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Histopathological Assessment of Primary Osteoarthritic Knees in Large Patient Cohort Reveal the Possibility of Several Potential Patterns of Osteoarthritis Initiation

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Abstract

Objective—The two main objectives of the study include 1) Test the hypothesis that the lateral femoral condyle (LFC) in patients with primary OA and varus knees undergoing total knee arthroplasty (TKA) can be used as a model to better characterize varying histological features of human OA. 2) Correlate characteristic OA features using the established histopathological scoring systems (HHGS and OARSI) to understand potential histopathological patterns of OA initiation.

Design—Two osteochondral specimens (4×4×8mm) were collected from fifty patient's LFC at the time of TKA (total 100 specimens), who presented preserved lateral knee compartment with joint space width >2mm. Three independent readers graded the sections on three different occasions using HHGS and OARSI systems. The correlation between individual parameters of the two scoring systems and their inter- and intra-reader variability, reliability and reproducibility were estimated.

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Author contributions

1. Conception of study: GM, RJM. Study design, acquisition of data, data analyses: VPM, NP, TZ, RJM. Statistical analysis and interpretation: NO, VPM.
2. Drafting of the article: VPM. Critical revising for important intellectual content: NP, RJM, GFM.
3. Final approval of version to be submitted: GFM, RJM, VPM, NP.

Conflict of interest

The authors declare no conflict of interest.

Results—All samples in this cohort showed abnormal histopathological features. Total histopathological scores of the LFC ranged from HHGS median=4.6 (range=0 to 11), and OARSI median=5.2 (range=0 to 19.5). The four individual sub-items of HHGS scoring system (structure, cells, safraninO staining, tidemark) were weakly correlated, with the correlation between structure and cellularity being the strongest ($r=0.40$). Both the scoring systems had similar repeatability and reproducibility coefficients of <21%.

Conclusions—OA changes in the LFC are not confined to any one region, and maybe seen in different regions of cartilage, tidemark, subchondral bone, and/or the marrow space vascularity. These variations may point to the possibility of several potential patterns of initiation in OA.

Keywords

Primary osteoarthritis; cartilage; histology; histopathology; grading

Introduction

Osteoarthritis (OA) of the knee is a very common and disabling form of joint disease.[1] OA is recognized to be a multifactorial disease that affects the whole-joint. It is associated with changes in local and systemic inflammatory mediators, genetic factors and innate immunity. [2–7]

OA changes in cartilage structure and function can be due to: chondrocyte phenotype and function modifications (proliferation, differentiation, survival and distribution); modifications in the synthesis and degradation of the proteoglycan rich extracellular matrix; cell migration from adjacent synovium; infiltration of blood vessels from the subchondral bone (SCB), and local changes in the concentration and distribution of inflammatory mediators.[8–12] The order in which these phenomena occur is still unclear. Validated cartilage histological scoring assessments can readily identify some of these changes.[13]

The histological assessment and grading of progression in human primary OA typically has been traditionally done using two scoring systems[13]. First the widely used histologic/histochemical grading system (HHGS) developed by Mankin et al.[14] which scores four cartilage parameters independently (structure, cellularity, safraninO staining and tidemark integrity). These independent scores are summed to provide a total score from 0 (normal) to 14 (end-stage OA). Second, over three decades later, a prestigious expert panel from the Osteoarthritis Research Society International (OARSI) developed a new Osteoarthritis Cartilage Histopathology Assessment System.[15] This OARSI system was designed based on five principles: simplicity, utility, scalability, extendibility and comparability.[15] In the OARSI system, the “grade” of OA assesses the depth of cartilage tissue loss from its apical surface from 0 (intact surface) to 6 (full thickness loss of cartilage with bone deformation); an advanced OARSI grade scores half-point “subgrades”. The OARSI “stage” evaluates the extent of the cartilage surface involved in OA from 0 (normal) to 4 (>50%). Thus, the total OARSI score, obtained by multiplying grade and stage, yields a range from 0 (normal) to 24 (end-stage OA).

