Osteoarthritis and Cartilage 18 (2010) S17–S23

Osteoarthritis and Cartilage

The OARSI histopathology initiative – recommendations for histological assessments of osteoarthritis in the mouse

S.S. Glasson †*, M.G. Chambers ‡, W.B. Van Den Berg §, C.B. Little ||

† *Pfizer, Tissue Repair, 200 CambridgePark Drive, Cambridge, MA 02140, USA*
‡ *Eli Lilly and Company, Musculoskeletal Drug hunting Team, Lilly Corporate Center, Building 98C/B, Indianapolis, IN 46285, USA*
§ *Nijmegen Centre for Molecular Life Sciences, Rheumatology Department, PO Box 9101, 6500 HB, Nijmegen, Netherlands*
|| *Raymond Purves Bone and Joint Research Laboratories, Kolling Institute of Medical Research, University of Sydney at Royal North Shore Hospital, Level 10 Kolling Building – B6, St Leonards, NSW 2065, Australia*

ARTICLE INFO

Article history:
Received 30 April 2010
Accepted 2 May 2010

Keywords:
Osteoarthritis
Histopathology
Animal model
Mouse

SUMMARY

Aim: To describe a histologic scoring system for murine osteoarthritis (OA) that can be applied universally to instability, enzymatic, transgenic and spontaneous OA models.
Methods: Scientists with expertise in assessing murine OA histopathology reviewed the merits and drawbacks of methods described in the literature. A semi-quantitative scoring system that could reasonably be employed in any basic cartilage histology laboratory was proposed. This scoring system was applied to a set of 10 images of the medial tibial plateau and femoral condyle to yield 20 scores. These images were scored twice by four experienced scorers (CL, SG, MC, TA), with a minimum time interval of 1 week between scores to obtain intra-observer variability. An additional three novice scorers (CR, CL and MM) with no previous experience evaluated the images to determine the ease of use and reproducibility across laboratories.
Results: The semi-quantitative scoring system was relatively easy to apply for both experienced and novice scorers and the results had low inter- and intra-scorer variability. The variation in scores across both the experienced and novice scorers was low for both tibia and femur, with the tibia always having greater consistency.
Conclusions: The semi-quantitative scoring system recommended here is simple to apply and required no specialized equipment. Scoring of the tibial plateaus was highly reproducible and more consistent than that of the femur due to the much thinner femoral cartilage. This scoring system may be a useful tool for both new and experienced scorers to sensitively evaluate models and OA mechanisms, and also provide a common paradigm for comparative evaluation across the many groups performing these analyses.

Introduction

The histologic evaluation of osteoarthritis (OA) in the mouse has increased exponentially in the past decade with the advent of transgenic animals being used to look for mechanisms involved in the development of OA. The first significant reports of mice developing OA were in the mid-1900s[1–3] and included studies showing that aged C57BL/6 mice developed spontaneous, idiopathic OA. Investigators noted that murine OA exhibited many of the same pathologic features as the approximately 2500-fold heavier human, including loss of proteoglycan (PG) staining, fibrillation, cloning, and erosion of cartilage matrix. Other spontaneous models were intermittently reported over the subsequent decades (STR/ort[4], STR-1 N[5]) and included a number of spontaneous mutations[6,7] that had human counterparts which also developed early OA. The observations that some inbred strains of mice had far greater incidence and severity of OA than others, in early adult-hood as well with advanced-age, were important as they indicated that murine OA was more than a "wear and tear" phenomenon and had a strong genetic basis.

As murine embryonic stem (ES) cells, transgenic, knock out (KO) and knock in (KI) techniques became widely available, mice have been extensively used to replicate the genetic defects and biochemical processes thought to be involved in the development

* Address correspondence and reprint requests to: S. Sonya Glasson Consulting, 2 Pinewood St, Lexington, MA 02421, USA.
E-mail addresses: ssglasson@gmail.com (S.S. Glasson), chambers_mark_geoffrey@lilly.com (M.G. Chambers), W.vandenBerg@reuma.umcn.nl (W.B. Van Den Berg), christopher.little@sydney.edu.au (C.B. Little).

1063-4584/$ – see front matter © 2010 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.joca.2010.05.025

of human disease. These mice also allow for a better understanding of the mechanisms of OA development and confirm the role of critical molecules, such as degradative enzymes[9], in the disease process. Therefore in the past two decades, the mouse is being used not only to replicate known human diseases, but also to examine the impact of deleting, over-expressing or altering critical enzymes or structural proteins that could impact OA pathogenesis. The replication of human pathology in the mouse following the targeting of the same human genetic mutation validates the utility of mouse models of genetic conditions[8].

