061	**<.0001**
	Tb.Sp, ROI = PM	Controls	−1.25 (−1.92, −0.59)	0.0002	–
	Tb.Sp, ROI = AM	OA-no BML	−2.16 (−2.87, −1.45)	**<.0001**	**<.0001**
	Tb.Sp, ROI = PM	OA-no BML	0.43 (−0.50, 1.37)	0.3637	–
	Tb.Sp, ROI = AM	OA-BML	0.55 (0.09, 1.00)	0.0182	0.1778
	Tb.Sp, ROI = PM	OA-BML	0.40 (−0.53, 1.33)	0.4015	–
SMI	SMI, ROI = AM	Controls	−0.13 (−0.87, 0.62)	0.7363	0.0243
	SMI, ROI = PM	Controls	0.85 (0.46, 1.25)	<.0001	–
	SMI, ROI = AM	OA-no BML	−1.28 (−3.68, 1.11)	0.2946	0.0024
	SMI, ROI = PM	OA-no BML	0.46 (−1.47, 2.39)	0.6414	–
	SMI, ROI = AM	OA-BML	−0.48 (−0.82, −0.14)	0.0061	0.0064
	SMI, ROI = PM	OA-BML	0.76 (−0.72, 2.24)	0.3128	–
DA	DA, ROI = AM	Controls	1.40 (0.90, 1.89)	<.0001	0.0001
	DA, ROI = PM	Controls	1.56 (−0.05, 3.17)	0.0569	–
	DA, ROI = AM	OA-no BML	0.54 (−1.58, 2.66)	0.6153	0.0061
	DA, ROI = PM	OA-no BML	−2.50 (−8.17, 3.18)	0.3886	–
	DA, ROI = AM	OA-BML	0.63 (−0.16, 1.41)	0.1162	0.0226
	DA, ROI = PM	OA-BML	0.43 (−0.77, 1.62)	0.4821	–
Tb.Pf	Tb.Pf, ROI = AM	Controls	0.04 (−0.05, 0.13)	0.3463	0.4286
	Tb.Pf, ROI = PM	Controls	0.07 (0.03, 0.11)	0.0010	–
	Tb.Pf, ROI = AM	OA-no BML	0.00 (−0.47, 0.47)	0.9976	0.6763
	Tb.Pf, ROI = PM	OA-no BML	0.13 (−0.05, 0.30)	0.1535	–
	Tb.Pf, ROI = AM	OA-BML	0.11 (0.03, 0.19)	0.0058	0.1120
	Tb.Pf, ROI = PM	OA-BML	0.04 (−0.10, 0.18)	0.5563	–

The critical value for statistical significance was chosen as $P < 0.0001$.

microstructural adaptation due to OA disease, in terms of significant loss of rod-like trabeculae and thickening of plate-like trabeculae in all regions of the human tibial plateau (with and without severe cartilage loss), providing a valuable insight into the dynamics of subchondral bone microstructural changes in OA. They also confirmed that similar changes preceded cartilage degeneration in the guinea pig model of spontaneous OA. These results suggested that specific trabecular changes might be important during the development and progression of OA[30]. Complementary to those findings, in our study SMI, DA and Tb.Pf, as nonmetric measures of topological structural features and useful determinants of mechanical strength[31], were significantly different in both OA-no BML and OA-BML compared to controls, confirming a lower bone quality of OA subchondral bone and its inability to resist overloading[32].

Collectively, structural alterations found in OA-no BML resemble changes that have been described previously in an early stage of OA[3–5,33–40]. The OA BML group was associated with the greatest degree of sclerosis, cartilage degeneration and loss of cartilage volume, which was consistent with advanced OA[9,10,41].

Presence of BMLs predicts more extensive microstructural differences in the tibial plateau

The presence and size of BMLs is strongly associated with clinical symptoms (pain)[42] and focal structural degeneration (loss of cartilage and bone sclerosis)[11–14]. The results of this study suggest that the presence of a BML might be associated with structural differences that extend beyond the BML, particularly in the medial compartment. Moreover, it seems that focal sclerosis of the BML