Arthroplasty procedure provides effective therapies for end stage OA. However, current practice demands a greater emphasis on OA prevention, early diagnosis and treatment to block or delay disease progression.[16] A better understanding of the patterns and variation in initiation and progression of OA could influence the design and patient specific selection of therapies to OA. Animal models have been developed to examine stages of OA that involve either cartilage injury, meniscal injury, ligamentous instability or chemically or pharmacological induction of inflammation.[17] While these models may provide insight into biological mechanisms that are relevant to human post-traumatic OA, they do not necessarily provide insight into the frequency and variation of pathological mechanisms that are present in the progressive human primary OA.

Previous studies have demonstrated that varus knee OA patients had significantly higher percent of load on the medial femoral condyle than on LFC, and that the JSW in the lateral compartment was preserved in many cases.[18] Therefore, we sought: 1) to test the hypothesis that the lateral femoral condyle (LFC) in patients with primary OA and varus knees undergoing total knee arthroplasty (TKA) can be used as a model to better characterize varying histological features of human OA; 2) to correlate characteristic OA features using the established histopathological scoring systems (HHGS and OARSI) to assess potential patterns of OA progression; 3) to define the intra- and inter-observer variations using these two scoring systems; and 4) to correlate joint space width determined from X-rays with Kellgren and Lawrence (K-L) scores and with HHGS and OARSI scores.

Methods

Recruiting – Patients Inclusion and Exclusion Criteria

This study was approved by the Institutional Review Board committee of the Cleveland Clinic (Protocol: 13641). Fifty patients with varus knees undergoing total knee arthroplasty (TKA) were recruited (Mean age: 63 years (range 37 to 80 years); Mean BMI: 31kg/m² (range: 18.2 to 48.9kg/m²); male=30, female=20). Inclusion criteria required a diagnosis of OA exhibiting a relatively spared lateral compartment (i.e. primarily medial compartment and/or patellofemoral disease) based on pre-operative weight-bearing anterior-posterior (AP) radiographs taken in full extension and 30° of flexion. Patients were excluded if they had weight-bearing AP radiographs with <2mm lateral compartment joint space width (JSW), secondary arthritis related to systemic inflammatory arthritis (e.g.: rheumatoid arthritis, psoriatic arthritis); current or previous treatment with systemic glucocorticoids or osteotropic medication (e.g. bisphosphonates); or known or suspected infection, osteonecrosis or neoplasm.

Radiographic evaluation

Weight-bearing AP radiographs were taken with the knee in 30° of flexion (Rosenberg radiograph).[19] Joint space width (JSW) in the lateral compartment was determined in a systematic manner following the midpoint technique described by Ravaut et al.[20] A digital calibrated scale was used to measure the distance in millimeters. The radiographs were graded in blinded fashion according to the K-L grading system (Supplementary Fig. 1). [21] The scoring of the radiographs was performed once by four independent scorers. For

the 50 patients, knee joint K-L scores of 1, 2, 3, and 4 yielded frequencies of 1.5%, 24%, 43% and 31.5%, respectively (Supplementary Fig. 1). Lateral compartment JSW ranged from 2mm to 10mm (median=6mm).

Human cartilage procurement

During TKA, the lateral femoral condyle (LFC) was collected after making the distal femoral cut (Figs. 1A). Two osteochondral specimens (4×4×8mm) were prepared from the weight-bearing center portion of the LFC; one was located medial (M) and one lateral (L) to the LFC midline (Figs. 1B,C). The centers of these two samples were separated by 10mm, including the 4mm thickness of the intervening sample and the associated kerf thickness of 1mm.