A universal system for histologic scoring of murine OA would allow comparison of the severity of cartilage destruction across different spontaneous, enzymatic, chemical or surgically-induced murine OA models. A universal system would also provide a more objective evaluation as to the relative level of disease acceleration or amelioration using a specific treatment or gene-deleted mouse. This could allow prioritization of resources to those targets found to be more critical to OA progression in the mouse. The proposed system is considered sufficiently resilient to be utilized for all the widely used models of murine OA[10], including surgical, intra-articular (IA) collagenase, and spontaneous models.

This paper will restrict itself to the description of histology of the knee only, since the knee is the predominant joint for spontaneous OA development and is sufficiently large for IA access (chemical, enzymatic and cruciate disruption models) and for microsurgery.

Anatomy and joint pathology

The anatomy of the mouse knee resembles that of other species and is only notable from other mammals by its extremely small size. Typical mice weigh only 20–40 g, more than 10-fold less than rats, and 2500-fold less than man. The cartilage of the mouse is only 30 μm thick, which is nearly 10-fold thinner than the rat and approximately 50-fold thinner than man[11]. The layer of calcified cartilage is nearly as thick as the non-calcified cartilage (or even thicker in some joint regions), which is in stark contrast to the thin calcified cartilage layers seen in larger animals and humans. The organization and pathology of cartilage degeneration in the mouse are largely related to the extremely thin cartilage. The cartilage is only several cell layers thick and does not have clearly distinguishable superficial, transitional and radial zones. It is rare to capture the pathology extending through different depths in the non-calcified cartilage, as non-calcified cartilage loss tends to be an all-or-none phenomenon. The pathology of cartilage degeneration tends to progress rapidly from a loss of PG, then mild fibrillation, through focal, extending to broader, full-thickness loss of non-calcified cartilage.

- Mouse cartilage is very thin and rapidly progresses to full-depth fibrillation, which starts as focal regions of non-calcified cartilage loss, and progressively involves larger areas.

Macroscopic scoring of mouse cartilage degeneration

Due to the extremely small size of the mouse, macroscopic staging of cartilage degeneration is difficult and should utilize dissecting microscopes, microsurgical dissection and potentially the use of dyes such as India ink to contrast the lesions. Due to the shallow nature of the lesions, depth information may not be available. We recommend preserving intact mouse joints for histology so that the intact joint can be evaluated without a concern for iatrogenic damage inflicted at dissection and kissing lesions can be appreciated between the tibia and femur. The entire mouse joint is small enough to be captured on a single microscopic section, decreasing sampling bias for histology.

- Macroscopic scoring of mouse OA is not routinely performed.

Microscopic scoring of mouse cartilage degeneration

Specimen preparation

Histology is the gold standard for evaluation of murine OA. Knee joints are dissected free of skin or excess muscle, and placed in a fixation solution. The patella (or other orienting region) may be stained with a tissue marker to aid in orientation to provide consistent embedding. Murine knee joints can undergo frozen sectioning or be embedded in plastic, but in most joints are decalcified and paraffin embedded. The paraffin methodology requires less specialized equipment, is cost-effective and provides high quality slides adequate for most purposes and will be the method described here. Twenty-four hours fixation in 10% formalin is utilized for routine histology, with 4% paraformaldehyde providing extra flexibility for immunohistochemistry. The samples are then transferred to a decalcification solution which may be a formic acid (10% v/v), commercially-available decalcification solution, or 20% ethylenediaminetetraacetic acid (EDTA). Seven days in 20% EDTA on a plate shaker at RT or 48 h in 10% formic acid is generally sufficient to decalcify adult mouse knees depending on the surrounding length of tibia and femur. Longer decalcification times are required for very large and/or aged mice such as the STR/ort mice. Formic acid or other rapid decalcification systems require less time but must be carefully optimized so that excessive decalcification, leading to decreased staining of PGs is avoided. Following decalcification, the samples are thoroughly rinsed and samples processed with graded alcohol dehydration and infiltration with paraffin. The knee joints are then embedded in paraffin blocks. We recommend frontal (coronal) embedding as it allows for concurrent evaluation of the medial and lateral tibio-femoral joints and fewer sections need to be evaluated in the anterior–posterior plane than in the medial to lateral plane to incorporate the whole joint. Since the pathology in novel murine OA models or in genetically modified animals cannot be predicted to occur in only the medial compartment, the evaluation of the entire joint is important. Embedding the joint to provide frontal sections is more difficult than that for sagittal sections. Accurate identification of the femur, tibia and patella is critical so that the patella can either be embedded uppermost or at the bottom of the paraffin mold.

