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Association between quantitatively measured infrapatellar fat pad high signal intensity alteration and MRI-assessed progression of knee osteoarthritis

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Competing interests

The authors declare that they have no competing interests.

Abstract

Objective: To describe the cross-sectional and longitudinal associations between quantitative measures of infrapatellar fat pad (IPFP) signal intensity alteration and knee structural abnormalities in patients with symptomatic knee OA.

Methods: 261 participants (mean age 63.0 ± 7.2 years) with symptomatic knee OA were selected from a randomized controlled trial with a follow up of 2 years. IPFP signal intensity alterations at baseline were quantitatively measured on T2-weighted fat-saturated MRI using MATLAB. These quantitative measures included the standard deviation [sDev (IPFP)] of whole IPFP signal intensity, the upper quartile value [UQ (H)] of high signal intensity, the ratio of volume of high signal intensity alteration to volume of whole IPFP [Percentage (H)] and Clustering factor (H) representing the clustering effect of high signal intensity. Cartilage volume and defects, and BMLs were assessed using validated measures

Results: Higher baseline sDev (IPFP), UQ (H) and Clustering factor (H) were associated with greater loss of tibial cartilage volume and larger increases in tibiofemoral cartilage defects over 2 years. Patients with high and medium tertiles of Clustering factor (H) had greater loss of cartilage volume per annum compared with those with low tertile (4.9% and 4.6% vs 3.3% p.a.). Baseline Percentage (H) and Clustering factor (H) were positively and significantly associated with increases in tibiofemoral BMLs over 2 years. Cross-sectional associations between IPFP measures and knee structures were similar but more consistent.

Conclusion: Quantitative measures of increased signal intensity in the IPFP were associated with knee structural abnormalities in tibiofemoral compartment, suggesting that these measurements could be used as an additional entry criteria in order to enrich for 'faster progressors' in studies of knee OA.

Significance and innovations:

1. Using novel quantitative measurements to assess the variation of IPFP signal intensity alterations as well as the extent, quantity and clustering effect of IPFP high signal intensity alterations;
2. These measurements were associated with radiographic osteoarthritis and MRI-assessed knee structural abnormalities, and predicted disease progression in tibiofemoral compartment over 2 years, suggesting they are clinically relevant.

Introduction

Osteoarthritis (OA) is a common cause of chronic disability in older adults and has been considered as a multifactorial condition with risk factors including genetics, aging, female sex, injury and obesity (1, 2). Obesity is a primary preventable risk factor which has effects partly through increasing loading in the knee joint (3). Obese adipose tissue also has endocrine function that can produce pro-inflammatory cytokines and adipokines affecting the process of joint degradation (4, 5). Similar to systemic adipose tissue, local adipose tissue such as infrapatellar fat pat (IPFP) has the ability of secreting pro- and anti-inflammatory cytokines and various adipokines, all of which may play roles in maintenance of cartilage and bone homeostasis in the knee joint (6-9).

IPFP is structurally similar to subcutaneous adipose tissue (10) with an abundance of adipocytes, immune cells, vessels and nerve fibres (11). Our previous studies reported that larger IPFP size had a potentially protective effect on knee symptoms and structural changes (12, 13). Despite its capabilities of reducing the impact loading and absorbing forces generated through the knee joint, recent evidence suggests that abnormal IPFP appears to play an important role in the initiation and progression of knee OA (14) (11). IPFP can be an important local source of age-related inflammation and a central contributor to the degradation of neighbouring tissues within the knee (15).

Inflammation within IPFP can be assessed using non-contrast enhanced magnetic resonance imaging (MRI) (16). High signal intensity alteration of IPFP has been regarded as a surrogate of peripatellar synovitis in epidemiological studies (17, 18). We used semi-quantitative measurements to assess signal intensity alterations in IPFP and reported that they were significantly associated with knee symptoms and structural abnormalities in older adults (19). There are currently few studies that have used quantitative measurements to evaluate IPFP signal intensity alterations and to describe their relationship with knee osteoarthritic abnormalities (20). We recently established a method to quantitatively assess IPFP signal intensity alterations and reported that this method was reproducible, and associated with knee structural changes (21). Therefore, our current study aims to describe the cross-sectional and longitudinal associations between quantitative measures of IPFP signal intensity alterations and knee structural abnormalities in patients with symptomatic knee OA.

Methods

Participants

This study was conducted as part of the Vitamin D Effect on Osteoarthritis (VIDEO) study, a multicentre randomized, double-blind and placebo-controlled clinical trial for evaluating the effects of vitamin D supplementation on knee pain and structural changes in knee OA patients with low 25-hydroxy vitamin D [25(OH)D] (22). Participants aged between 50 to 79 years with symptomatic knee OA for at least 6 months assessed according to American College of Rheumatology (ACR) criteria for clinical knee OA (23), visual analogue scale (VAS) scores of more than 20 mm and serum 25 (OH)D levels between 12.5 nmol/L and 60 nmol/L were included. Exclusion criteria included grade 3 radiographic OA according to the Altman's atlas (24), severe knee pain on standing (more than 80 mm on a 100 mm VAS), contraindication to MRI, other forms of arthritis such as rheumatoid or psoriatic arthritis, severe renal impairment, and others (25). Outcomes were measured at baseline and 24-months follow-up. The VIDEO study was approved by the Tasmania Health and Human Medical Research Ethics Committee (reference number H1040). Written informed consent was obtained from

all participants. Our current study consisted of a sample of 261 participants who had sagittal T2-weighted MRI scans in Tasmania. The sample of 152 participants who had coronal T2-weighted MRI scans in Melbourne was not included in this study. Treatment and placebo groups were combined as a cohort.