Histological sample processing

A total of 100 osteochondral specimens were processed from LFCs of 50 patients. Immediately after surgical retrieval, specimens were collected in 10% neutral buffered formalin containing 0.5% cetylpyridium chloride (preserves proteoglycan and hyaluronan contents in cartilage tissues)[22] and were fixed for 48h at 4°C. They were subsequently decalcified for 5 weeks at 4°C using Cal-rite (ThermoScientific, MA). The decalcified tissue was dehydrated in an alcohol series into a xylene-substitute and embedded in paraffin with a consistent spatial orientation. Five-micron thick paraffin sections were cut and stained with freshly prepared hematoxylin and eosin (H&E) or SafraninO and fast green (SafO-FG). The embedded tissue was cut in the plane perpendicular to the surface of the cartilage to obtain a representative overview of the tissue structure and thickness. Two adjacent sections per each stain were used for scoring using HHGS and advanced OARSI systems.[14,15]

Digital imaging

Color images of the entire stained sections were acquired using a Leica DM6000 inverted microscope (Leica Microsystems, Wetzlar, Germany) with a 10X objective (pixel resolution: 1.72 microns/pixel) equipped with a H101ANN1 Prior motorized stage (Prior Scientific Inc., Rockland, MA) and QICAM camera (QImaging, Surrey BC, CA). Higher magnification imaging (20X objective) was used to confirm fine histological details.

Histological scoring

Using HHGS and advanced OARSI systems, stained section images were graded by three blinded readers (VM, NSP, RJM) to assess inter-reader agreement: senior investigator (A), orthopedic surgery fellow (B) and post-doctoral fellow (C). Each reader performed blinded grading on three occasions, with a time difference of at least two months in between rescoring to assess intra-reader agreement. Prior to initiating the review, reader-A was experienced in using HHGS, however reader B and C were not. All readers were inexperienced using OARSI and underwent a training period using self-education[15] and discussions with colleagues in this field to become familiar with this scoring system. The total samples (n=100) were randomly divided into subsets for scoring each time with HHGS and OARSI.

During the training period, a consensus needed to be established with respect to several feature descriptions and definitions in order to standardize HHGS scoring criteria. First, distinctions between zones (e.g.: transitional and radial zones) were sometimes obscured by extensive surface remodeling of the cartilage tissue making interpretation about a cleft's depth problematic based on classical spatial zones classification.[23] In these cases, the length of the cleft was assessed as a ratio of the cartilage tissue height.[24] Second, hypercellularity was scored based on the prevalence of doublets/triplets along the width of the hyaline cartilage[25] and distinguished from cloning by a 6-cell cluster cutoff.[26] Third, a cloning score did not consider cell numbers sharing the same territorial matrix above the cutoff, nor did it consider a single clonal unit distinct from several clonal units. Fourth, SaFO-FG staining reduction was assessed as the percentage of total tissue area devoid of stain: slight <33%, moderate 33–66% and severe 66–99%. Fifth, many tissue sections exhibited multiple tidemarks and a tidemark breach was scored based on blood vessels and bone tissue that crossed the most superior tidemark.[27]

Statistical analysis

The intra-reader variability was characterized by the repeatability coefficient (RC) and the inter-reader variability by the reproducibility coefficient (RDC).[28] The RC and RDC are the minimum expected differences between two measurements on the same subject by the same reader or by different readers, with 95% confidence. The lower the RC and RDC value, the better is the repeatability and reproducibility, respectively. For each of the sub-items of HHGS scoring system (structure, cellularity, SaFO-FG staining and tidemark integrity) and OARSI (grade and stage), the RCs and RDCs were determined using the methods described above. Correlation coefficient were estimated to assess the association of: i) x-ray findings (K-L score and JSW) with mean histological scores, and ii) the sub-items in each scoring system to total histology score. 95% confidence intervals were estimated using bootstrap and Fisher's z-transformation. We used SAS Version 9.4 for all statistical analyses.

Results

Histopathology Scoring

Fig. 2 demonstrates this study's osteochondral histology quality and each HHGS category scoring criteria [14]; some noteworthy observations are provided below. First, the presence of a pannus-like synovial tissue overgrowth of the cartilage surface was infrequent (2%). Second, the incidence of hypocellularity was relatively low (6%) and often involved loss of deep-zone chondrocytes even when upper-zone cells were reasonably well preserved (Fig. 2F). Third, hypercellularity was observed frequently in the topmost third of the hyaline cartilage tissue though it was not uncommon to see hypercellularity in the middle or deep-zones as well. Fourth, some specimens exhibited relatively good surface structure scores and just a moderate loss of SaFO-FG staining in this upper-zone, but exhibited a large loss of SaFO-FG staining in the deep-zone juxtaposed to the calcified cartilage region (Fig. 2H, right-bottom panel). Fifth, many tissue sections exhibited loss of SaFO-FG staining at both the apical and basal hyaline cartilage areas (Figs. 2H and 2I). In one extreme case (Fig. 2J) the surface structure was reasonably well preserved, but the top two-thirds of the hyaline cartilage did not stain positive for SaFO-FG, while the bottom-third maintained good SaFO-