- Joints are usually fixed for 24 h in formalin, decalcified for 7 days in 20% EDTA, then embedded frontally in paraffin.

Sectioning

The method of sectioning in the literature varies greatly. Many groups utilize serial sections through the entire knee[12] while others restrict the evaluation to a focal region. Some studies describe the methodology of sectioning through the entire joint and stipulate the location of lesions[4]. Lapveteläinen *et al.*[13] utilize frontal sections located at the insertion of the anterior cruciate ligament to examine the four quadrants there and at two more 200 μm intervals, to cover approximately the central third of the volume of the knee. In many papers, the location of sections for analysis is not always clearly disclosed.

Since the whole mouse joint is small and harvested intact, rather than a specific gross lesion or joint region, it is feasible to section the entire joint. Sectioning requires a trained histologist to recognize the start and stop landmarks in a joint, usually confirmed on

Table I
Comparison of the commonly referenced mouse OA scoring paradigms contrasting the regions analyzed and complexity of the scoring

Scoring system	Embedding	Interval of section evaluation	Region scored	System	Add-on systems	Reported score
Walton[4]	Frontal	25 μm	MTP	0–4	–	0–4
Lapveteläinen *et al.*[13]	Frontal, 90° angle	3 central 200 μm levels	MTP, LTP, MFC, LFC	0–4	–	0–4 (4 separate scores)
Chambers *et al.*[12]	Frontal		MTP, LTP	0–6	–	0–6 (2 separate scores)
Visco *et al.*[15]	Sagittal	Medial only	MTP (MFC?)	0–5	Osteophytes; bone; synovitis; PG Staining etc.	0–16 (with add-ons)
Brewster *et al.*[16]	Frontal	Score single central section from 5 × 60 μm slides	MTP, LTP, MFC, LFC	0–4	–	0–16 (whole joint)
Mahr *et al.*[17]	Frontal	5 semi-serial	MTP, LTP, MFC, LFC	0–6	–	0–6 (whole joint)
Rudolphi *et al.*[18]	Frontal	5 × 70 μm levels	MTP, LTP, MFC, LFC	0–8	PG staining; bone; cellularity	0–32 (with add-ons; 4 separate scores)
Glasson *et al.*[19]	Frontal	80 μm	MTP, LTP, MFC, LFC	0–3	–	0–12 (maximal score); 0 to >200 (Summed score)
Kamekura *et al.*[20]	a. Frontal b. Sagittal	a. Through joint b. Medial only	MTP (LTP?) MTP	0–4	Osteophytes	0–4
Bomsta *et al.*[21]	Frontal 120° angle	6 × 210 μm levels	MTP, LTP, MFC, LFC	0–6	PG staining; cellularity	0–6 (average rather than maximal)

unstained slides, or otherwise take excessive numbers of sections for staining and scoring. The landmarks we employ for the posterior aspect of the joint are the appearance of the flattened tibial plateau (usually prominent for some distance beyond the femoral condyles). For the anterior margin, the entry of significant amounts of synovial tissue in the joint space with flattening of the femur and loss of cartilage on the tibia is an appropriate position to stop collecting sections.

Three 4–6 μm sections can be placed on each slide to allow for redundancy in sections in case of histologic artifacts on any particular section. It is usual to obtain 13–16 slides, harvested at approximately 80 μm intervals, for histologic scoring of the entire articular surface of a mouse knee joint embedded frontally. Intervening sections can either be discarded or placed on slides, and stored for additional stains or immunohistochemistry.

- Serial step frontal sections are recommended to encompass lesions in all weight-bearing areas of the femoro-tibial joint.

Staining

The primary slides are stained for cartilage PGs using either a Safranin-O Fast-Green technique or Toluidine blue Fast-Green. Either staining technique can be utilized with the scoring paradigm described here.