Anthropometrics

Height was measured to the nearest 0.1 cm (with shoes, socks, and headgear removed) using a stadiometer. Weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed) using a single pair of electronic scales (Model 707; Seca Delta, Hamburg, Germany) that were calibrated using a known weight at the beginning of each clinic. Body mass index (BMI) [weight (kg)/height^2 (m^2)] was calculated.

Knee radiographic assessment

A standing anteroposterior view of the symptomatic knee with 15 degrees of flexion was performed for each participant at baseline, and joint space narrowing (JSN) and osteophytes were individually assessed on a scale of 0 to 3 (0=normal and 3=most severe) according to the Osteoarthritis Research Society International (OARSI) atlas developed by Altman *et al* (24). JSNs were assessed at medial tibiofemoral, lateral tibiofemoral, medial patellofemoral and lateral patellofemoral compartments, while osteophytes were assessed at medial tibial, medial femoral, lateral tibial, lateral femoral, medial patellar and lateral patellar sites. We summed the osteophyte and JSN scores as total knee radiographic OA (ROA) score (range, 0-30), as previously described (26).

MRI protocol

MRI scans of the study knee were obtained according to a standardized protocol using a 1.5 T whole-body MRI unit with a commercial transmit-receive extremity coil. Fat-suppressed (FS) T2-weighted fast spin echo (FSE) sequences were used as described previously (22). The following sequences were used: sagittal FS T2-weighted 3D FSE sequence, flip angle 90º, repetition time 3,067 ms, echo time 112 ms, FOV 16 cm, 15 slices, 228 × 256-pixel matrix slice thickness of 2 mm with no gap.

Measurements of high signal intensity in IPFP

High signal intensity in IPFP was assessed (by WH) on sagittal planes of FSS T2-weighted images using MATLAB as described previously (Figure 1) (21). The segmentation of IPFP was performed at ten intermediate slices in whole IPFP in order to avoiding the interference from other tissues (i.e. synovium, ligaments and subcutaneous fat) and effusion which were hard to distinguish in the beginning and ending slices. This measurement is a semi-automated procedure. An initial lasso was automatically created by a set of points selected manually near the outer contour of IPFP and then contracted inward to approximate the real boundary of IPFP automatically. High signal intensity alterations were obtained by newly developed algorithm. Data were output automatically. We previously reported (21): among these measurements, Clustering factor (H) and sDev (IPFP) were consistently and significantly associated with all joint structural measurements; Percentage (H) and Volume (H) were consistently and significantly associated with knee cartilage defects and ROA but not with BMLs, while Median (H) and UQ (H) were only significantly associated with tibiofemoral cartilage defects; Mean (IPFP) was not significantly associated with any knee structural measurements.

All these measures can be classified as four categories: signal intensity of whole IPFP, high signal intensity of IPFP, volume of high signal intensity and clustering effect of high signal intensity. Based on the concurrent validity and the clinical construct validity we reported, we selected one measure from each category into the current study: sDev (IPFP), UQ (H), Percentage (H) and Clustering factor (H). Of them, sDev (IPFP), being introduced to represent signal intensity variation of IPFP, is the standard deviation of whole IPFP signal intensity; UQ (H), representing the value of high signal intensity, is the upper quartile value of high signal intensity; Percentage (H), being calculated as the ratio of volume of high signal intensity region to whole IPFP volume, represents the adjusted quantity of high signal intensity; and Clustering factor (H) is calculated as:

$$\text{Clustering factor (H)} = \frac{\sum_{i=1}^{m} Vol_i / \sum_{j=1}^{n} Vol_j}{m/n}$$

where,

$$m = \begin{cases} n * 10\% & n > 10 \\ 1 & n \leq 10 \end{cases}$$

n is the number of high intensity regions. Vol_j is the volume of seed group P_j. $\sum_{j=1}^{n} Vol_j$ is the total volume of high intensity regions. $\sum_{i=1}^{m} Vol_i$ is the volume of the top m largest high intensity regions. Clustering factor (H) reflects the actual clustering effects, the bigger $Factor_{(H)}$, the greater clustering effects. Intraobserver and interobsever reliabilities of these quantitative measurements ranged from 0.92 to 0.96 (intraclass correlation coefficient, ICC) and from 0.90 to 0.94, respectively.

Measures for knee structural abnormalities

Cartilage volume was assessed using the previously described image processing techniques (27). The volumes of individual cartilage plates (medial tibial, lateral tibial and patellar) were isolated by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis then resampled by means of bilinear and cubic interpolation for final 3D rendering using OsiriX Lite imaging software (32-bit version 5·9, Pixmeo SARL, Geneva, Switzerland). Total tibial cartilage

volume was summed, and change in cartilage volume per annum was defined as (follow-up – baseline)/interval. The coefficient of variation (CV) was 2·1% for medial tibia and 2·2% for lateral tibia (28).