FG staining. Fig. 3 demonstrates the scoring criteria for each advanced OARSI grade and stage based on Pritzker et al.[15]

Correlation of HHGS and OARSI scoring sub-items

Consistent with the relative weighing of its variables, the HHGS structure sub-item score had the strongest correlation ($r=0.806$), while tidemark integrity had the weakest correlation ($r=0.391$) with the total HHGS score. Cellularity and SafO-FG staining were moderately correlated with the total HHGS score ($r=0.54$ and $r=0.55$, respectively). The four individual sub-items were weakly correlated with each other, with the correlation between structure and cellularity being the strongest ($r=0.40$). The grade sub-item of the OARSI system had the strongest correlation to the total OARSI score ($r=0.89$), while the stage sub-item had a weaker correlation ($r=0.47$).

Intra-reader variability

HHGS 95% CI RC estimates for the three readers were similar (1.73, 2.29), (2.08, 2.75), (1.69, 2.24), but more variable for OARSI (2.40, 3.18), (4.50, 5.95), (3.21, 4.24). Estimated RC values were adjusted for differences in the range of the scores (0–14 for HHGS, and 0–24 for OARSI), and with this adjustment, the HHGS and OARSI systems had %RC values of 14–17%, and 11–21% respectively, which do not differ significantly.

RC values for the four sub-items of HHGS were: 1.13 (structure); 0.91 (cellularity); 0.89 (SafO-FG staining); and 0.65 (tidemark integrity). When differences in the ranges for each sub-item were normalized, the corresponding sub-items had %RC of 18.8% (structure), 30.3% (cellularity), 22.2% (SafO-FG) and 65% (tidemark), suggesting structure is most reliably scored. RC values for OARSI were: 1.05 (grade) and 1.51 (stage). Normalizing for the differences in the range for each sub-item, %RC was 17.5% (stage) and 37.7% (grade).

Inter-reader variability

The RDC estimated for third scoring of HHGS and OARSI was 2.31 (95% CI 2.03, 2.69) and 4.52 (95% CI 3.97, 5.25), respectively. Estimated RDCs were adjusted for differences in the range of each scoring system: HHGS and OARSI systems had %RDCs of 17%, and 19% respectively (not significantly different).

The RDC for all four sub-items of HHGS are: 1.41 (structure), 1.28 (cellularity), 1.03 (SafO-FG staining) and 0.85 (tidemark integrity). Sub-item scores were normalized for their differences in range yielding %RDC of: 23.5% (structure), 42.7% (cellularity), 25.7% (SafO-FG staining), and 85% (tidemark integrity), suggesting structure is most reproducibly scored. The RDC for the two sub-items of OARSI are: 1.28 (grade) and 2.15 (stage). Sub-item scores were normalized for their differences in range yielding %RDC of 21.3% (stage) and 53.7% (grade).

Radiograph Analysis

Correlation of K-L scores with HHGS scores was $r=0.20$ (95% bootstrap CI $-0.04, 0.40$), and with OARSI scores it was $r=0.16$ (95% bootstrap CI $-0.08, 0.38$). Correlation of JSW scores with HHGS scores was $r=-0.21$ (95% bootstrap CI $-0.44, 0.05$), and with OARSI

scores it was $r=-0.18$ (95% bootstrap CI $-0.42, 0.10$). The 95% CI in all the correlations included zero, indicating no association between K-L score, JSW and the two scoring systems (Supplementary Figure 2 AC).