Histologic scoring

Multiple scoring systems exist in the literature and those that have been applied to the mouse have a dramatic range in complexity and are almost exclusively restricted to evaluation of the knee joint. Some scoring paradigms restrict evaluation of OA to cartilage destruction[4,12,14], while others involve multiple aspects of OA including bone, osteophyte and synovial changes[15]. A summary of the some of the published histologic scoring systems[4,12–21] is provided in Table I, but is far from exhaustive. In the majority of cases, a single maximal score is reported. Many of the most widely used histologic scoring systems are the simplest and the best described. These include the 0–4 score of Walton[4], Wilhelmi and Faust[14], or the 0–6 score of Chambers *et al.*[12].

Traditional scoring systems utilized for human OA, such as the Mankin system[22], have been applied to the mouse knee[7,23] although its relevance must be questioned given that the zonal structure in the mouse is not easily identified (due to the extremely thin[11] cartilage). The loss of PG staining and fibrillation, clefts and erosion through the cartilage zones are also not as gradual as in the human or larger animal species. Histologic sections can be evaluated at frequent intervals to determine the extent of area involved, and not just the single most severe lesion as for the Mankin system.

The recommended scoring system (Table II) is a modification from Chambers *et al.*[12] and is recommended to apply to all four quadrants of the joint: medial femoral condyle (MFC), medial tibial plateau (MTP), lateral femoral condyle (LFC), lateral tibial plateau (LTP). A score of 0 represents normal cartilage, 0.5 = loss of PG with an intact surface, 1 = superficial fibrillation without loss of cartilage, 2 = vertical clefts and loss of surface lamina (any % or joint surface area), 3 = vertical clefts/erosion to the calcified layer lesion for 1–25% of the quadrant width, 4 = lesion reaches the calcified cartilage for 25–50% of the quadrant width, 5 = lesion reaches the calcified cartilage for 50–75% of the quadrant width, 6 = lesion reaches the calcified cartilage for >75% of the quadrant width. If scores are kept separately, it is possible to identify where in the joint the most severe lesions occur as well as the global extent of damage (Supplemental Table S1). This method focuses on the structural changes observed and the lesions of fibrillation are generally preceded or co-incide with losses in PG staining.

- For simple histologic scoring of OA in the mouse, we recommend a 0–6 subjective scoring system (Table II), to be applied to all four quadrants and through multiple step sections through the joint. The OA severity is expressed as summed and/or maximal scores which can be combined for the entire joint, or split out for MTP, MFC, LTP or LFC.

Table II
The recommended semi-quantitative scoring system

Grade	Osteoarthritic damage
0	Normal
0.5	Loss of Safranin-O without structural changes
1	Small fibrillations without loss of cartilage
2	Vertical clefts down to the layer immediately below the superficial layer and some loss of surface lamina
3	Verical clefts/erosion to the calcified cartilage extending to <25% of the articular surface
4	Vertical clefts/erosion to the calcified cartilage extending to 25–50% of the articular surface
5	Vertical clefts/erosion to the calcified cartilage extending to 50–75% of the articular surface
6	Vertical clefts/erosion to the calcified cartilage extending >75% of the articular surface

Microscopic scoring of synovial, bone or PG alterations

Additional scoring parameters can be utilized as separate scoring criteria for the presence of osteophytes, subchondral bone changes, and synovitis. It is recommended that each of these be scored and reported separately using a 0–3 scoring paradigm where 0 is normal, 1 = mild, 2 = moderate and 3 = severe changes. In general, osteophytes and subchondral bone thickening are observed in concert with cartilage degradation, and the subchondral bone changes co-localize with the cartilage lesion. MicroCT can also be utilized to quantitate the bone changes associated with OA[24]. Synovitis is not an appreciable feature of the milder surgical models of OA such as the DMM model. Notes should be made on any anomalies such as blood presence in the joint, deposits, abnormal appearance of ligaments (chondrogenesis, degradation, etc.), meniscii, and subchondral bone cysts, etc. if they are present.

PG depletion can be measured subjectively with either Safranin-O or Toluidine blue staining and a semi-quantitative scoring system (Supplemental Table S2) used as an adjunct to the structural cartilage damage system (described above). It is recognized that in the face of significant loss of non-calcified cartilage the PG score becomes less meaningful. Nevertheless, significant progression of PG loss may still be observed extending beyond the erosive lesions.