Cartilage defects were evaluated using a modified Outerbridge classification (29) at medial tibial, lateral tibial, medial femoral, lateral femoral, femoral trochlear, and patellar sites: grade 0, normal cartilage; grade 1, focal blistering and intracrtilaginous hyperintensity with an normal contour; grade 2, irregularities on the surface and loss of thickness of less than 50%; grade 3, deep ulceration with loss of thickness of more than 50% without exposure of subchondral bone; grade 4, full thickness chondral wear with exposure of subchondral bone. A total score was calculated as the total of subregional scores with a maximum score of 24. Presence of tibiofemoral cartilage defects was defined as any tibial or femoral cartilage defects of >= grade 2, and an increase in cartilage defects was defined as the value from (follow-up cartilage defects – baseline cartilage defects) of >= 1. Intra-observer reliabilities (expressed as ICCs) ranged from 0.89 to 0.94 for different compartments. Inter-observer reliabilities were assessed in 50 MR images and yielded correlation coefficients of 0.85 to 0.93 for different compartments (30).

Subchondral bone marrow lesions (BMLs) were defined as discrete areas of increased signal adjacent to the subchondral bone. They were measured semi-quantitatively using the modified Whole-Organ Magnetic Resonance Imaging Score (WORMS) method (31). BMLs were scored from 0 to 3 based on the extent of subregional involvement (0 = none; 1 = < 25% of the subregion; 2 = 25-50%; 3 = >50%). The ICCs were 0.93-0.98 for this scoring system (32). Any presence of tibiofemoral BMLs was defined as a BML score of >= grade 1, and an increase in BMLs was defined as the value from (follow-up BMLs – baseline BMLs) of >= 1.

Data analysis

Baseline characteristics were summarised using descriptive statistics. Univariable and multivariable linear regression analyses were used to examine the associations between baseline IPFP signal intensity alteration measures (independent variables) and baseline ROA score, and baseline and change in tibial cartilage volume (dependent variables). The associations between baseline IPFP signal intensity alteration measures (independent variables) and the presence of or increase in tibiofemoral cartilage defects and BMLs (dependent variables) over 2 years were assessed using logistic regression analyses. The associations were adjusted for age, sex and BMI in cross-sectional analyses, and adjusted for age, sex, BMI and vitamin D treatment allocation in longitudinal analyses. Interactions between the measurements of IPFP high signal intensity alterations and sex or BMI on knee structural abnormalities were assessed by testing the statistical significance of the coefficient of a (sex or BMI × measures of IPFP high signal intensity alteration).

Multivariable linear/logistic regression was used to analyse the difference in change in tibial cartilage volume or an increase in tibiofemoral cartilage defects/BMLs over 2 years among tertiles of Clustering factor (H), in Figure 2, which visualised the association between knee structural changes and Clustering factor (H).

A p-value < 0.05 (2-tailed) or a 95% confidence interval (CI) not including the null (for linear regression) or 1 (for logistic regression) point was considered as statistical significance. All statistical analyses were performed on SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL).

Results

Characteristics of the study population are presented in Table 1. A total of 261 participants (49.4% females) between 50 and 79 years of age (mean, 63.0 years) took part in the present study. There were no significant differences in demographic factors (age, sex, and BMI) between these participants and those who were excluded from this study due to no sagittal knee MRI scans available (n = 152) (data not shown). The mean baseline total score of radiographic OA was 6.9, and the mean tibial cartilage volume was 3.7 ml. Tibiofemoral cartilage defects and any tibiofemoral BMLs were present in 53.3% and 65.3% of participants, respectively. Using quantitative measurements, the values of sDev (IPFP), UQ (H), Percentage (H) and Clustering factor (H) were presented in Table 1.

Cross-sectional associations

All baseline IPFP signal intensity alteration measures were significantly and positively associated with total radiographic OA score and presence of tibiofemoral cartilage defects and BMLs, but not significantly with presence of patellar cartilage defects and BMLs, in both univariable and multivariable analyses (Table S1-S3). Baseline Percentage (H) and Clustering factor (H) were significantly and negatively associated with tibial cartilage volume, while sDev (IPFP) and UQ (H) were significantly and negatively associated with patellar cartilage volume, in multivariable analyses (Table S4).

Longitudinal associations

Cartilage volume loss

Higher baseline sDev (IPFP), UQ (H) and Clustering factor (H) were significantly associated with greater tibial cartilage loss, but not associated with patellar cartilage loss, over 2 years before and after adjustment for covariates (Table 2). Patients with low tertile of Clustering factor (H) had 3.3% loss of

cartilage volume per annum (p.a.), while those with medium tertile had 4.6% loss of cartilage volume p.a., and with high tertile had 4.9% loss of cartilage volume p.a. (Figure 2a). The results were similar when medial and lateral tibial cartilage volumes were analysed individually (data not shown).

Increase in cartilage defects

Higher baseline sDev (IPFP), UQ (H) and Clustering factor (H) were significantly associated with higher risks of an increase in tibiofemoral cartilage defects over 2 years in both univariable and multivariable analyses, while all baseline IPFP signal intensity alteration measures were not associated with increase in patellar cartilage defects (Table 3). There was a significant and positive association between tertiles of Clustering factor (H) and an increase in tibiofemoral cartilage defects (Figure 2b).