Discussion

Histological assessment is an essential tool and metric to assess osteoarthritis, providing insight into the process of disease development and progression. Standard and reliable histopathological assessment systems are essential tools for guiding and standardizing the assessment and comparison of histological features. This study used both HHGS and advanced OARSI systems, which have emerged as the two most relevant and validated histopathologic scoring systems for grading severity of primary OA cartilage in humans.[13] Herein, we characterized osteochondral specimens from human LFC present in joints with an average JSW of 5.5 ± 1.7 mm (patient's age: 50 to 72years). These findings were similar to what Beattie KA et al. reported for healthy individuals between the age of 50 to 70 years, with K-L grade 0, where the average JSW on the lateral compartment was 5.05mm.[29] Only three studies to date have compared the HHGS and OARSI in human knee samples. Two of these had a patient cohort of <20 patients.[30,31] The largest study included 65 patients with a wide spectrum of cartilage pathology.[32] However, this study presents 50 patients with stringent inclusion/exclusion criteria, which provided a larger set of cartilage samples, which might resemble early, mild or moderate phases of knee OA, rather than late stages of OA, since they had preserved JSW.

Structure modifying treatments for OA have been disappointing.[17,33] There is a need to segregate patients with different OA subtypes to pair them with an optimal mode of treatment to yield an effective intervention.[34,35] In order to do so, a better understanding of the patterns of change in primary OA may be needed to predict response to specific intervention. Furthermore, pre-clinical OA studies have indicated relatively poor translation to clinical practice.[17] A possible reason for this result is that many pre-clinical studies involve posttraumatic OA, which accounts for only 12% of symptomatic OA,[36] and exhibits a different pathophysiology from primary OA.[17] This study provides a robust and consistent model to study human primary OA in the knee to contribute to the present challenges.

In support of the OA sub-type contention, the correlation of HHGS sub-items with each other (particularly structure with other components), was found to be relatively poor and not very predictive. This became even more striking when one narrows the focus of interest on the 50% of our samples that exhibited the least histopathology. This suggests that the key sub-items in the OARSI and the HHGS system may change independently, with no one consistent as a "first hit". In this light, a strong emphasis of the OARSI grading system on sequential pattern of events, where structural alterations are followed by, or associated with changes in cellularity, and SaFO-FG stain may be of limited use for scoring primary OA specimens exhibiting early, mild or moderate OA changes. We found that nearly half of our specimens exhibited reasonably intact surfaces, but exhibited significant histological changes within the middle and/or deep zones of the hyaline cartilage.[37]

As a recognition of inflammation in primary OA pathology emerges, different pathways of cartilage damage are beginning to be elucidated: cell death, cell activation, abnormal differentiation of the remaining cartilage cells, degradation of extracellular matrix, changes in the SCB, and production of inflammatory mediators.[8–12] Both HHGS and OARSI histological scoring systems are heavily weighted towards structural changes in articular cartilage (mostly surface changes), following the traditional theories that focus on mechanical and structural failure as a central etiological process. Waldstein et al. have demonstrated in a large cohort of patients, that biomechanical properties of human articular cartilage are directly proportional to the degree of severity of the disease according to OARSI system.[38] However, OA progression with structural changes is only one of the many possible phenotypes of disease progression. There is a need to better understand the complete spectrum of characteristics involved in this heterogeneous disease to improve characterization of knee OA sub-types.[39] In order to do so, we need to clearly document, define and analyze changes in cellular response,[40,41] extracellular matrix,[42–44] tidemark and SCB [45–48] that are important to the structure and function of cartilage. This is an important aspect from therapeutic perspective also, in order to improve the efficacy of the treatments, by identifying various possible OA sub-groups for targeted treatment procedures.