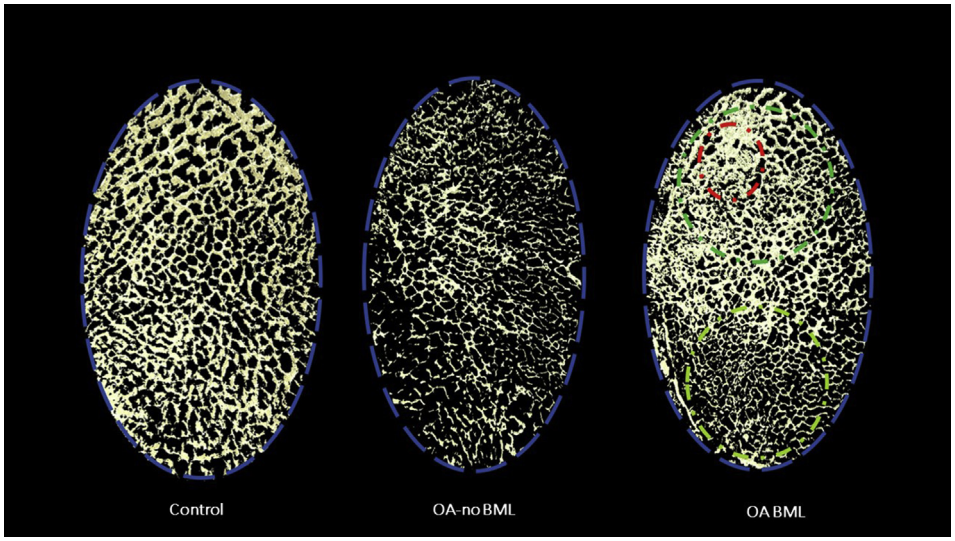

Fig. 4. Representative image of subchondral trabeculae in the medial compartment (transaxial view) of control, OA-no BML and BML groups. In OA-BML, approximate location of BML is shown by a red circular shape, AM-anterior medial (dark green round shape), PM-posterior medial (light green round shape). Purple solid line showing extent of sclerotic appearance of trabecular bone.

area expands radially to the adjacent subregions (Fig. 4). As BML sclerotic changes in a specific compartment are closely related to loss of cartilage volume and higher OARSI grade in the same compartment, BMLs could represent an epicentre of the structural change in the osteochondral unit of the tibial plateau. Findings from previous clinical studies have indicated that bone altering therapies reduce the size of BML and thus promote healing within affected tissues[17,18]. While results from larger studies are awaited, this study supports the notion that BMLs might provide valid biomarkers in the development of new therapies.

Sclerotic changes are widely accepted as a key feature of advanced OA progression, but it is important to point out that these findings have not been consistently reported for end stage OA[43,44]. A possible explanation for the different observations between studies might be the presence or absence of BMLs, since these subchondral bone features appear to segregate with more severe disease. Recently, Steinbeck *et al.* described two subtypes within a KOA population, defined according to microstructural features of subchondral bone (sclerotic and non-sclerotic trabecular bone), and suggested different mechanisms of disease progression[45]. Finnila *et al.* also analysed the tibial plateau in late stage of OA and found that bone volume fraction, trabecular thickness and trabecular number increase with OARSI grade, while trabecular separation and SMI decrease, suggesting that sclerosis might potentially be used in radiological assessment of OA severity[46]. Neither of these studies considered the presence or absence of a BML in the tissue. Our study suggests that OA subjects without a BML belong to a non-sclerotic OA subtype, while those with BML belong to a sclerotic OA subtype. This conclusion will require greater numbers for confirmation.

Our study has several limitations. Firstly, the relatively small and heterogeneous OA-no BML group limits the strength of comparisons with other groups to investigate in more detail the relationship between BML and subchondral bone structure. Further studies are recommended to investigate these relationships. Secondly, as this study is cross-sectional in design, the differences in subchondral bone structure characteristic of early stage disease cannot be confirmed, nor can mechanisms of BML genesis in the tissue be identified. Longitudinal studies would help to clarify this sequence of events. We believe the strength of this study is that we have analysed subchondral bone microstructure for the entire human tibial plateau, while previous studies have mainly investigated bone microstructural changes in specific sub-regions.

In conclusion, this study showed that the presence of a BML defines the changes in both the subchondral bone and cartilage, which in turn relate to the severity of the disease. BMLs may therefore provide surrogate biomarkers that can discriminate OA subtypes or severity, for example helping to triage candidates for joint replacement surgery or conservative, non-surgical treatment, or be used as therapeutic targets, and response to treatment.

Authors contributions

All authors meet the criteria for authorship. DM designed the study, performed the experiments and analysis of the results, interpreted the data and wrote the manuscript. FC, AW, DF and JK designed the study, interpreted the data, provided overall supervision and wrote the manuscript. YL contributed to the collection of specimens from patients, performed the experiments and critically revised the manuscript. All authors read and approved the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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Supplementary data

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