Increase in BMLs

Higher baseline Percentage (H) and Clustering factor (H) were significantly associated with higher risks of an increase in tibiofemoral BMLs over 2 years in both univariable and multivariable analyses. In contrast, there were no significant associations between all baseline IPFP signal intensity alteration measures and an increase in patellar BMLs in both analyses (Table 4). Tertiles of baseline Clustering factor (H) were positively associated with an increase in tibiofemoral BMLs over 2 years (Figure 2c).

The results were similar when tertiles of other baseline IPFP signal intensity measures were used as independent variables (data not shown). Baseline IPFP signal intensity alteration was positively associated with baseline effusion-synovitis but not with changes in effusion-synovitis (data not shown). There were no significant interactions between the measurements of IPFP signal intensity

alterations and sex or BMI on knee structural abnormalities (data not shown); therefore, men and women, and those who were normal weight, overweight or obese were combined for analyses.

Discussion

This is the first study to describe the longitudinal associations between quantitative measures of IPFP signal intensity alteration and knee structural abnormalities in patients with symptomatic knee OA. We found that, quantitative measurements of IPFP high signal intensity alteration were significantly associated with increased ROA, tibiofemoral cartilage defects and BMLs, and reduced cartilage volume at baseline. Furthermore, most of higher baseline IPFP high signal intensity alteration measures were significantly associated with greater tibial cartilage volume loss and increases in tibiofemoral cartilage defects and BMLs over 2 years. In contrast, these quantitative measurements were not significantly associated with knee structural abnormalities at patellar site.

IPFP is the biggest adipose tissue structure within the knee. Abundant with adipocytes, immune cells and vessels (11), IPFP is an important source of inflammatory cytokines and adipokines (5, 7-9), which can be a key contributor to knee OA. A pathological study illustrated that there were vascular neoformations, fibrosis, and chronic inflammation in IPFP obtained from end-stage OA patients (33). T2-weighted FSE MRI can be used to assess IPFP signal intensity alterations (16), and these high signal intensity alterations may represent inflammation and vascular neoformations in IPFP. Several clinical and epidemiological studies used high signal intensity alteration of IPFP as a surrogate for peripatellar synovitis (17, 18); however, the measurement was semi-quantitative. Our study uses a quantitative assessment of IPFP signal intensity alteration, which is more objective and includes more details of IPFP signal intensity changes. In addition to the rare report of associations between IPFP signal intensity alterations and knee structural changes in patients with knee OA, the results of the current study suggested that IPFP signal intensity quantitative measurements could be considered as outcome measures in future knee OA research.

Routine radiography is considered as the gold diagnostic standard of OA in clinical practice due to the ability of revealing joint space loss and osteophytes at the joint margin, and this method provides an indirect measurement of cartilage loss (34). The OARSI atlas developed by Altman *et al* (24) is frequently used to assess features of radiographic OA (35). Our current study found that all measures of signal intensity alteration were positively associated with radiographic knee OA in patients with symptomatic knee OA. Our findings are consistent with two recent publications using semi-quantitative assessments of IPFP signal intensity alteration. Atukorala *et al* reported that Hoffa-synovitis (assessed as high signal intensity alterations in IPFP) strongly predicted the incidence of radiographic knee OA (36). We recently reported signal intensity alteration score was significantly associated with radiographic knee OA in population-based older adults (19). We were unable to evaluate the temporal relationship between IPFP signal intensity alteration and ROA, as X-ray assessment was only performed at baseline as an exclusion criteria in the original clinical trial.

Cartilage loss is generally considered as a major feature of OA progression. Inflammatory and metabolic factors, such as interleukin 6 (IL-6), tumor necrosis factor α (TNFα) and leptin, have been involved in cartilage breakdown and eventually cartilage loss(37, 38). Klein-Wieringa *et al* reported that IPFP in patients with knee OA could secrete higher levels of inflammatory mediators (IL-6, adipsin, adiponectin, and visfatin) that contributed to the pathogenesis of cartilage loss (6). Other studies also showed that IPFP in knee OA could release pro-inflammatory factors and adipokines that could induce cartilage degradation (7, 39). Our previous observational study illustrated that semi-quantitative measure of IPFP signal intensity alteration was significantly and positively associated with tibiofemoral cartilage loss in older adults (19). Consistent with these reports, we found that baseline sDev (IPFP), UQ (H) and Clustering factor (H) were associated with tibiofemoral cartilage volume loss and cartilage defects (focal cartilage loss) in patients with symptomatic knee OA over 2 years in the current study. Both studies did not find significant associations between IPFP signal

intensity alterations and patellar cartilage volume loss. In this study, nearly half of the participants were with grade 4 patellar cartilage defects, and only 15% of participants had an increase in patellar cartilage defects. Moreover, the change in patellar cartilage volume was only one third of change in tibiofemoral cartilage volume. The patellofemoral compartment had more severe disease and less changes than the tibiofemoral compartment, and this may be the reason that we did not find significant associations with changes in patellar compartment. This may also explain why we only found significantly cross-sectional associations with cartilage defects and BMLs in tibiofemoral compartment. Moreover, we found that, cross-sectionally, some measures (Percentage (H), Clustering factor (H)) were related tibial cartilage volume, while others (sDev (IPFP), UQ (H)) were significantly related to patellar cartilage volume. The reasons underlying these discrepancies are unclear but the cross-sectional findings were not the focus of our current study.

