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# Osteoarthritis cartilage histopathology: grading and staging[1,2]

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## Summary

*Objective*: Current osteoarthritis (OA) histopathology assessment methods have difficulties in their utility for early disease, as well as their reproducibility and validity. Our objective was to devise a more useful method to assess OA histopathology that would have wide application for clinical and experimental OA assessment and would become recognized as the standard method.

*Design*: An OARSI Working Group deliberated on principles, standards and features for an OA cartilage pathology assessment system. Using current knowledge of the pathophysiology of OA morphologic features, a proposed system was presented at OARSI 2000. Subsequently, this was widely circulated for comments amongst experts in OA pathology.

*Results*: An OA cartilage pathology assessment system based on six grades, which reflect depth of the lesion and four stages reflecting extent of OA over the joint surface was developed.

*Conclusions*: The OARSI cartilage OA histopathology grading system appears consistent and simple to apply. Further studies are required to confirm the system's utility.


*Key words*: Osteoarthritis, Cartilage, Histopathology, Grading.

## Introduction

A major obstacle to understanding osteoarthritis (OA) natural history and its modifications by therapy has been lack of consensus concerning the order and role of cartilage pathologic features characteristic for OA biologic activity and progression. Further, common histopathologic assessment methods (Grade) under both clinical and experimental conditions reflect poorly mild (or early) phases of disease, have wide interobserver variation and are very non-linear over the range from mild to advanced disease[1–3]. Recent biologic advances have enabled classification of OA cartilage pathology features into those of activity and progression and have facilitated functional correlation of osteoarthritic cartilage morphologic changes[4].

Most current methods for OA histopathologic assessment are based on macroscopic assessment grading system

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devised by Collins[5,6] and a microscopic Histologic Histochemical Grading System (HHGS) developed in 1971 by Mankin *et al.*[7].

Collins, in his pioneering work, had as his aim to identify macroscopic OA pathology and to classify the observations into lesser (earlier) changes and more advanced OA[5,6,8]. The Collins system was based on observation of knee tissues, usually distal femur removed at incidental surgery or at autopsy. Collins graded OA severity as Grades I–IV using extensive qualitative descriptions of cartilage surface texture, lesion size and bony changes. Of this system, Collins[5], wrote "This grading system is, of course, arbitrary and in no way represents fixed stages in the progress of the disease". The first step towards an osteoarthritic cartilage grading system based on biologic disease mechanisms was the work of Collins and McElligott[8] who assessed patellas removed at autopsy from 34 patients. They studied $^{35}SO_4$ uptake by chondrocytes and correlated these findings to histologic changes of OA. Chondrocytes in osteoarthritic cartilage showed increased $^{35}SO_4$ uptake, and osteoarthritic cartilage from higher Collins grades (Grades III and IV) showed more $^{35}SO_4$ uptake than chondrocytes from lesser lesions (Grades I and II). This work demonstrated that hyaline cartilage chondrocytes in OA were hyperactive, not inert or effete. These studies, showing that osteoarthritic chondrocytes were very metabolically active, changed the concept of OA from a mechanical condition of cartilage "wear and tear" to a disease of biologic reaction.

Mankin *et al.*[7], in an equally pioneering work, developed an OA pathology grading system using femoral heads removed at arthroplastic surgery. The objectives of their study were to provide a histopathologic correlation with cartilage biochemical changes associated with OA progression. Mankin's histopathology grading system was based on microscopic evaluation of decalcified sections of surgically removed osteoarthritic femoral heads stained by Safranin O with Light Green counterstain. This system used a 14 point score based on a composite of cellular changes, histochemical presence of Safranin O matrix staining and architectural changes (erosion, vessel penetration through tidemark). This system is known eponymously as the Mankin System or, more recently, as the HHGS[1,2]. In the following years, this method was adapted and modified by most investigators. The HHGS has been applied to study OA spontaneously arising in humans, non-human primates and other animals, as well as to investigations of experimental OA models, generated by surgical lesions to induce instability or by other means[9].

Both these grading systems were based on study of specimens with very advanced OA. It is not surprising that these grading systems were not linear for mild or earlier phases of the disease typical of the pathology observed in many OA model systems.

The reproducibility and the validity of the HHGS for osteoarthritic cartilage has been questioned formally[1,2].

In common with the trend to standardize the clinical, radiologic and arthroscopic assessment of OA[10,11], there are growing needs to standardize the assessment of OA histopathology. To address this need, in 1998 OARSI established an OA Working Group to devise a standard OA grading system based on current pathophysiologic knowledge. It was recognized at the outset that human OA affects all joint tissues with subchondral bone having a prominent role in some OA subsets, as well as some experimental models[12]. For practical reasons, it was decided to confine the grading system to one target tissue, articular cartilage.

First, the Working Group established a set of target principles to guide the development of the grading system. Second, it was agreed that the grading system should be designed for broad but defined applications in OA assessment. Third, the Working Group recognized the desirability to standardize conditions under which OA cartilage pathology is assessed. The principles, applications and proposed standards for the grading system are outlined below.

## PRINCIPLES

The five principles for an *ideal* cartilage histopathology system are: simplicity, utility, scalability, extendability and comparability (Table I).

1. *Simplicity*—The system should be simple and reproducible. Therefore, the system should be able to be applied easily by investigators with varying levels of histopathology experience. At the very least, the system should be superior to the HHGS or other OA histopathology systems in current use, particularly in assessment of early disease.
2. *Utility*—The system should be equally useful for assessment of both clinical OA and experimental OA models. While assessing morphologic changes, the grading system should reflect biochemical and molecular indicators of OA progression. The grading system should be applicable with conventional histologic preparations and stains.
3. *Scalability*—The system should be scalable such that the cartilage macroscopic appearance can be correlated with the cartilage histopathology. Further, it would be desirable to have the system scalable such that the cartilage histopathology could be related linearly to morphologic appearances seen on diagnostic imaging and/or arthroscopy.
4. *Extendability*—The grading system design should be capable of being applied successfully by an observer's qualitative observation of microscopic slides. However, some research applications may benefit from more detailed grading/staging or by morphometric analysis of the osteoarthritic features. Ideally, the system should be capable of accommodating the needs of most observers and, as well, be extendable to the few advanced applications that require more morphologic detail and/or morphometry.
5. *Comparability*—The OA histopathology grading system should be capable of being harmonized eventually with histological assessment systems for other cartilage

Table I
*OA cartilage histopathology assessment—principles*

| | Principles | |
|---|---|---|
| 1 | Simplicity | Simple, reproducible |
| 2 | Utility | Useful for both clinical and experimental OA |
| 3 | Scalability | Direct extension from macroscopic assessment to microscopic histopathology |
| 4 | Extendability | Extendable to more detailed grading/staging or to morphometric methodology |
| 5 | Comparability | Comparable to pathology grading/staging systems for other cartilage disorders and to pathology grading systems for other diseases |

disorders or, at least, those assessment systems associated with cartilage repair[13,14].

## APPLICATIONS

Applications for the OARSI osteoarthritis pathology assessment system include distinguishing OA subsets, defining endpoints for clinical trials, evaluating new OA features or biomarkers and evaluating animal models of OA.

1. *Distinguishing OA subsets*—Long-standing historical controversy surrounds the nomenclature of OA itself (OA vs osteoarthrosis) and whether OA is primarily a ''degenerative'' joint disease[15–23]. Similarly, the spectrum or range of disease encompassed by the term OA varies widely[21]. Historically, this variation has been dependent both on medical discipline (orthopaedic surgeons, rheumatologists, pathologists) and on geography, Europe (narrow definitions), North America (broader definitions). Currently, OA is considered a group of joint diseases characterized within articular cartilage, in part, by simultaneous presence of cartilage matrix degradation and regeneration/repair, as well as chondrocyte death, chondrocyte replication and proliferation[4]. OA may have several subsets, some of which have cartilage as a primary lesion, others bone, others soft tissue laxity or still others, a combination of target tissues. Whatever the subsets, these forms of OA, in their later stages, come to resemble each other closely. A standard histopathology assessment system that could identify early OA may well identify groups which have differential features in either lesion location or OA progression that permits subset identification.
2. *Defining clinical trial endpoints*—A major issue in OA clinical trials is the need to define endpoint criteria for disease progression or modification of progression by therapy. Although all tissues of the joint are involved in OA, the weight bearing cartilage surface is the critical tissue affected. Whether the endpoint is viewed by imaging techniques or, as has been done in a few trials, by clinical biopsies, a standard histopathologic assessment system for OA cartilage pathology is required for clinical trial endpoints.
3. *Evaluating new OA features or disease markers characteristic of OA*—As the tissue composition of cartilage becomes better known, there are many different kinds of molecules which undergo change in quality or quantity in OA. These may be directly observed as a histopathologic feature or may be present in synovial fluid, blood or urine as surrogate biomarkers. The need exists to define a standard histologic system by which candidate surrogate biomarkers of OA progression can be evaluated.
4. *Evaluating animal models of OA*—Histopathologic assessment systems have been used extensively to evaluate animal models of OA. Unfortunately, because of the varied assessment systems employed, many of these papers are very difficult to compare to each other.