Computerized systems can also be utilized with single-stained (no counter-stain) sections[25] to quantitatively measure PG loss, but this method, while very objective for PG retention, requires more specialized equipment and is dependent on the quantity or selection technique of regions analyzed. Other computerized systems are also available for measurement of many parameters including cartilage volume, cell number, etc. These are very sensitive tools and much additional information can be extracted from these analyses, the major drawbacks being the time to select, capture and analyze each image. Given that the method recommended here is

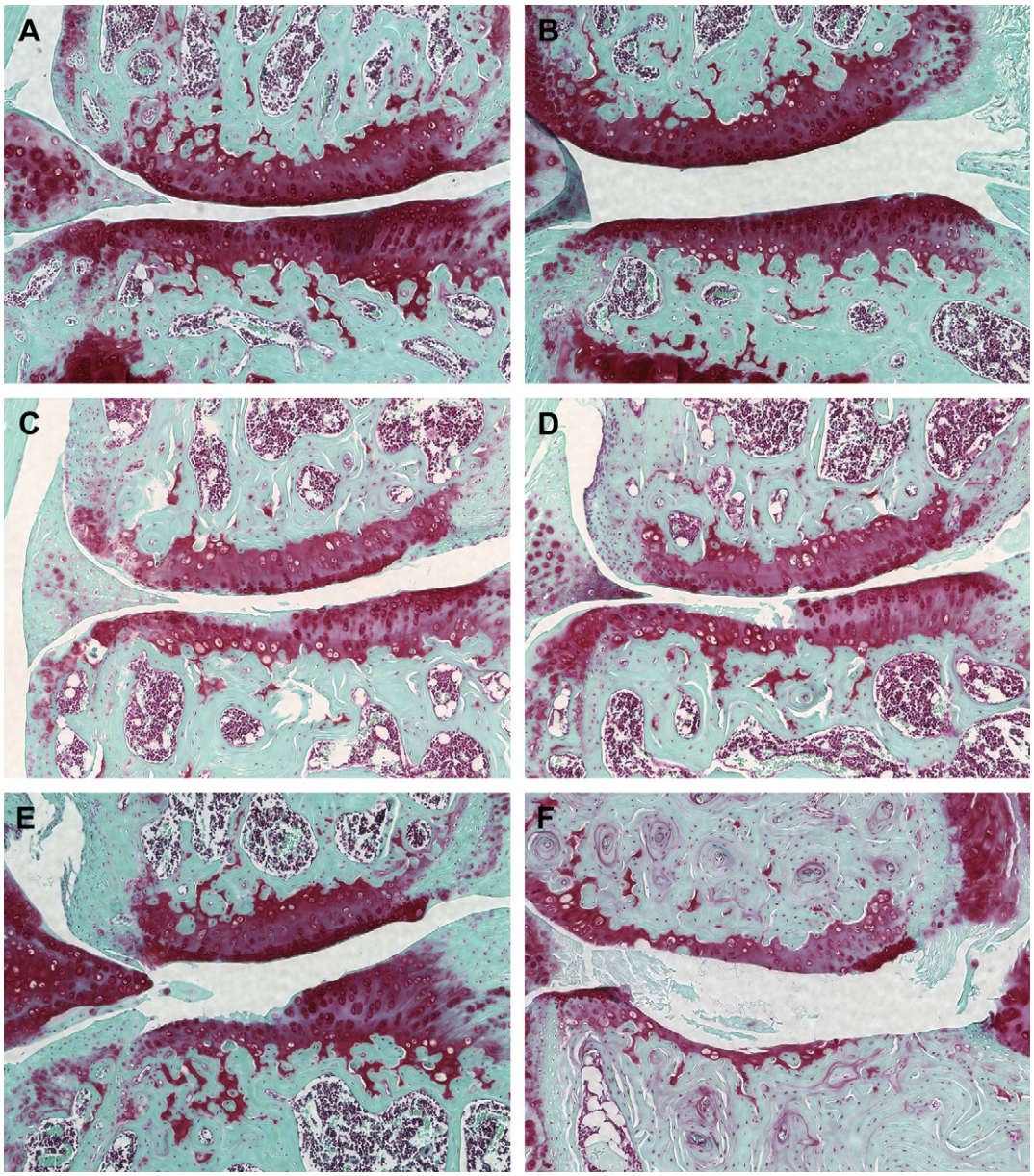

Fig. 1. Safranin-O photomicrographs showing the MFC (above) and MTP (below) and the medial meniscus (left), displaying a variety of OA severity and semi-quantitative scores. First score represents MFC, second score is MTP; (A) 0, 0.5; (B) 0, 1; (C) 0.5, 2; (D) 3, 3; (E) 0, 4; (F) 5, 6.