Subchondral BMLs have been considered as a hallmark of OA and been related with cartilage damage and pain in the initiation and progression of OA (40, 41). These subchondral pathological lesions may be influenced by inflammatory mediators (42) and may have a cross-talk with IPFP pathological changes through inflammatory pathways. Our previous study reported that high signal intensity alteration score in IPFP was significantly and positively associated with BMLs in older adults, suggesting there may be a link between IPFP and subchondral bone changes in the process of knee OA (19). Consistently, our current study reported that baseline Percentage (H) and Clustering factor (H) were associated with tibiofemoral BMLs in patients with symptomatic knee OA, while there were no significant associations between these quantitative measures and patellar BMLs.

Our current study used a novel and a semi-automatic method to assess signal intensity alterations in IPFP with the output of quantitative measures. Using this new method, our results were consistent with previous semi-quantitative methodology, suggesting the pathology of IPFP signal intensity alteration may play an important role in the progression of knee OA. Furthermore, these quantitative

measures are more objective and the results showed that Clustering factor (H), sDev (IPFP) and UQ (H), but not Percentage (H), had stronger associations with knee structure changes. These measures may reflect different magnitudes of pathological changes of IPFP, and this is why they were associated with different MRI-assessed knee structural changes of knee OA. Further pathological studies are needed to identify these differences. Overall, Clustering factor (H) was more consistently associated with all MRI-assessed knee structural changes, suggesting it may be a more useful biomarker for future research. Although this quantitative methodology focused on high rather than low signal intensity alterations in IPFP, it included more details of IPFP signal intensity alteration than previous semi-quantitative methodology and had more advantages over this semi-quantitative one. It will enrich the MRI-based whole joint assessment of knee OA, and could be included in selection of participants for future clinical trials in knee OA as by excluding those in the low tertile of increased signal intensity in the IPFP thus enriching for those with faster progression of knee OA. This measure appears to provide an additional selection method, independent of age, gender, radiological grade and other structural changes.

The main strength of this study is that we used a novel quantitative measurement to assess the signal intensity alterations in IPFP. Additionally, this study included blind readings and the standardized methods used for data acquisition with high intra- and inter- reader reliabilities. This study has several potential limitations. First, as this study is conducted as a post-hoc analysis within a subsample of a RCT it may not generalizable to general population of knee OA and needs further confirmations in the further studies. Second, MRI coronal planes were used at Melbourne so the quantitative measures in these participants were unable to be performed; however, there were no significant differences in demographic factors between participants included and excluded from this study. Third, histological examinations were not able to perform in our study so the pathological changes of IPFP signal intensity alterations are unknown. Fourth, more severe structural abnormalities in patellofemoral compartment at baseline would result in “ceiling effects” for the changes, which could be the reason why we did not detect significantly longitudinal associations in patellofemoral compartment. Future

studies are required to examine these in patients without advanced disease in this compartment. Last, measurement error may influence results. However, all measures were highly reproducible suggesting this is unlikely.

In conclusion, the quantitative measures of IPFP signal intensity alteration were significantly and positively associated with knee structural abnormalities in tibiofemoral compartment over 2 years in patients with symptomatic knee OA, suggesting that the pathology of IPFP may play an important role in knee OA progression. Additionally, these quantitative measurements of IPFP signal intensity alterations could be used as an additional entry criteria in order to enrich for 'faster progressors' in studies of in knee OA.

Reference

1. Garner M, Alshameeri Z, Khanduja V. Osteoarthritis: genes, nature-nurture interaction and the role of leptin. International orthopaedics. 2013;37(12):2499-505.

2. Roos EM, Arden NK. Strategies for the prevention of knee osteoarthritis. Nature reviews Rheumatology. 2016;12(2):92-101.

3. Wluka AE, Lombard CB, Cicuttini FM. Tackling obesity in knee osteoarthritis. Nature reviews Rheumatology. 2013;9(4):225-35.

4. Messier SP, Mihalko SL, Legault C, Miller GD, Nicklas BJ, DeVita P, et al. Effects of intensive diet and exercise on knee joint loads, inflammation, and clinical outcomes among overweight and obese adults with knee osteoarthritis: the IDEA randomized clinical trial. Jama. 2013;310(12):1263-73.

5. Presle N, Pottie P, Dumond H, Guillaume C, Lapicque F, Pallu S, et al. Differential distribution of adipokines between serum and synovial fluid in patients with osteoarthritis. Contribution of joint tissues to their articular production. Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society. 2006;14(7):690-5.

6. Klein-Wieringa IR, Kloppenburg M, Bastiaansen-Jenniskens YM, Yusuf E, Kwekkeboom JC, El-Bannoudi H, et al. The infrapatellar fat pad of patients with osteoarthritis has an inflammatory phenotype. Annals of the rheumatic diseases. 2011;70(5):851-7.

7. Distel E, Cadoudal T, Durant S, Poignard A, Chevalier X, Benelli C. The infrapatellar fat pad in knee osteoarthritis: an important source of interleukin-6 and its soluble receptor. Arthritis and rheumatism. 2009;60(11):3374-7.

8. Clockaerts S, Bastiaansen-Jenniskens YM, Feijt C, De Clerck L, Verhaar JA, Zuurmond AM, et al. Cytokine production by infrapatellar fat pad can be stimulated by interleukin 1beta and inhibited by peroxisome proliferator activated receptor alpha agonist. Annals of the rheumatic diseases. 2012;71(6):1012-8.