## STANDARDIZATION OF OA CARTILAGE PATHOLOGY ASSESSMENT

Prerequisites for grading and staging of OA cartilage histopathology include both standardization of the assessment system itself and, also, the conditions under which the histopathology is assessed. These latter conditions include:

1. *Standard definitions of OA cartilage histopathology features*—The nomenclature of OA histopathology features has evolved historically over the past 200 years with contributions by investigators in several languages, principally Latin, Greek, German, French and English. In the assessment system presented here, an attempt has been made to use terms in current, common use and to use the terms within a precise and narrow definition. The definitions for cartilage histology features and their modification in OA used in this paper are tabulated in the glossary, Appendix A.
2. *Standard exclusion criteria of other forms of arthritis*—While it is recognized that the features of OA are different from those of inflammatory arthritis, there is considerable overlap of primary OA histopathology features with those of other forms of arthritis, which have separate etiology and pathogenesis. This may be particularly true in latter phases of the diseases where different forms of arthritis may share some morphologic features. In the absence of agreed exclusionary criteria, the system should be applied principally to primary OA and experimental OA. If the system is to be used to assess OA features in other forms of arthritis, this should be clearly specified.
3. *Standard sampling for each joint and each joint compartment (standard topology)*—While sampling of each joint at a standard anatomical plane may be ideal, as yet there is no consensus on optimal sampling planes. Indeed, different sample planes may be required for different studies, depending on the purpose, e.g., correlation with magnetic resonance imaging; correlation with arthroscopy. While standard topology may be ideal, in each study it is critically important that the anatomical sampling plane be defined and described with precision, and that samples with consistent specifications are taken throughout the study. As lesions vary in location, it is not useful to sample blocks in a precise anatomical plane irrespective of lesion location. Rather, the lesion should be sampled where the plane of the block transverses the lesion to the greatest extent. Where the lesion is very small, the sections should be cut into the block until the maximal extent of the lesion is reached. For small animals such as mice sections at successive, different deeper levels may be required to obtain appropriate phase to score OA optimally. For assessment of human joints, there are particular challenges. Ideally, there should be one sample block which extends from one joint margin to the other through the maximal extent of OA. This can be accomplished at autopsy and with standard surgical excisions of femoral head and knee joint tissues. As these blocks may be large, special embedding techniques may be required. However, as minimally invasive surgery becomes more widely deployed, smaller, more fragmented samples will likely result. In these situations, display of the fragments in original anatomical relationships will be necessary to obtain the appropriate samples for staging and scoring. Regarding directed cartilage biopsies which may be indicated to assess disease modifying therapies, a valid grading assessment can be made provided

that the biopsy is directed macroscopically to the most advanced portion of the arthritic lesion. Staging and scoring would not be possible in these circumstances, unless staging was based on ancillary criteria, such as arthroscopy and imaging.

4. *Standard histologic preparation and staining of standard sections*—As tissues shrink differentially in different fixatives and as histologic technique is important to reduce staining variation, it is important, at least within each study, to define and describe the precise conditions for histologic preparation[24,25]. The histologic preparation technology, currently used at Mount Sinai Hospital, Toronto, Canada, is presented in Appendix B.

While standard disease exclusion criteria, standard sampling and standard histology preparation are all needed, consensus on these standards is not yet available.

The tissues should be processed and stained according to a standard protocol such as in Appendix B.

## The OARSI osteoarthritis cartilage histopathology assessment system

A draft OA histopathology assessment system incorporating the principles and design for the applications above was presented to OARSI in October 2000[26]. The system follows an analogy of the concept widely used in cancer pathology assessment. Increasing grade indicates a more biologically aggressive disease; increasing stage indicates greater disease extent. For OA, lesion depth into cartilage area represents more severe arthritis; lesion extent over cartilage surface represents extent of disease. Subsequently, through feedback from the OARSI Working Group and other interested members of the OA community, the cartilage histopathology assessment system was refined as described below.

The OARSI Osteoarthritis Cartilage Histopathology Assessment System is based on histologic features of OA progression. Prior OA diagnosis and recognition of OA activity within cartilage, such as chondrocyte clustering, is assumed. All the grades and stages assume that the tissue reaction observed has microscopic features characteristic of OA activity[4]. The system employs analysis of a standard block/section assessment by grade, stage of arthritis with subsequent calculation of an arthritis score.

### STANDARD HISTOLOGICAL BLOCK/SECTION FOR GRADE AND STAGE ASSESSMENT METHODOLOGY

Each standard block/section for grade/stage assessment is confined to one articular surface and subjacent tissues from one joint compartment, e.g., medial femoral condyle. The section must extend horizontally from one edge of the joint to the other and in depth from the articular surface to below the articular bone plate. For small animals such as mice, multiple sections of the block at defined different levels may be required to assess OA score appropriately.

Microscopic sections are assessed at low power magnification. The OARSI template to assess histopathology grade (Table II and Figs. 1 and 2) can be used as a guide to identify features and a reference scale marker line (on microscope stage or eyepiece) can be used to estimate the extent of OA. More elaborate morphometric methods for staging can be used if more precision is required.

Table II
*A cartilage histopathology grade assessment—grading methodology*

| Grade (key feature) | Associated criteria (tissue reaction) |
|---|---|
| Grade 0: surface intact, cartilage morphology intact | Matrix: normal architecture<br>Cells: intact, appropriate orientation |
| Grade 1: surface intact | Matrix: superficial zone intact, oedema and/or superficial fibrillation (abrasion), focal superficial matrix condensation<br>Cells: death, proliferation (clusters), hypertrophy, superficial zone<br>Reaction must be more than superficial fibrillation only |
| Grade 2: surface discontinuity | As above<br>+Matrix discontinuity at superficial zone (deep fibrillation)<br>±Cationic stain matrix depletion (Safranin O or Toluidine Blue) upper 1/3 of cartilage<br>±Focal perichondronal increased stain (mid zone)<br>±Disorientation of chondron columns<br>Cells: death, proliferation (clusters), hypertrophy |
| Grade 3: vertical fissures (clefts) | As above<br>Matrix vertical fissures into mid zone, branched fissures<br>±Cationic stain depletion (Safranin O or Toluidine Blue) into lower 2/3 of cartilage (deep zone)<br>±New collagen formation (polarized light microscopy, Picro Sirius Red stain)<br>Cells: death, regeneration (clusters), hypertrophy, cartilage domains adjacent to fissures |
| Grade 4: erosion | Cartilage matrix loss: delamination of superficial layer, mid layer cyst formation<br>Excavation: matrix loss superficial layer and mid zone |
| Grade 5: denudation | Surface: sclerotic bone or reparative tissue including fibrocartilage within denuded surface. Microfracture with repair limited to bone surface |
| Grade 6: deformation | Bone remodelling (more than osteophyte formation only). Includes: microfracture with fibrocartilaginous and osseous repair extending above the previous surface |

II. Grade = depth progression into cartilage.

### *Grade, stage and score*

Grade is defined as OA depth progression into cartilage. Grade is an index of the *severity* or biologic progression of the osteoarthritic process. This assumes that OA involvement of deeper cartilage is a more advanced disease and a good indicator of progressive disease. Grade is assessed by noting the most advanced grade present within the cartilage, irrespective of its horizontal extent. Stage is defined as the horizontal *extent* of cartilage involvement within one side of a joint compartment irrespective of the underlying grade. Score is defined as assessment of combined OA grade and OA stage. Therefore, score represents a combined assessment of OA severity and extent.