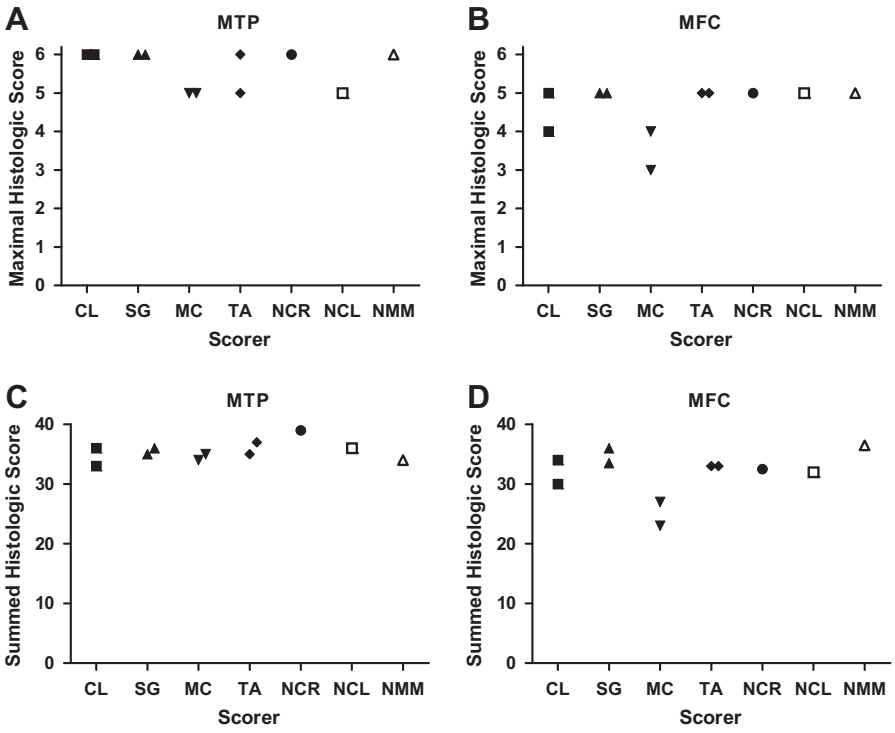

Fig. 2. Variation across experienced and novice scorers for maximal histologic score across the MTP (A), MFC (B), and summed MTP (C) and MFC (D) scores. The greatest consistency in scores was observed for the summed MTP data sets.

very quick to implement and the number of animals in mouse studies usually great, the use of computerized methodologies will not be explored here.

- Additional scores for synovial, bone or PG alterations can complement the basic summed or maximal scores.

Intra- and inter-observer validation study

The OA grading table (Table II) along with representative images from spontaneous and surgically-induced OA [Fig. 1(A–E)] was sent to all scorers. Ten frontal Safranin-O/Fast-green sections through a surgically-induced OA knee of moderate to severe severity were digitally captured using a 20× objective (total of 10 images) [Supplemental Fig. 1(A–I)]. All images were stored as JPEG files and sent concurrently to the assigned four experienced (CL, SG, MC, TA) and three novice (CR, CL, MM) scorers, along with an electronic scoring sheet which recorded each score given and the location for comparison across scorers. At a minimum time interval of 1 week later, the same slides were read by the experienced scorers and entered into a separate worksheet. This data was analyzed to obtain the reproducibility between experienced and non-experienced scorers, as well as the intra-observer reproducibility of experienced scorers.

All scorers were able to complete the scoring with no further instruction than that provided from the table and representative images. The inter-observer reproducibility of scores for both experienced and novice scorers was excellent [Fig. 2(A–D)] and was the most uniform with MTP summed scores (1C). A statistician performed a Pearson's correlation coefficient and the more appropriate intra-class correlation coefficient (Supplementary Table S3) which confirmed reproducibility. One scorer