9. Eymard F, Pigenet A, Citadelle D, Flouzat-Lachaniette CH, Poignard A, Benelli C, et al. Induction of an inflammatory and prodegradative phenotype in autologous fibroblast-like synoviocytes by the infrapatellar fat pad from patients with knee osteoarthritis. Arthritis & rheumatology. 2014;66(8):2165-74.

10. Vahlensieck M, Linneborn G, Schild H, Schmidt HM. Hoffa's recess: incidence, morphology and differential diagnosis of the globular-shaped cleft in the infrapatellar fat pad of the knee on MRI and cadaver dissections. Eur Radiol. 2002;12(1):90-3.

11. Clockaerts S, Bastiaansen-Jenniskens YM, Runhaar J, Van Osch GJ, Van Offel JF, Verhaar JA, et al. The infrapatellar fat pad should be considered as an active osteoarthritic joint tissue: a narrative review. Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society. 2010;18(7):876-82.

12. Han W, Cai S, Liu Z, Jin X, Wang X, Antony B, et al. Infrapatellar fat pad in the knee: is local fat good or bad for knee osteoarthritis? Arthritis research & therapy. 2014;16(4):R145.

13. Pan F, Han W, Wang X, Liu Z, Jin X, Antony B, et al. A longitudinal study of the association between infrapatellar fat pad maximal area and changes in knee symptoms and structure in older adults. Annals of the rheumatic diseases. 2015;74(10):1818-24.

14. Ioan-Facsinay A, Kloppenburg M. An emerging player in knee osteoarthritis: the infrapatellar fat pad. Arthritis research & therapy. 2013;15(6):225.

15. Greene MA, Loeser RF. Aging-related inflammation in osteoarthritis. Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society. 2015;23(11):1966-71.

16. Roemer FW, Jarraya M, Felson DT, Hayashi D, Crema MD, Loeuille D, et al. Magnetic resonance imaging of Hoffa's fat pad and relevance for osteoarthritis research: a narrative review. Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society. 2016;24(3):383-97.

17. Roemer FW, Guermazi A, Zhang Y, Yang M, Hunter DJ, Crema MD, et al. Hoffa's Fat Pad: Evaluation on Unenhanced MR Images as a Measure of Patellofemoral Synovitis in Osteoarthritis. AJR American journal of roentgenology. 2009;192(6):1696-700.

18. Hill CL, Hunter DJ, Niu J, Clancy M, Guermazi A, Genant H, et al. Synovitis detected on magnetic resonance imaging and its relation to pain and cartilage loss in knee osteoarthritis. Annals of the rheumatic diseases. 2007;66(12):1599-603.

19. Han W, Aitken D, Zhu Z, Halliday A, Wang X, Antony B, et al. Signal intensity alteration in the infrapatellar fat pad at baseline for the prediction of knee symptoms and structure in older adults: a cohort study. Annals of the rheumatic diseases. 2016;75(10):1783-8.

20. Steidle-Kloc E, Wirth W, Ruhdorfer A, Dannhauer T, Eckstein F. Intra- and inter-observer reliability of quantitative analysis of the infra-patellar fat pad and comparison between fat- and non-fat-suppressed imaging--Data from the osteoarthritis initiative. Ann Anat. 2016;204:29-35.

21. Lu M, Chen Z, Han W, Zhu Z, Jin X, Hunter DJ, et al. A novel method for assessing signal intensity within infrapatellar fat pad on MR images in patients with knee osteoarthritis. Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society. 2016;24(11):1883-9.

22. Jin X, Jones G, Cicuttini F, Wluka A, Zhu Z, Han W, et al. Effect of Vitamin D Supplementation on Tibial Cartilage Volume and Knee Pain Among Patients With Symptomatic Knee Osteoarthritis: A Randomized Clinical Trial. Jama. 2016;315(10):1005-13.

23. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum. 1986;29(8):1039-49.

24. Altman RD, Gold GE. Atlas of individual radiographic features in osteoarthritis, revised. Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society. 2007;15 Suppl A:A1-56.

25. Cao Y, Jones G, Cicuttini F, Winzenberg T, Wluka A, Sharman J, et al. Vitamin D supplementation in the management of knee osteoarthritis: study protocol for a randomized controlled trial. Trials. 2012;13:131.

26. Jones G, Ding C, Scott F, Glisson M, Cicuttini F. Early radiographic osteoarthritis is associated with substantial changes in cartilage volume and tibial bone surface area in both males and females. Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society. 2004;12(2):169-74.

27. Ding C, Cicuttini F, Parameswaran V, Burgess J, Quinn S, Jones G. Serum levels of vitamin D, sunlight exposure, and knee cartilage loss in older adults: the Tasmanian older adult cohort study. Arthritis Rheum. 2009;60(5):1381-9.

28. Jones G, Glisson M, Hynes K, Cicuttini F. Sex and site differences in cartilage development: a possible explanation for variations in knee osteoarthritis in later life. Arthritis Rheum. 2000;43(11):2543-9.

29. Baysal O, Baysal T, Alkan A, Altay Z, Yologlu S. Comparison of MRI graded cartilage and MRI based volume measurement in knee osteoarthritis. Swiss medical weekly. 2004;134(19-20):283-8.

