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Original article

# Histopathological assessment of primary osteoarthritic knees in large patient cohort reveal the possibility of several potential patterns of osteoarthritis initiation

suppl. Informations

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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 ($4 \times 4 \times 8$ mm) 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 $> 2$ mm. 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.
*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.


## 1. 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

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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 "sub-grades". 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 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;
- Correlate characteristic OA features using the established histopathological scoring systems (HHGS and OARSI) to assess potential patterns of OA progression;
- Define the intra- and inter-observer variations using these two scoring systems;
- Correlate joint space width determined from X-rays with Kellgren and Lawrence (K-L) scores and with HHGS and OARSI scores.

## 2. Methods

### 2.1. 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: 31 kg/m$^2$ (range: 18.2 to 48.9 kg/m$^2$); 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 < 2 mm 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.

### 2.2. 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 Ravaud 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 2 mm to 10 mm (median = 6 mm).

### 2.3. Human cartilage procurement

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

### 2.4. 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 48 h at 4 °C. They were subsequently decalcified for 5 weeks at 4 °C using Calrite (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].

### 2.5. Digital imaging

Color images of the entire stained sections were acquired using a Leica DM6000 inverted microscope (Leica Microsystems, Wetzlar, Germany) with a 10× 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 (20× objective) was used to confirm fine histological details.

### 2.6. 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 re-scoring to assess

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