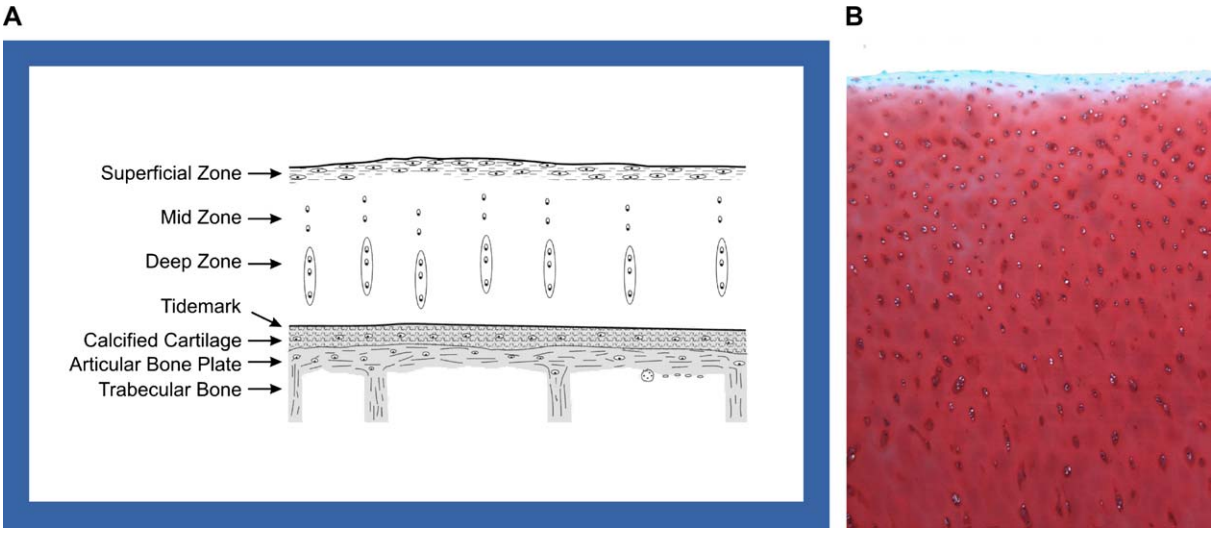


Fig. 1. (A & B) Normal articular cartilage: histologic features, grade 0. The cartilage surface is smooth. The matrix and chondrocytes are organized into superficial, mid and deep zones. (B) Safranin O stain, original magnification ×5.

*OA grade*. A key concept underlying the grading system proposed is that whatever the biologic mechanisms, the earliest cartilage changes in OA are observed near the cartilage surface. As OA becomes more severe, increasingly deeper cartilage becomes involved. Ultimately, the cartilage becomes eroded completely and the subjacent bone becomes the articular surface. The key features of each OA grade are described in Table II and shown graphically in Figs. 1 and 2.

*Recommended OA grading method*. With normal cartilage as grade 0, OA severity is divided into six grades. Grades 1–4 involve articular cartilage changes only, whereas grades 5 and 6 involve subchondral bone as well. The morphologic features of the six grades are as follows:

*Grade 0*—Grade 0 is hyaline articular cartilage uninvolved with OA. In grade 0 (Fig. 1), the cartilage surface is smooth. The matrix and associated chondrocytes are organized in three appropriately oriented, well ordered zones. No enlargement/distortion of chondrons and no proliferative changes of chondrocytes are observed.

*Grade 1*—By definition, grade 1 is the threshold for OA in cartilage. Grade 1 OA is characterized by retention of the articular surface layer. However, mild abrasion, termed ''superficial fibrillation'', characterized by microscopic cracks into the superficial zone, may be present. As this type of fibrillation is known to be directly mechanically induced without biologic reaction[27], this criterion alone is insufficient for OA. Other histology features in grade 1 OA include focal or generalized cartilage matrix swelling (oedema), which in extreme form, leads to cartilage hypertrophy. Cartilage oedema may be reflected by matrix changes such as focal rarefaction and condensation of collagen fibres in the superficial zone or upper mid zone or variable matrix cationic staining (Safranin O or Toluidine Blue). Proliferation of chondrocytes in the superficial zone, characterized by chondrocyte clusters and/or disorientation of chondrocytes, may be seen. Chondrocyte death is identified by absence of chondrocytes within the chondron or, more specifically, by presence of chondrocytes with a cell membrane ''ghost'' and with nucleus lacking basophilic staining. Apoptosis can be inferred by observing fragmentation of chondrocyte nuclei. Chondrocyte hypertrophy can be recognized by the relative increase of chondrocyte cytoplasm compared to other chondrocytes in the histologic cartilage layer.

Within this grade, lack of matrix colouration by cationic stains such as Safranin O or Toluidine Blue has been deliberately omitted as a defining feature. Under specified conditions, these stains can be indicative of long-standing cartilage matrix proteoglycan depletion. However, loss of matrix staining can be attributed to transient endogenous proteoglycan degradation[28] or to histologic technique, such as proteoglycan diffusion into fixative during prolonged storage in aqueous fixatives (e.g., formalin). With an intact superficial zone, reversible loss of matrix staining can be seen following acute traumatic injury. Permanent matrix cationic staining loss is associated with extensive superficial zone cell death. Reversible matrix staining loss is characteristic of the effects of proteolytic enzymes which may be derived either from cartilage or from synovium[28]. Permanent matrix staining loss is a result of cell death (necrosis and apoptosis) coupled with inhibition of chondrocyte proliferation. In contrast, focal increased matrix staining in the superficial zone may be a feature of grade 1 OA.

*Grade 2*—Grade 2 OA is characterized by focal discontinuity of the cartilage superficial zone. Within this grade, abrasion from shear forces leads to loss of small portions of superficial matrix parallel to the surface. The exfoliated fragments may appear as matrix ''flakes'' or ''fibrils'' in synovial fluid. Formally, using material science nomenclature, the loss of superficial matrix fragments parallel to the surface, ''flaking'', is known as spallation (exfoliation). Within this grade, one or more matrix cracks may extend completely through the superficial zone (deep fibrillation).

With the establishment of superficial zone discontinuity, matrix staining depletion within the upper one-third of cartilage may be present. As a reaction, focal increased staining about the chondrons, particularly in the upper mid zone, may be seen. Chondrocytes within the mid zone may show reactive changes demonstrated by clustering, as well as focal expansion and/or loss of orientation of the chondrons.