30. Ding C, Garnero P, Cicuttini F, Scott F, Cooley H, Jones G. Knee cartilage defects: association with early radiographic osteoarthritis, decreased cartilage volume, increased joint surface area and type II collagen breakdown. Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society. 2005;13(3):198-205.

31. Peterfy CG, Guermazi A, Zaim S, Tirman PF, Miaux Y, White D, et al. Whole-Organ Magnetic Resonance Imaging Score (WORMS) of the knee in osteoarthritis. Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society. 2004;12(3):177-90.

32. Raynauld JP, Martel-Pelletier J, Berthiaume MJ, Abram F, Choquette D, Haraoui B, et al. Correlation between bone lesion changes and cartilage volume loss in patients with osteoarthritis of the knee as assessed by quantitative magnetic resonance imaging over a 24-month period. Annals of the rheumatic diseases. 2008;67(5):683-8.

33. Macule F, Sastre S, Lasurt S, Sala P, Segur JM, Mallofre C. Hoffa's fat pad resection in total knee arthroplasty. Acta Orthop Belg. 2005;71(6):714-7.

34. Attur M, Krasnokutsky-Samuels S, Samuels J, Abramson SB. Prognostic biomarkers in osteoarthritis. Current opinion in rheumatology. 2013;25(1):136-44.

35. Culvenor AG, Engen CN, Oiestad BE, Engebretsen L, Risberg MA. Defining the presence of radiographic knee osteoarthritis: a comparison between the Kellgren and Lawrence system and OARSI atlas criteria. Knee surgery, sports traumatology, arthroscopy : official journal of the ESSKA. 2015;23(12):3532-9.

36. Atukorala I, Kwoh CK, Guermazi A, Roemer FW, Boudreau RM, Hannon MJ, et al. Synovitis in knee osteoarthritis: a precursor of disease? Annals of the rheumatic diseases. 2016;75(2):390-5.

37. Tetlow LC, Adlam DJ, Woolley DE. Matrix metalloproteinase and proinflammatory cytokine production by chondrocytes of human osteoarthritic cartilage: associations with degenerative changes. Arthritis Rheum. 2001;44(3):585-94.

38. Posever J, Phillips FM, Pottenger LA. Effects of basic fibroblast growth factor, transforming growth factor-beta 1, insulin-like growth factor-1, and insulin on human osteoarthritic articular cartilage explants. Journal of orthopaedic research : official publication of the Orthopaedic Research Society. 1995;13(6):832-7.

39. Hui W, Litherland GJ, Elias MS, Kitson GI, Cawston TE, Rowan AD, et al. Leptin produced by joint white adipose tissue induces cartilage degradation via upregulation and activation of matrix metalloproteinases. Annals of the rheumatic diseases. 2012;71(3):455-62.

40. Dore D, Martens A, Quinn S, Ding C, Winzenberg T, Zhai G, et al. Bone marrow lesions predict site-specific cartilage defect development and volume loss: a prospective study in older adults. Arthritis research & therapy. 2010;12(6):R222.

41. Foong YC, Khan HI, Blizzard L, Ding C, Cicuttini F, Jones G, et al. The clinical significance, natural history and predictors of bone marrow lesion change over eight years. Arthritis research & therapy. 2014;16(4):R149.

42. Geurts J, Patel A, Hirschmann MT, Pagenstert GI, Muller-Gerbl M, Valderrabano V, et al. Elevated marrow inflammatory cells and osteoclasts in subchondral osteosclerosis in human knee osteoarthritis. Journal of orthopaedic research : official publication of the Orthopaedic Research Society. 2016;34(2):262-9.

Table 1. Baseline characteristics of participants

Characteristic	**Values*(n = 261)**
Age (years)	63.0 (7.2)
Female sex (%)	49.4
BMI (kg/m^2)	29.7 (4.9)
ROA (grade, 0 - 30)	6.9 (5.0)
Tibial cartilage volume (ml)	3.7 (1.1)
Tibiofemoral cartilage defects present (%)	53.3
Any tibiofemoral BMLs present (%)	65.3
sDev (IPFP)	8.4 (2.9)
UQ (H)	4.2 (1.4)
Percentage (H)	6.9 (1.3)
Clustering factor (H)	6.3 (0.9)

*Mean (SD) or percentage of patients. BMI: body mass index; IPFP: infrapatellar fat pad; ROA: radiographic osteoarthritis; BMLs: bone marrow lesions; sDev (IPFP): standard deviation of IPFP signal intensity values; UQ(H): upper quartile value of high signal intensity region; Percentage (H): ratio of volume of high signal intensity region/whole IPFP volume; Clustering factor(H): clustering factor of high signal intensity. Presence of tibiofemoral cartilage defects was defined as any tibial or femoral cartilage defects of >= grade 2. Any presence of tibiofemoral BMLs was defined as a BML score of >= grade 1.