We found that both the HHGS and OARSI systems exhibited some limitations in accurately assessing very early OA pathological features, which could be the subject of further refinement. Several areas of improvement might be proposed. A broader array of staining techniques may be considered to enhance the depth and granularity of data regarding underlying biological function at a cell and matrix level. Quantitative imaging and image analysis tools may be considered to provide more quantitative and less subjective metrics regarding the number, concentration and distribution of cells and matrix components within defined regions of the articular cartilage.[49] Conventional images, like those illustrated in Fig. 2&3 could be used to develop an extended online image library of consensus-based images. This type of resource could enable a new observer to see as well as read about the meaning of terms in the classification system. Moreover, an expanded series of images could be provided as a visual reference scale to improve standardization and interpretation of various parameters, especially for in-experienced scorers.[50] It will also help consequently in better comparison and interpretation of various studies, as it has become increasingly challenging, given the number of modifications of these scoring system, to accommodate their specimen assessments.[13]

Conclusions

Although characteristics of late stages of OA in the human knee with structural changes are well characterized, the characteristics and patterns seen in earlier phases of disease when cartilage does not present substantial structural surface changes are less well characterized. These data suggest that systematic study of osteochondral tissue obtained from LFC in patients with primary OA and varus knees undergoing TKA (JSW in lateral compartment >2mm) is a practical and valuable model for investigation of different phases of primary OA progression. In order to improve the reliability and reproducibility of the histopathological scoring systems and to better correlate with clinical outcomes, we need to better identify and

define the changes of human primary OA which 1) are not confined to any one region, and may be seen in superficial and deep regions in cartilage, 2) may manifest as changes in the cell population, 3) loss of cartilage extracellular matrix in different patterns, 4) may also involve the tidemark, subchondral bone and/or the marrow space. These variations may point to the possibility of several potential patterns of initiation in OA, which may contribute to variation in the onset, progression and response to therapy. Moreover the existing histological scoring systems may require refinement to better quantify and define features of OA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Sample of lateral femoral condyle was obtained (A) and cut into 4 mm thick arches through the region of the femur that is weight bearing in extension(B). The central region of one arch was cut into five specimens (4mmx4mm), the second (Lateral) and fourth (Medial) were processed for histology (C). Each specimen was paraffin embedded, sectioned and stained with H&E (E), and SafO-FG (F) for analysis.

Fig. 2.

HHGS histological Grading: A. Normal surface (structure=0) and normal cell distribution (cells=0). B. Surface irregularities (structure =1) and diffuse hypercellularity (cells=1). C. Pannus and surface irregularities (structure=2) and hypercellularity (cells=1). D. Clefts to transitional zone (structure=3) with cloning (cells=2). E. Clefts to radial zone (structure=4) with chondrocyte cloning (cells=2). F. Surface irregularities (structure =1) with region of hypocellularity (cells=3). G. Normal even distribution of Safranin O stain throughout the cartilage section except for in the superficial zone (safraninO staining=0). H. Slight reduction of stain area observed near the superficial zone as well as the deep zone above calcified cartilage region (safraninO staining=1). I. Moderate reduction of the stain area detected in superficial, mid and deep zone (safraninO staining=2). J. Severe reduction in stain area in the superficial and mid zone, with loss of intra-territorial matrix in the deep zone (safraninO staining=3). K. Intact single tidemark (tidemark integrity=0). L. Tidemark breached by two blood vessels (tidemark integrity=1). Note: All structure, cell and tidemark features of HHGS were detected using H&E sections.

Fig. 3.

OARSI histological Grading: A. Intact cartilage with intact surface (grade=0, stage=0) B. Surface fibrillation with cell proliferation and minor cationic stain depletion (grade=1, stage=2). C. Severe surface fibrillation with cell proliferation and stain loss (grade=1.5, stage=3). D. Discontinuity in superficial zone along with cell proliferation and greater cationic stain loss (grade=2, stage=2). E. Surface abrasion with matrix loss in the superficial zone with predominant cell proliferation (grade=2.5, stage=2). F. Simple clefts through superficial zone to mid zone with cationic stain loss seen till the mid zone (grade=3, stage=3). G. Wider, branched clefts seen to the mid zone with cationic stain loss seen till mid-zone (grade=3.5, stage=3). H. Superficial cartilage delamination with matrix stain loss (grade=4, stage=3). I. Cartilage excavation in the mid-zone along with matrix stain loss and cell cloning (grade=4.5, stage=3). Note: The OARSI scoring was done using SaO-FG sections.