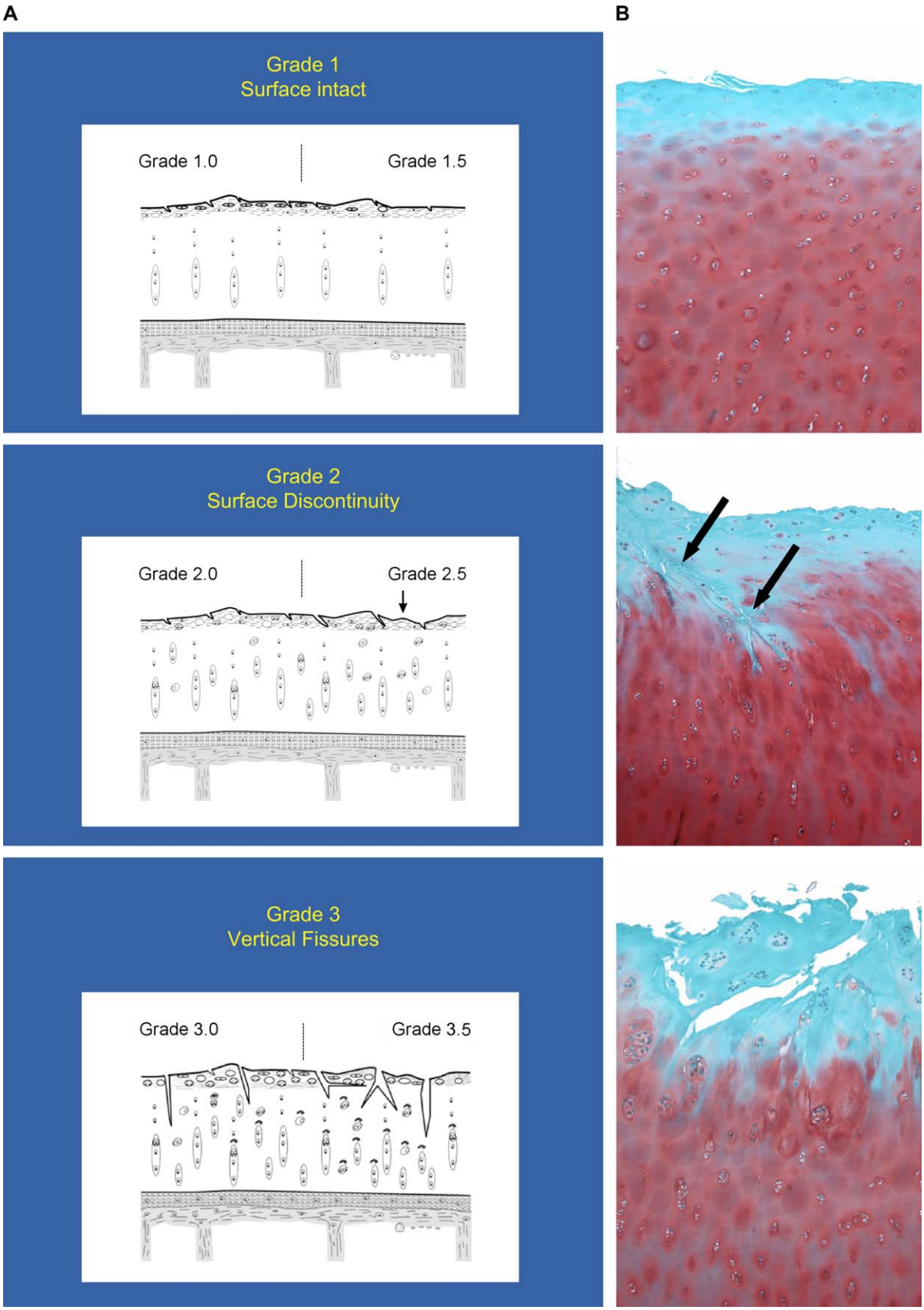


Fig. 2. OA cartilage pathology, OARSI grades 1–6, histologic features. (A & B): OA cartilage pathology: grades 1–6. Grade 1: surface intact. The articular surface is uneven and can demonstrate superficial fibrillation. This may be accompanied by cell death or proliferation. The mid zone and deep zone are unaffected. Grade 2: surface discontinuity. Focally fibrillation extends through the superficial zone to the superficial zone–mid zone portion ↓. This may be accompanied by cell proliferation, increased or decreased matrix staining and/or cell death in mid zone. Grade 3: vertical fissures extending into mid zone. The matrix fibrillation extends vertically downward into the mid zone. As the OA

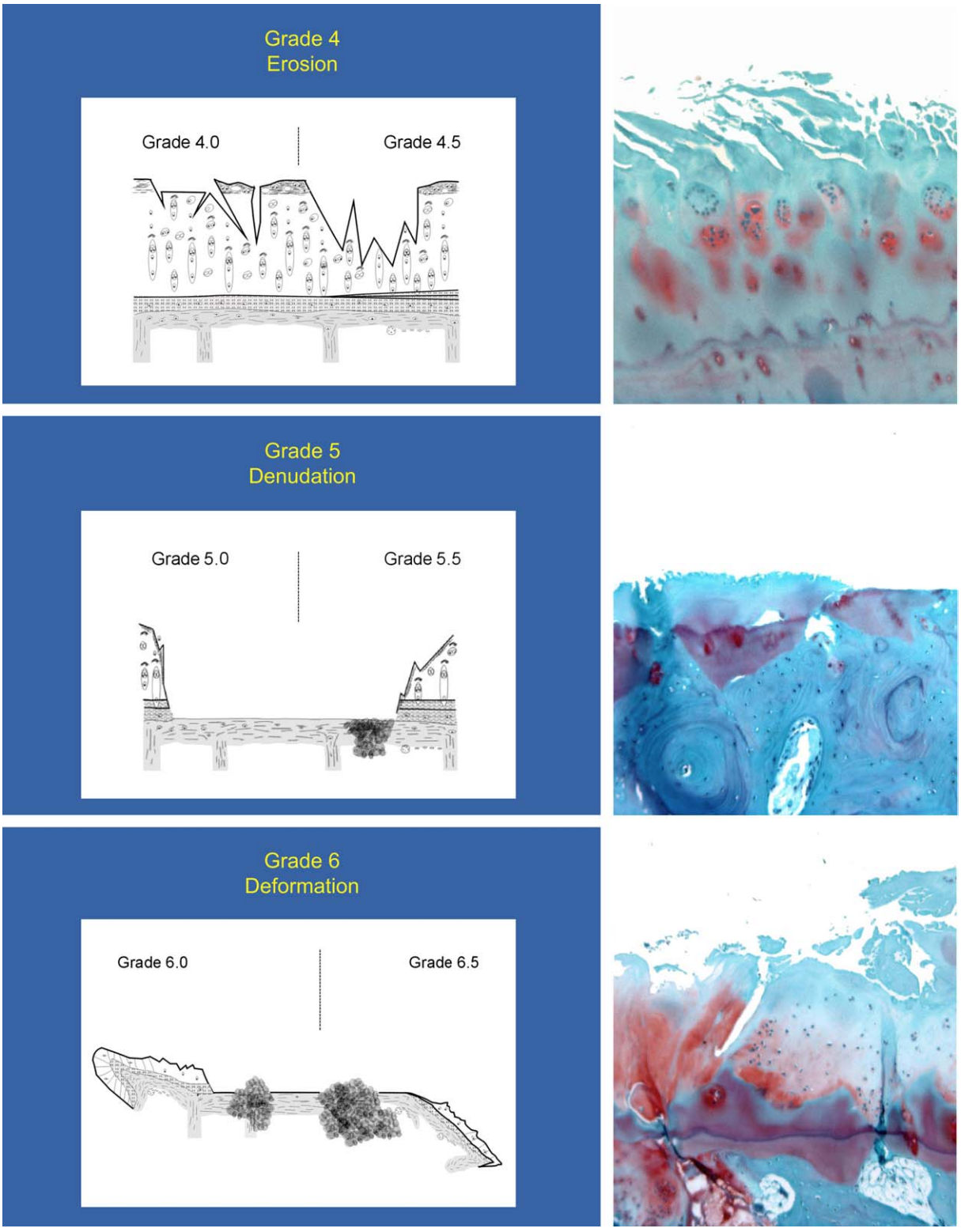


Fig. 2. (*continued*)

becomes more extensive, the fissures may branch and extend into the deep zone. Cell death and all proliferation may be observed most prominently adjacent to fissures. Grade 4: erosion. Cartilage matrix loss is observed which in earliest stage may be only delamination of superficial zone cartilage. More extensive erosion results illustrated in excavation, loss of matrix in fissured domains. Grade 5: denudation. The unmineralized hyaline cartilage is completely eroded. The articular surface is mineralized cartilage or bone. Microfracture through the bone plate may result in reparative fibrocartilage occupying gaps in the surface. Grade 6: deformation. The processes of microfracture, repair and bone remodelling change the contour of the articular surface. At the earliest phase, illustrated, fibrocartilage has grown along the level of the previously eroded and denuded surface. Fibrocartilaginous articular surfaces, marginal and central osteophytes are processes associated with more extensive articular contour deformation. (B) Safranin O stain, original magnification $\times 5$.

*Grade 3*—In grade 3 OA, extension of matrix cracks into the mid zone to form vertical fissures (clefts) is observed. As the process advances within this grade, the vertical fissures tend to extend and branch at angles from the vertical. The matrix texture is likely to become more heterogeneous, with adjacent domains of proteoglycan depletion and increased staining observed. Polarized light microscopy and/or Picro Sirius red staining after papain digestion[29] may demonstrate focal rarefaction and condensation of collagen fibres in the mid zone. Chondrocyte death, complex chondrons (chondrons a feature of chondrocyte proliferation) containing multiple chondrocytes and disoriented chondrocytes are likely to become more prominent. This may be particularly evident in the chondrons immediately adjacent to the fissures.