Table 2. Longitudinal associations between baseline IPFP signal intensity alteration and changes in cartilage volume

	Univariable	Multivariable*
	β (95% CI)	β (95% CI)
Change in tibial cartilage volume		
sDev (IPFP)	**-0.34 (-0.61, -0.08)**	**-0.37 (-0.65, -0.09)**
UQ (H)	**-0.90 (-1.51, -0.29)**	**-0.93 (-1.56, -0.31)**
Percentage (H)	-0.13 (-0.54, 0.28)	-0.15 (-0.56, 0.26)
Clustering factor (H)	**-1.01 (-1.66, -0.35)**	**-1.41 (-2.25, -0.57)**
Change in patellar cartilage volume		
sDev (IPFP)	-0.10 (-0.45, 0.25)	-0.05 (-0.41, 0.32)
UQ (H)	-0.21 (-0.92, 0.51)	-0.11 (-0.85, 0.62)
Percentage (H)	-0.02 (-0.58, 0.54)	-0.04 (-0.61, 0.53)
Clustering factor (H)	-0.59 (-1.73, 0.56)	-0.47 (-1.63, 0.70)

Dependent variable: cartilage volume; Independent variable: IPFP signal intensity alteration.

*Adjusted for age, gender, BMI, tibial bone area and treatment allocation.

IPFP: infrapatellar fat pad; sDev (IPFP): standard deviation of IPFP signal intensity values; UQ(H): upper quartile value of high signal intensity region; Percentage (H): ratio of volume of high signal intensity region/whole IPFP volume; Clustering factor(H): clustering factor of high signal intensity.

Table 3. Longitudinal associations between baseline IPFP signal intensity alteration and increase in cartilage defects

	Univariable	Multivariable*
	RR (95% CI)	RR (95% CI)
Increase in tibiofemoral cartilage defects		
sDev (IPFP)	**1.33 (1.16, 1.53)**	**1.37 (1.18, 1.58)**
UQ (H)	**2.00 (1.46, 2.74)**	**2.10 (1.51, 2.92)**
Percentage (H)	0.90 (0.76, 1.08)	0.89 (0.74, 1.07)
Clustering factor (H)	**2.15 (1.44, 3.22)**	**2.19 (1.46, 3.30)**
Increase in patellar cartilage defects		
sDev (IPFP)	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.03)
UQ (H)	0.03 (-0.01, 0.06)	0.03 (-0.01, 0.06)
Percentage (H)	-0.02 (-0.04, 0.01)	-0.03 (-0.05, 0.01)
Clustering factor (H)	0.03 (-0.02, 0.09)	0.03 (-0.02, 0.08)

Dependent variable: cartilage defects; Independent variable: IPFP signal intensity alteration.

*Adjusted for age, gender, BMI and treatment allocation.

IPFP: infrapatellar fat pad; sDev (IPFP): standard deviation of IPFP signal intensity values; UQ(H): upper quartile value of high signal intensity region; Percentage (H): ratio of volume of high signal intensity region/whole IPFP volume; Clustering factor(H): clustering factor of high signal intensity.

An increase in cartilage defects was defined as the value from (follow-up cartilage defects – baseline cartilage defects) of >= 1.

Table 4. Longitudinal associations between baseline IPFP signal intensity alteration and increase in BMLs

	Univariable	Multivariable*
	RR (95% CI)	RR (95% CI)
Increase in tibiofemoral BMLs		
sDev (IPFP)	1.09 (0.96, 1.23)	1.09 (0.96, 1.24)
UQ (H)	1.10 (0.83, 1.45)	1.08 (0.81, 1.44)
Percentage (H)	**1.28 (1.06, 1.55)**	**1.28 (1.05, 1.55)**
Clustering factor (H)	**1.47 (1.00, 2.17)**	**1.52 (1.02, 2.26)**
Increase in patellar BMLs		
sDev (IPFP)	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)
UQ (H)	-0.01 (-0.04, 0.02)	-0.01 (-0.05, 0.03)
Percentage (H)	0.02 (-0.01, 0.04)	0.02 (-0.01, 0.05)
Clustering factor (H)	0.00 (-0.06, 0.06)	0.00 (-0.06, 0.06)

Dependent variable: BMLs; Independent variable: IPFP signal intensity alteration.

*Adjusted for age, gender BMI and treatment allocation.

BMLs: bone marrow lesions; IPFP: infrapatellar fat pad; sDev (IPFP): standard deviation of IPFP signal intensity values; UQ(H): upper quartile value of high signal intensity region; Percentage (H): ratio of volume of high signal intensity region/whole IPFP volume; Clustering factor(H): clustering factor of high signal intensity.

An increase in BMLs was defined as the value from (follow-up BMLs – baseline BMLs) of >= 1.

Figure legends

Figure 1. Measurements of signal intensity alteration in IPFP using MATLAB.

Semi-automatic segmentations of IPFP were performed first. Then, high signal intensity regions, showing as the red areas, were obtained by newly developed algorithm

Figure 1A has the lowest Percentage (H) and Clustering factor (H), and Figure 1B has the highest sDev (IPFP) and UQ (H). Although Figure 1B and Figure 1C have similar Percentage (H) and Clustering factor (H), they have different sDev (IPFP) and UQ (H). Figure 1D has the biggest Percentage (H), but its sDev (IPFP) and UQ (H) are lower than Figure 1B.

Figure 2. Longitudinal associations between Clustering factors of the high signal intensity alterations in IPFP and knee structural changes over 2 years.

(A) Change of tibial cartilage volume per annum (%); (B) Increase in tibiofemoral cartilage defects (%); (C) Increase in tibiofemoral BMLs (%).

**p values were those after adjustment for baseline age, gender, BMI, and treatment allocation.*