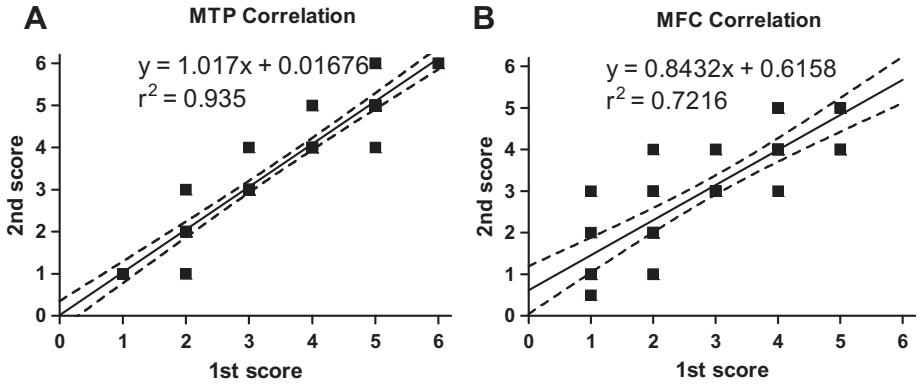

Fig. 3. Intra-observer reproducibility over time was evaluated by comparing the scores for the same images at two different times at a minimum of 1 week apart. Both the MTP (A) and MFC (B) data sets, each with 40 paired scores, had a high correlation. The highest reproducibility was observed with the MTP lesions. The dotted lines indicate the 95% confidence intervals.

consistently had lower MFC scores than the other scorers and it was noted that scoring of the femoral condyle was more difficult than the tibial plateaus due to the much thinner cartilage, difficulty in identifying the tidemark and convexity of the condyles making it difficult to determine the area to evaluate. The low variability across all the scores is apparent in the low variability in data displayed in Supplemental Table S1. The mean ± standard deviations for the summed MTP and MFC scores were 35.5 ± 1.6 and 31.8 ± 3.9. The mean ± standard deviations for the maximal MTP and MFC scores were 5.6 ± 0.5 and 4.6 ± 0.7. The correlation of the pairs of 40 raw scores following repeat scoring to assess the intra-observer reproducibility was excellent on the MTP [Fig. 3(A)] and sufficient on the MFC [Fig. 3(B)].

Discussion

The semi-quantitative scoring system proposed in this study was relatively easy to apply for both experienced and novice scorers, and the final version was not present in any of the laboratories before this scoring exercise took place. The reproducibility in scores for a first time deployment was excellent as no scorer received training or retraining following any of their scores. It is anticipated that a common series of images could be utilized for training or retraining of individuals performing these studies, so that uniformity in application of the scoring system could be further optimized.

This methodology allows for a rapid, yet thorough evaluation of histologic changes through a murine OA knee joint. This method should be sufficiently sensitive for selection of the more promising models or drug targets, as earlier (less optimized) versions of this system were. Any joints of interest can undergo further detailed histologic analyses. Given that the OA field is relatively small and the distribution of models, KO mice and DMOAD agents is limited, it is very important that we obtain a common paradigm to quantitate the magnitude of change observed with any one of these. This will enable our community to appreciate the factors driving major and minor changes associated with the progression of murine OA.

Conflict of interest

No author has any conflict of interest surrounding this work and no external sources of funding were provided. Sonya Glasson was an employee and holds stock in Pfizer; Mark Chambers is an employee and holds stock in Eli Lilly and Company; Wim Van Den Berg receives project funding from Schering Plough; Christopher Little receives project funding from Pfizer Inc. and Fidia Farmaceutici S.p.A.

Acknowledgements

Many thanks are extended to Shuguang Huang for performing the Pearson's and intra-class correlation coefficients and the new scorers Christine Resmini, Charles Lin and Margaret McNalty who evaluated this scoring paradigm. We appreciate the advice of Anna-Marja Säämänen and Cathy Carlson and recognize the valuable contributions of Diane Peluso for histologic expertise and Thomas Aigner (TA) for co-evaluating the histologic slides.

Printing costs were supported by an unrestricted educational grant to OARSI by Bayer, Expanscience, Genzyme, Lilly, MerckSerono, Novartis, Pfizer, SanofiAventis, Servier, and Wyeth. The work performed was not influenced at any stage by the support provided.

Supplementary material

Supplementary data associated with this article can be found in online version at doi:10.1016/j.joca.2010.05.025.