*Grade 4*—Cartilage erosion is the principal incremental feature of grade 4 OA. Two distinct processes can be distinguished: delamination and excavation. Delamination involves the loss of a superficial zone fragment related to the action of shear forces. Antecedent events include circumferential extension of matrix cracks from the surface through to the mid zone and tissue reaction, which degrades cartilage matrix preferentially at the superficial zone/mid zone junction or within an oedematous upper mid zone. For delamination to occur, the matrix subjacent to the reactive domain must be relatively intact, such that a tissue interface with different mechanical compliance (soft/hard) develops. Crack propagation likely occurs first by curvilinear extension of the crack. Similar to the conditions beneath the superficial zone, at the outer vertical edge of the crack, there is relatively slight reaction. As the crack expands, the fragment becomes less mechanically stable, generating more matrix reaction at the edge of the crack and at the superficial zone/mid zone interface. Further extension dislodges the fragment resulting in delamination. Delamination is recognized as the cartilage surface is relatively smooth, yet the subjacent tissues have the appearance (and reality) of mid zone cartilage organization.

Excavation represents cavity formation related to matrix loss in a circumscribed cartilage volume. This is likely to result from the extension and coalescence of branch fissures. Once again, this is not a purely mechanical phenomenon. Necrosis and chondrocyte regeneration, as well as matrix degradation (loss of matrix stain, collagen fibre rarefaction/collagen fibre condensation) may be observed in the matrix domains adjacent to the fissures. As the fissures extend and coalesce, the fragment becomes more unstable, increasing the reaction in adjacent, lateral and deeper tissues. Finally, the fragment is set free by mechanical forces as a microscopic ''loose body''. A variant process, usually at the edges of the erosion contributing to excavation, is mechanical dislodgement of deeply fissured cartilage matrix domains with release of matrix fibrils into the synovial fluid. Presumably, several conditions including pre-existing cartilage histologic structure, the rate of OA progression in cartilage and the uniformity of the reaction are determinants of the modes of excavation and the size of the released matrix fragments. Cartilage matrix at a distance from the fissures may show increased collagen condensation and new collagen fibre formation. The new collagen fibres may be thicker and more birefringent than pre-existing fibres. These collagen fibres are usually type I fibres. These fibres are produced by pre-existing chondrocytes that have undergone metaplasia to fibrochondrocytes or by chondrocytes that have migrated from reparative cartilage present in the microfracture reparative tissue in the articular plate[4,27,30].

Another process which may be present in grade 4 OA, particularly in thick cartilage, is mid zone cyst formation such as is found in chondromalacia patellae. Cyst formation results from persistent oedema, with eventual loss of matrix. This interior hollowing out process can be described as cavitation. Extension of this process can lead to delamination of the superficial and a portion of the upper zones. On a smaller length scale, yet another process in which there is loss of matrix, has been termed ''lacunar resorption''[16,17]. This represents loss of fibrillar matrix in a perichondronal area, without collagen condensation or further repair.

The area of ''resorption'' consists of fluid containing predominantly amorphous proteoglycan. This usually represents OA, which in the affected domains, has been arrested in a state of incomplete repair.

*Grade 5*—Grade 5 OA is recognized by denudation, complete erosion of hyaline cartilage to level of mineralized cartilage and/or bone, whether or not the bone surface is accompanied by fibrocartilaginous repair. Typically, the bone at the denuded surface appears denser than adjacent bone. As well, the articular bone plate is thicker, not only at the exposed area, but also beneath the adjacent cartilage. While this bone is thicker (bone/marrow ratio), this bone plate is usually less mineralized than deeper trabecular bone[31] and is more metabolically active[32].

This results in microfractures through the bone plate with fibrocartilage repair. Thus, a portion of the denuded surface may be fibrocartilage. Under some circumstances, the reparative fibrocartilage is capable of growth and filling in of part or the entire excavated hyaline cartilage volume. Another common reaction is for appositional new bone to form on the bone present at the denuded surface. Typically, this bone can form a pattern of ridges and grooves aligned in the direction of the joint motion. This pattern is still considered within grade 5 OA.

More problematic is articular plate microfracture with reparative fibrocartilage from the fracture extending upward into the deep layer of cartilage, while intact hyaline cartilage or cartilage with lower osteoarthritic grades remains above the fibrocartilage. At the level of microfracture, a simple discontinuity of the articular bony plate, this is likely a common occurrence. This repair may proceed to completion, restoring the bone plate without further cartilage disruption. Accordingly, the presence of fibrocartilage in the deep zone, under this circumstance, does not alter the OA grade. To repeat, grade 5 OA requires denudation of hyaline cartilage from the affected articular surface. In this grade, fibrocartilage may be present on the surface but no deformation of joint surface geometry is acceptable.

*Grade 6*—Grade 6 OA is characterized by deformation, change in the contour of the articular surface. This results not only from articular plate fractures, but also from increased metabolic activity of the articular bone plate, as well as from activation of connective tissue at the lateral and, sometimes, central cartilage/bone interfaces. The earliest deformative change is growth of reparative fibrocartilage focally above the level of the previously eroded and denuded articular surface. Deformation itself is a result of articular bone plate microfracture and repair often frequently repeated at adjacent sites. Articular plate fractures may lead to successive, more lateral bone microfractures in which the more central portion of the articular plate gradually slides under the more marginal plate, displacing the marginal portion outwards. Eventually, the submerged central hyaline cartilage fragment becomes bound by cancellous bone

to the marginal fragment above. Second, activation of connective tissue at the joint margin gives rise to fibrocartilaginous proliferation and, subsequently, osseous metaplasia to form an osteophyte.

In this grading system for purposes of defining grade, osteophytes as a feature by themselves are excluded from consideration.

Under some conditions, such as Diffuse Intervertebral Skeletal Hyperostosis, connective tissue at the cartilage bone joint margin becomes activated and osteophytes form without cartilaginous features of OA[33]. These types of osteophytes are excluded from consideration in this OA grading system. Similarly, osteophytes may form at earlier grades of OA where deformation is absent. As noted, osteophyte formation, at first, tends to affect the margins of articular surfaces. Osteophytes adjacent to ligamentous structures, such as ligament teres of the femoral head, are considered topologically as marginal osteophytes.

Under some circumstances, osteophytes form more centrally in the joint. These osteophytes arise from reparative fibrocartilage, which has filled space resulting from cartilage excavation, a cartilage crack and microfracture. These central osteophytes are usually associated with articular surface deformation and are considered to be a feature of grade 6 OA.

*Advanced Grading Methodology*—The six OA grades noted above can be used for most purposes. Advanced grading methodology may be required for specific research purposes, such as searching for additional features with a specific histologic grade. For these restricted purposes, it is possible to extend and subdivide the grades for more detailed examination. The advanced subgrade is indicated by adding 0.5 to the grade (Table III).

Specific features within each OA grade indicate that the lesion is more advanced. An advanced grade within the primary grade is denoted by adding 0.5 to the primary grade. Therefore, grade 1.0 would have intact chondrocytes, whereas in grade 1.5, cell death by apoptosis or necrosis would be seen. Similarly, in grade 2.0, the surface discontinuity would consist of fibrillation only, whereas in grade 2.5, additionally, abrasion of the surface with loss of a portion of the superficial cartilage zone would be seen. In grade 3, penetration into the mid zone is seen by the presence of simple fissures or clefts. In grade 3.5, extension of fissures to become branched or complex fissures is noted. In grade 4, where the key feature is erosion, grade 4.0 represents superficial zone delamination (loss of the superficial zone only), whereas grade 4.5 shows excavation into the mid zone. In grade 5, where denudation is a key feature, grade 5.0 represents presence of a bone surface which consists of intact calcified cartilage or sclerotic bone, whereas in grade 5.5 at the denuded surface, reparative fibrocartilaginous tissue or new bone formation is observed. In grade 6, grade 6.0, deformation of the joint geometry at the joint margins is seen, whereas in grade 6.5, the extent of remodelling is such that both the joint margins and force-bearing areas show deformation changes.

*OA stage*. Initially, OA progression involves focal involvement of cartilage usually subjacent to the surface affected most by mechanical force. Other portions of the articular cartilage and other cartilages in the joint remain structurally intact. With progression, osteoarthritic changes are

Table III
*OA cartilage histopathology grade assessment—advanced grading methodology*

| Grade (key feature) | Subgrade (optional) | Associated criteria (tissue reaction) |
|---|---|---|
| Grade 0: surface intact, cartilage intact | No subgrade | Intact, uninvolved cartilage |
| Grade 1: surface intact | 1.0 Cells intact<br>1.5 Cell death | Matrix: superficial zone intact, edema and/or fibrillation<br>Cells: proliferation (clusters), hypertrophy<br>Reaction must be more than superficial fibrillation only |
| Grade 2: surface discontinuity | 2.0 Fibrillation through superficial zone<br>2.5 Surface abrasion with matrix loss within superficial zone | As above<br>+Discontinuity at superficial zone<br>±Cationic stain matrix depletion (Safranin O or Toluidine Blue) upper 1/3 of cartilage (mid zone)<br>±Disorientation of chondron columns |
| Grade 3: vertical fissures | 3.0 Simple fissures<br>3.5 Branched/complex fissures | As above<br>±Cationic stain depletion (Safranin O or Toluidine Blue) into lower 2/3 of cartilage (deep zone)<br>±New collagen formation (polarized light microscopy, Picro Sirius Red stain) |
| Grade 4: erosion | 4.0 Superficial zone delamination<br>4.5 Mid zone excavation | Cartilage matrix loss, cyst formation within cartilage matrix |
| Grade 5: denudation | 5.0 Bone surface intact<br>5.5 Reparative tissue surface present | Surface is sclerotic bone or reparative tissue including fibrocartilage |
| Grade 6: deformation | 6.0 Joint margin osteophytes | Bone remodelling. Deformation of articular surface contour (more than osteophyte formation only) |
| | 6.5 Joint margin and central osteophytes | Includes: microfracture and repair |

I. Grade = depth progression into cartilage.

observed in contiguous portions of cartilage until, in advanced OA, the entire joint surface is involved.

OA stage represents the proportion of articular surface that is involved with OA compared to the total surface length, irrespective of the subjacent OA grade or whether the OA involvement is discontinuous (multiple lesions). As the grade may be variable, typically low at the lesion edge and rising higher at the centre, this method biases the histopathology assessment towards more advanced arthritis when an OA score is used.

*Recommended OA staging method*. Four OA stages are defined, depending on the horizontal extent of the involved cartilage surface irrespective of underlying OA grade. As seen in the microscopic section, stage 1 represents less than 10% involvement. Stage 2 represents 10−<25% involvement. Stage 3 represents 25−50% involvement. Stage 4 represents more than 50% involvement (Table IV). Each successive stage represents an exponential rather than linear progression from the previous stage. While arbitrary, this methodology appears more sensitive to early or mild OA than using a linear scale. Most OA lesions are continuous, although the grade within the lesion might vary. For discontinuous lesions, the stage is considered as the sum of the total surface length involved with OA to the total surface length.

*Alternative staging methods*. Staging by surface length is suitable for most applications. For specialized applications including different types of microscopy, OA stage can also be assessed as proportion of area or volume. These assessments can be denoted as follows: stage (length) ($S_l$); stage (area) ($S_a$) and stage (volume) ($S_v$). For staging according to length criteria, microscopic visualization is adequate. For staging according to area or volume assessments, morphometric techniques are recommended.

*Morphometric assessment of grade and stage*. For more specialized research applications, if desired, both grading and staging technology can be extended to incorporate morphometric technology. Stage can be assessed as percent of joint surface. Typically, these measurements are time consuming and require high expertise to define the borders of cartilage domains. Therefore, these methods should be reserved for those studies where qualitative methods are insufficiently precise to distinguish different OA groups.

*OA score*. As stated previously, OA cartilage score represents a combined assessment, based on both the severity (grade) and extent (stage) of OA in the articular cartilage. An OA cartilage score can be determined using either qualitative, semi-quantitative or quantitative methods. In considering scoring methodology, the method selected should be as simple as that required to obtain the information needed to separate OA from controls or to compare one OA set with another.

To compare an OA test group to controls, qualitative or semi-quantitative methods are usually sufficient. To compare the extent of OA between cartilage domains within a model, quantitative methods are recommended as a first choice.

*Recommended OA score method*. The recommended score is an index of combined grade and stage. The simple formula: score = grade × stage is recommended. This method produces an OA score with a range of 0−24 based on the most advanced grade and most extensive stage present (Table V). This method provides equal ordinal number weight to severity (grade) and to extent (stage). This method continues to bias OA assessment towards the most advanced disease observed.

Because this scoring method is dependent on multiples of primes, there are ''voids'' or gaps in the scoring system, representing prime numbers or multiples of primes above the primes used to assess grade for an individual specimen. Because of this mathematical anomaly for individual scores, this simple scoring system omits the OA scores 7, 11, 13, 14, 17, 21, 22 and 23. From Table V, it can be seen that this loss of linearity mostly involves OA at high grade and extensive stage. However, depending on the number of samples involved and the extent of OA, scores within these numbers may be seen in group scores (which are averages of individual scores). In the absence of an absolute standard for OA progression, this mathematical anomaly is not relevant. If required, this difficulty can be circumvented by assessing score based on measuring stage as the percentage of cartilage surface involved with OA.

*Alternative OA score methods*. (1) Qualitative Score, Simple Method. This indicates the most advanced OA grade/stage observed in the section, e.g., grade 2, stage 3. This method has the advantage of providing one grade and one stage, which is indicative of the most advanced grade and most advanced stage present. With this method, OA severity is distinguished from OA extent. (2) Qualitative Score, Complex Method. This measures the most advanced stage for each grade, e.g., G1S4, G2S3, G3S2. This method may give a more accurate portrayal of how much OA is in the joint. However, this requires much more assessment time.

The alternative methods may be useful to indicate the amount of OA in special situations, for example, individual clinical samples. With these methods, comparison between patient samples is more cumbersome and must be restricted

Table IV
*OA cartilage histopathology—stage assessment*

| Stage | % Involvement (surface, area, volume) |
|---|---|
| Stage 0 | No OA activity seen |
| Stage 1 | <10% |
| Stage 2 | 10−25% |
| Stage 3 | 25−50% |
| Stage 4 | >50% |

Stage = extent of joint involvement.

Table V
*OA score—semi-quantitative method*

| Grade | Stage | | | |
|---|---|---|---|---|
| | S1 | S2 | S3 | S4 |
| G1 | 1 | 2 | 3 | 4 |
| G2 | 2 | 4 | 6 | 8 |
| G3 | 3 | 6 | 9 | 12 |
| G4 | 4 | 8 | 12 | 16 |
| G5 | 5 | 10 | 15 | 20 |
| G6 | 6 | 12 | 18 | 24 |

Score = grade × stage.

Table VI
*OA cartilage histopathology grade assessment—comparison of methods*

| Feature | OARSI system | HHGS[7] |
|---|---|---|
| 1. Principle | Assumes histologic features present<br>Grading = vertical depth<br>Staging = surface, area or volume extent | Characteristic OA histologic features, presence and amount |
| 2. Quantification | Grading: score is progressive with depth | Specific feature or score = combination of features |
| | Staging: score is progressive with length, area or volume involved<br>Total score: qualitative and quantitative options | Total score = sum of feature scores |
| 3. Macroscopic assessment correlation (gross pathology) | Direct | Indirect |
| 4. Arthroscopy correlation | Direct | Indirect |
| 5. Imaging correlation | Direct | Indirect |
| 6. Expandable | | |
| 6.1. Subdivide grade if required | Yes | No |
| 6.2. Can include new features in grade | Yes | No |
| 6.3. Morphometry for staging | Yes | No |
| 7. Score range | 0–24 | 0–14 |
| 8. Identify early OA | Expanded proportional score 0–12 of 24 | Limited: score 0–4 of 14 |
| 9. Validity | Requires further validation | Not valid |

to counting the number of samples which are assigned to each score category.