References

1. Sokoloff L. Natural history of degenerative joint disease in small laboratory animals. I. Pathological anatomy of degenerative joint disease in mice. AMA Arch Pathol 1956;62:118–28.
2. Silberberg M, Silberberg R. Age changes of bones and joints in various strains of mice. Am J Anat 1941;68:69–95.
3. Silberberg M, Silberberg R. Osteoarthrosis and osteoporosis in senile mice. Gerontologia 1962;6:91–101.
4. Walton M. Degenerative joint disease in the mouse knee; histological observations. J Pathol 1977;123:109–22.
5. Jay Jr GE, Sokoloff L. Natural history of degenerative joint disease in small laboratory animals. II. Epiphyseal maturation and osteoarthritis of the knee of mice of inbred strains. AMA Arch Pathol 1956;62:129–35.
6. Silberberg R. Epiphyseal growth and osteoarthrosis in blotchy mice. Exp Cell Biol 1977;45:1–8.
7. Glasson SS, Trubetskoy OV, Harlan PM, Chavarria AE, Haimes HB, Jimenez PA. Blotchy mice: a model of osteoarthritis associated with a metabolic defect. Osteoarthritis Cartilage 1996;4:209–12.
8. Reginato A, Olsen B. The role of structural genes in the pathogenesis of osteoarthritic disorders. Arthritis Res 2002;4:337–45.
9. Glasson SS, Askew R, Sheppard B, Carito B, Blanchet T, Ma HL, *et al*. Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. Nature 2005;434:644–8.
10. Smith M, Little C. In: Moskowitz Rea, Ed. Experimental models of osteoarthritis. Philadelphia: Lippincott Williams & Wilkins; 2007:107–25.
11. Simon WH. Scale effects in animal joints. I. Articular cartilage thickness and compressive stress. Arthritis Rheum 1970;13:244–56.
12. Chambers MG, Bayliss MT, Mason RM. Chondrocyte cytokine and growth factor expression in murine osteoarthritis. Osteoarthritis Cartilage 1997;5:301–8.
13. Lapveteläinen T, Nevalainen T, Parkkinen JJ, Arokoski J, Kiraly K, Hyttinen M, *et al*. Lifelong moderate running training increases the incidence and severity of osteoarthritis in the knee joint of C57BL mice. Anat Rec 1995;242:159–65.
14. Wilhelmi G, Faust R. Suitability of the C57 black mouse as an experimental animal for the study of skeletal changes due to ageing, with special reference to osteo-arthrosis and its response to tribenoside. Pharmacology 1976;14:289–96.
15. Visco DM, Orevillo CJ, Kammerman J, Kincaid SA, Widmer WR, Christen AJ, *et al*. Is the murine partial medial meniscectomy model a useful model? Trans Orthop Res Soc 1998;23:876.
16. Brewster M, Lewis EJ, Wilson KL, Greenham AK, Bottomley KM. Ro 32-3555, an orally active collagenase selective inhibitor, prevents structural damage in the STR/ORT mouse model of osteoarthritis. Arthritis Rheum 1998;41:1639–44.
17. Mahr S, Menard J, Krenn V, Muller B. Sexual dimorphism in the osteoarthritis of STR/ort mice may be linked to articular cytokines. Ann Rheum Dis 2003;62:1234–7.
18. Rudolphi K, Gerwin N, Verzijl N, van der Kraan P, van den Berg W. Pralnacasan, an inhibitor of interleukin-1beta converting enzyme, reduces joint damage in two murine models of osteoarthritis. Osteoarthritis Cartilage 2003;11:738–46.
19. Glasson SS, Askew R, Sheppard B, Carito BA, Blanchet T, Ma HL, *et al*. Characterization of and osteoarthritis susceptibility in ADAMTS-4-knockout mice. Arthritis Rheum 2004;50:2547–58.

20. Kamekura S, Hoshi K, Shimoaka T, Chung U, Chikuda H, Yamada T, *et al*. Osteoarthritis development in novel experimental mouse models induced by knee joint instability. Osteoarthritis Cartilage 2005;13:632–41.
21. Bomsta BD, Bridgewater LC, Seegmiller RE. Premature osteoarthritis in the Disproportionate micromelia (Dmm) mouse. Osteoarthritis Cartilage 2006;14:477–85.
22. Mankin HJ. Biochemical and metabolic aspects of osteoarthritis. Orthop Clin North Am 1971;2:19–31.
23. Neuhold LA, Killar L, Zhao W, Sung ML, Warner L, Kulik J, *et al*. Postnatal expression in hyaline cartilage of constitutively active human collagenase-3 (MMP-13) induces osteoarthritis in mice. J Clin Invest 2001;107:35–44 [see comment].
24. Botter S, Glasson SS, Hopkins B, Clockaerts S, Weinans H, van Leeuwan J, *et al*. ADAMTS-5−/− mice have less subchondral bone changes after induction of osteoarthritis through surgical instability: implications for a link between cartilage and subchondral bone changes. Osteoarthritis Cartilage 2009;17:636–45.
25. Saamanen AM, Hyttinen M, Vuorio E. Analysis of arthritis lesions in the Del1 mouse: a model for osteoarthritis. Methods Mol Med 2007;136:283–302.