## Discussion

A comparison of the OARSI Scoring System proposed above and the HHGS is shown in Table VI. The HHGS assigns grades to histologic features characteristic of OA, independent of the location or extent. The HHGS develops the combined score based on qualitative assignment of numbers as specific OA histologic features. At least one major feature, matrix cationic stain depletion may be physiologically transient under some conditions and, technically, is highly subject to the vagaries of histological preparation. Further, the HHGS cannot distinguish low grade OA present over a small portion of the surface from more extensive OA of the same grade. In contrast, the OARSI system assumes prior recognition that osteoarthritic histologic features are present. Where defined OA histology features are present, grade is assigned to the vertical depth within cartilage (reflecting the biologic aggressiveness of the lesion) and stage to the horizontal, two-dimensional or three-dimensional extent (surface or volume), irrespective of particular OA features. As depth and horizontal extent are simpler features to assess than differences amongst particular OA features, it is likely that the OARSI system can be applied more consistently by less experienced observers than the HHGS.

The simplest application of the OARSI system can be assessed using grade only (six grades) or stage only (four stages). Use of a cartilage OA score permits more complete assessment of the cartilage histopathology. The simple semi-quantitative score method, score = grade $\times$ stage, even with its ordinal gaps of potential scores for individual compartment assessment is the most practical for most studies. As noted above, the mathematical anomaly disappears when OA stage is assessed morphometrically.

The OARSI system also permits direct correlation of OA stage with macroscopic assessment (gross pathology), arthroscopy and imaging (X-rays, magnetic resonance imaging) or other techniques. The present system is also expandable in that the grade can be subdivided. New features can be included in the grade as they become validated. The OARSI system is also very amenable for morphometry.

A particular feature of the OARSI system is its capacity to identify differences within early or mild OA (grades 1–3). By defining earlier or mild OA features more precisely, a proportionally expanded score for early or mild OA is available. It is anticipated that the OARSI system will result in less variation amongst observers and proportionally a wider range of scores in early or mild OA than the HHGS system.

### NEXT STEPS

At this early stage, the OARSI criteria for OA histopathology assessment appear superior to previous methods. Preliminary assessment amongst multiple observers indicates that the OARSI osteoarthritis cartilage histopathology scoring system is reproducible and useful[34–36]. Much work remains to develop standards for pathologic assessment, sample preparation and staining, as well as, where needed, exclusionary criteria for other clinical forms of arthritis. Further, more extensive validation is required in both clinical and experimental arthritis before this methodology can truly be accepted as the standard with widespread consensus. While further assessment will be required, the OARSI Cartilage Histopathology Grading/Staging System is an instrument that does show considerable promise to become a standard tool for OA cartilage histopathology assessment.

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## Appendix A. OARSI cartilage pathology glossary

OA CARTILAGE: STRUCTURAL FEATURES

This glossary contains terms used in this article, together with synonyms and comments. References are to use of the terms in earlier publications, but may not be the first application of each term.

The terms are classified as follows:

A.1 Articular cartilage cells (chondrocytes) and the chondron
A.2 Articular cartilage matrix and bone: structures (matrix compartments)
A.3 OA: articular lesions
A.3.1 Cartilage matrix texture/composition
A.3.2 Cartilage matrix disruption
A.3.3 Cartilage matrix loss
A.3.4 Bone features

A.1
*Articular cartilage cells (chondrocytes) and the chondron*

| Preferred term | Definition | Synonym | Reference no. |
|---|---|---|---|
| Chondrocyte | Cartilage cell within cartilage matrix | • Chondrocyte<br>• Cell | (37) |
| Chondrocyte hypertrophy | Increase in cell size (increased cytoplasm) | | |
| Chondrocyte necrosis | Cell death (usually increased membrane permeability) | • Cell death<br>• Empty lacuna | (38) |
| Chondrocyte apoptosis | Programmed (controlled) cell death | • Pyknotic cell<br>• Fragmented nuclei | (38,39) |
| Chondrocyte atrophy | Decrease in cell size (decreased cytoplasm) | | |
| Chondrocyte density | Chondrocytes/unit area cartilage | • Chondrocyte density | (40) |
| Increased chondrocyte density | Increased chondrocytes/unit area cartilage | • Hypercellularity<br>• Increased cellularity | |
| Decreased chondrocyte density | Decreased chondrocytes/unit area cartilage | • Hypocellularity | (7) |
| Chondron | Cartilage cell including pericellular matrix<br>The chondron is a functional structure. | • Cell and pericellular matrix<br>• Chondron<br>• Lacuna | (37)<br>(4,41–45)<br>(46–49) |
| Chondron hypertrophy | Increase in chondron size | • Chondrocyte hypertrophy | |
| Chondron atrophy | Decrease in chondron size | | |
| Complex chondron | Multiple chondrocytes sharing pericellular matrix within a chondron | • Brood capsule<br>• Clone<br>• Synthetic clone<br>• Degenerative clone<br>• Cluster<br>• Clustered chondron clumps<br>• Focal cell proliferation<br>• Chondrocyte clusters<br>• Hypercellularity<br>• Diffuse hypercellularity<br>• Cell aggregates | (50)<br>(7,46,51)<br>(52)<br>(52)<br>(46,50,53)<br>(37,51)<br>(4,51)<br>(4,51)<br><br><br>(54) |
| Chondron density | Chondrons/unit area cartilage | • Chondron density | (40) |
| Chondrocytes/chondron | | • Chondrocytes/chondron cells/clusters | |

A.2
*Articular cartilage matrix and bone: structures (matrix compartments)*

| Preferred term | Definition | Synonym | Reference no. |
|---|---|---|---|
| Cartilage matrix | | | |
| Zone | Volume of cartilage parallel to joint surface with similar composition and architecture. | • Lamina<br>• Domain<br>• Layer | (51,55,56) |
| Superficial zone | Cartilage zone at joint surface. Collagen fibres are aligned parallel to surface. Chondrocytes, elongated and flattened, are aligned parallel to collagen fibres and to joint surface. | • Lamina splendens<br>• Horizontal zone<br>• Tangential zone<br>• Zone 1<br>• Superficial layer | (56–58) |
| Mid zone | Zone subjacent to superficial zone. Collagen fibres are aligned intermediately between superficial and deep zone alignments. Chondrocytes present in groups (chondrons) aligned parallel to collagen fibres. | • Transitional zone<br>• Intermediate zone<br>• Zone 2 | (56,57) |

A.2
(*continued*)

| Preferred term | Definition | Synonym | Reference no. |
|---|---|---|---|
| Deep zone | Zone subjacent to mid zone and above calcified cartilage. Collagen fibres are aligned predominantly perpendicular to joint surface. Chondrocytes within chondrons are aligned parallel to collagen fibres and perpendicular to joint surface. | • Radial zone<br>• Zone 3 | (56,57) |
| Tidemark | Zone of increased calcification at border of uncalcified and calcified cartilage. | • Calcification front<br>• Ligne bordant (line of increased basophilic stain) | (56,57) |
| Calcified cartilage | Calcified cartilage matrix. Collagen fibres and chondrocytes are aligned similar to deep zone cartilage. | • Zone 4<br>• Zone calcifié | (55,57,59) |
| Cement line | Zone of increased calcification between calcified cartilage and bone.<br>Line of increased basophilic stain. | • Cement line | |
| Bone structures | | | |
| Articular bone plate | Bone subjacent to articular cartilage. Collagen fibres aligned predominantly parallel to articular surface. | • *Os sous-chondral* | (56) |
| Subchondral trabecular bone | Bone subjacent to articular bone plate. Collagen fibres are aligned predominantly perpendicular to articular surface. | | |
| Chondron capsule | Domain of increased collagen including collagen VI surrounding chondron. Capsule is arranged spherically or in a vertical ovoid around the chondron. | | (41) |
| Chondron perilacunar matrix | Extracellular matrix within chondron | • Lacunar matrix | (41,43,45) |
| Territorial matrix | Extracellular matrix surrounding chondron. Usually, this matrix shows more intense cationic stain than the interterritorial matrix. Staining is aligned circumferentially around chondron. | | (45) |
| Interterritorial matrix | Extracellular matrix between domains of territorial matrix. Cationic staining is amorphous, not aligned to chondrons. | | (45) |

## A.3 OA: articular lesions

A.3.1
*Cartilage matrix texture/composition*

| Preferred term | Definition | Synonym | Reference no. |
|---|---|---|---|
| Matrix domain | Circumscribed area or volume of cartilage matrix with similar matrix texture/composition | • Area, volume | |
| Matrix oedema | Matrix oedema. Increase in matrix thickness | • Matrix oedema<br>• Oedema<br>• Matrix swelling<br>• Matrix hypertrophy<br>• Chondromalacia | (4) |
| Increased matrix proteoglycan | Enhanced cationic staining (Safranin O, Toluidine Blue) | • Hyperchromasia<br>• Metachromasia (Toluidine Blue)<br>• Increased charge density | |
| Decreased matrix proteoglycan | Decrease or absence of matrix cationic staining (Safranin O, Toluidine Blue) | • Staining loss<br>• Decreased charge density<br>• Glycosaminoglycan depletion<br>• Matrix proteoglycan depletion | |
| Matrix atrophy | Decrease in matrix thickness without disruption of surface layer | | |
| Collagen condensation | ↑ collagen fibre density. No alteration in collagen fibre size or orientation. Decreased space between fibres | • Fibrosis<br>• "Matrix streaks" | (60) |
| Collagen rarefaction | ↓ collagen fibre density. No alteration in collagen fibre size. Increased space between collagen fibres | • Oedema | |
| Increased collagen formation | ↑ collagen fibres with changes in fibre thickness, length (usually longer, thicker, increased heterogeneity) | • Fibrosis<br>• Amianthoid fibres | (61) |
| Collagen resorption | ↓ or absent collagen fibres. Adjacent fibres, no changes | | |

A.3.2
*Cartilage matrix disruption*

| Preferred term | Definition | Synonym | Reference no. |
|---|---|---|---|
| Fibrillation | Vertical cracks, irregularities or discontinuities of cartilage matrix confined to superficial zone | • Fibrillation<br>• Superficial fibrillation<br>• Mild fibrillation<br>• Abrasion<br>• Cleft<br>• Crack<br>• Discontinuity<br>• Minimal fibrillation<br>• Flaking, fraying, irregularity<br>• Split | (37,46)<br>(4)<br>(46)<br>(37)<br>(4,46,62)<br>(37)<br>(37)<br>(46,53)<br>(7,37)<br>(37) |
| Superficial fibrillation | Matrix discontinuity (crack) into the superficial zone | • Abrasion<br>• Crack | |
| Deep fibrillation | Matrix discontinuity through the superficial zone | • Crack<br>• Fissure | |
| Fissure | Vertical (60−90° to articular surface) matrix separation extending into mid and deep cartilage | • Fissure<br>• Simple fissure<br>• Branched fissure<br>• Cleft<br>• Crack<br>• Crevice<br>• Deep fibrillation<br>• Late fibrillation<br>• Overt fibrillation<br>• Ravine<br>• Split | (37)<br><br><br>(4,7,37,46,51,62,63)<br>(51)<br><br>(4,46,53,62)<br>(4,50)<br>(64,65)<br>(64,65)<br>(63) |
| Simple fissure | Unbranched fissure | | |
| Branched fissure | Fissure with one or more branch extensions | | |
| Complex fissure | Fissure with secondary branches propagating inward towards original fissure as well as outward | | |
| Split | Horizontal (0−60° to cartilage surface) matrix separation at junction of superficial and mid zone or to within mid or deep cartilage | | (63,66) |

A.3.3
*Cartilage matrix loss*

| Preferred term | Definition | Synonym | Reference no. |
|---|---|---|---|
| Perichondronal matrix disruption | Resorption of pericellular matrix adjacent to chondron with replacement by loose fibrous tissue ± fibrocytes | • Lacunar resorption<br>• Cellular resorption<br>• Pericellular lacunar wall<br>• Pericellular lacunar lesion<br>• Chondron enlargement | (16,52)<br>(63)<br>(49,56)<br>(48) |
| Abrasion | Focal loss of surface portion of superficial zone leaving rough surface | | |
| Delamination | Focal superficial zone matrix loss extending to mid zone | • Flaking<br>• Spallation | (67) |
| Spallation | Mechanical activity which results in loss of matrix fragment from cartilage | • Exfoliation | |
| Excavation | Activity that results in loss of cartilage fragment deep to superficial layer<br>Usually, intact cartilage is present laterally | | |
| Erosion | Loss of articular cartilage tissue including superficial and at least portions of deeper cartilage layers | • Ulceration | (68) |
| Cavitation | Formation of a cyst or fluid-filled space within cartilage<br>This process, representing extreme oedema of mid zone, may be seen in chondromalacia | • Cyst | (69) |
| Denudation | Matrix loss extending to calcified cartilage interface | • Ulceration | (68) |

A.3.4
*Bone features*

| Preferred term | Definition | Synonym | Reference no. |
|---|---|---|---|
| Tidemark | Thin layer (line) detected by increased basophilic staining demarcating transition from uncalcified to calcified cartilage | Tidemark ligne bordant | (56,70) |
| Tidemark penetration | Extension of subchondral blood vessel through the tidemark | Subchondral bone resorption<br>Subchondral bone resorption pit<br>Microcracks<br>Vascular channels<br>Vascular penetration | (45)<br>(4,44)<br>(43) |
| Tidemark advancement | Extension of calcified zone with replication of tidemark at new calcified/uncalcified cartilage boundary | | |
| Tidemark duplication | Two or more tidemarks indicative of multiple episodes of tidemark advancement | | |
| Articular plate fracture | Discontinuity through subchondral bone plate | | |
| Deformation | Distortion of contour of articular surface secondary to bone remodelling | | |
| Eburnation | Smooth shiny ''burnished'' articular surface indicative of exposed bone at articular surface | Ulceration | (68) |
| Corrugated bone articular surface | Eburnated bone surface with ridges and grooves aligned in plane of joint motion | | |
| Osteophyte | Growth of bone structure usually from joint margin (peripheral osteophyte). Osteophytes can also grow from subchondral bone into cartilage during repair of microfracture (central osteophyte) | | (50,51) |

## Appendix B. OARSI cartilage pathology

Histological preparations for OA pathology assessment

1. *OA lesion registration and block selection*: To locate the block with reference to the osteoarthritic lesion, a photograph and drawing of the articular surface is made including the lesion. A line is drawn on the document at plane and location of histologic block sample, usually through the maximal cross-section of the lesion in the plane of section.
2. *Specimen X-ray*: To record the original dimensions, as well as determine bone architecture and density. The sample is X-rayed using a fine focus equipment in an enclosed X-ray system.
3. *Fixation and decalcification*: The sample which includes articular cartilage and subjacent bone is fixed in 50 volumes of 10% neutral buffered formalin for a period of 72 h. All samples undergo macroscopic lesion identification and subsequent marking on the sample. The specimen is decalcified in 10% formic acid or equivalent ethylene diamine tetra-acetic acid solution in a container placed on an orbital shaker at 150 revolutions per minute. The decalcifying solution is changed everyday until decalcification is achieved. Completion of decalcification is monitored by performing chemical endpoint assay. Once decalcification is complete, the samples are rinsed thoroughly in distilled water to achieve neutral pH.
4. *Block processing, embedding and cutting*: The block is dehydrated in graded alcohols, cleared in xylene and embedded in Paraplast using an automated tissue processor using a schedule of 58 h. The samples are embedded with the marked lesion area closest to the surface that has the undergone microtomy.
5. *Microtomy and staining*: The blocks aligned in the same plane are cut at 5 μm and subsequently stained with Safranin O.
   To ensure consistency of staining, a control section of a block with known staining characteristics is stained with each sample. In experimental studies, both control and test slides are stained in each batch.
   Duplicate slides of selected samples are stained. If there is substantial variation in staining intensity, staining processes and solutions are reviewed. A common cause of sample variation is misalignment of microtome. This can result in a differing thickness across slide resulting in different staining densities.

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