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A comparison of radiographic, arthroscopic and histological measures of articular pathology in the canine elbow joint

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ABSTRACT

Validation of radiographic and arthroscopic scoring of joint pathology requires their comparison with histological measures of disease from the same joint. Fragmentation of the medial coronoid process (FMCP) is a naturally occurring disease of the canine elbow joint that results in osteoarthritis, and the objectives of this study were to compare the severity of histopathological changes in the medial coronoid process (MCP) and medial articular synovial membrane with gross radiographic scoring of elbow joint osteophytosis and the arthroscopic assessment of the MCP articular cartilage surface.

Radiographic scoring of osteophytosis and the arthroscopic scoring of visual cartilage pathology of the MCP correlated moderately well with the histopathological evaluation of cartilage damage on the MCP and synovial inflammation in the medial part of the joint, but not with bone pathology in the MCP. Marked cartilage pathology on the MCP was identified in joints with either no radiographic evidence of osteophytosis or with mild cartilage damage that was evident arthroscopically.

Introduction

Fragmentation of the medial coronoid process of the ulna (FMCP) is a common developmental disease of the canine elbow joint most frequently observed in young medium to large breed dogs (Mason et al., 1980; Meyer-Lindenberg et al., 2002; Moores et al., 2008). The aetiology of FMCP is undetermined at present (Danielson et al., 2006; Haudiquet and Rochereau, 2007). Several factors have been implicated in the past, such as radioulnar joint incongruity (Wind, 1986; Wind and Packard, 1986; Samoy et al., 2006; Gemmill and Clements, 2007), trauma (Guthrie et al., 1992; Haudiquet and Rochereau, 2007), genetics (Ekman and Carlson, 1998), growth rate (Ekman and Carlson, 1998), nutrition, ischaemia (Ekman and Carlson, 1998) and osteochondrosis (Grondalen and Grondalen, 1981; Wolschrijn et al., 2005). Direct identification of MCP disease, as well as the assessment of the articular surface, can be achieved by arthroscopic (van Ryssen et al., 1993; Bardet, 1997; van Ryssen and van Bree, 1997; van Bree and van Ryssen, 1998) or open arthrotomy evaluation of the elbow joint.

Osteophytosis is the radiographic hallmark of osteoarthritis (OA), and is often the only radiographic finding associated with MCP disease (Carpenter et al., 1993). Osteoarthritis is characterised by destruction of articular cartilage, but the involvement of other tissues in the pathogenesis of the disease process such as bone (Rogers et al., 2004), synovium (Dingle, 1981), fat (Ushiyama et al., 2003) and ligaments (Hill et al., 2005) is recognised. The radiographic changes which characterise OA are delayed relative to histological (Gilbertson, 1975) and gene expression changes (Stoker et al., 2006) in experimental canine OA. The histological changes reported with MCP disease in dogs include fatigue microdamage of the bone, increased bone porosity, a reduction in osteocyte density, and thickening of articular cartilage (Danielson et al., 2006). Changes in gene expression have been identified in cartilage and bone of the diseased MCP which correlate with the radiographic grade of OA (Clements et al., 2009). However, the utility of clinical measures as an indicator for disease severity at a microscopic level has not been evaluated in naturally occurring canine joint disease.

In this study we evaluated the clinical measures of osteoarthritis (radiographic and arthroscopic changes) in elbows affected by MCP disease and related them to the histological changes observed on the surgically removed MCP. All findings were compared to a control group of dogs without evidence of elbow osteoarthritis or MCP disease. We hypothesised that arthroscopic changes of the articular cartilage surface of the MCP would be closely related to

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the histological changes observed in cartilage of the MCP, but not to the bone of the MCP or the synovium of the medial aspect of the joint. We further hypothesised that these changes would not correlate with the severity of the radiographic changes identified on each elbow joint.

Material and methods

Samples

The MCP was evaluated by arthroscopy using a 2.7 mm 30° oblique arthroscope (Hopkins) in 53 elbows of 34 dogs [Labrador (22), cross breed (3), German Shepherd (2), Golden Retriever (2), Rottweiler (2), Mastiff (1), Newfoundland (1), Hungarian Viszla (1)] with lameness and elbow discomfort attributable to FMCP. Fragmentation of the medial coronoid process was diagnosed by arthroscopic and arthrotomy evaluation, as previously described (Danielson et al., 2006). The mean age of the affected dogs was 11 months (±4.5 months, range 5–23 months) with a mean bodyweight of 30.7 kg (±12.1 kg, range 7.2–70.0 kg).

Orthogonal radiographs were obtained from each elbow. The visual changes identified in the articular cartilage were scored at the time of surgery (Modified Outerbridge Score; Schulz, 2003) by a single experienced observer (NF). Subtotal coronoid ostectomy was performed by removing the MCP at its base perpendicular to its long axis by using an oscillating saw (Danielson et al., 2006). Synovial membrane was taken from the medial aspect of the joint at the arthrotomy incision in cases ($n = 40$) where resection of the redundant joint capsule was required for accurate closure of the medial arthrotomy.

Control samples (MCP and synovium) were collected from 10 dogs that were euthanased for reasons other than fore limb lameness. Five Beagles, two crossbreeds, two Labradors and one German Shepherd dog were evaluated. None showed clinical or radiographic evidence of elbow disease. In each case no evidence of elbow disease was evident upon visual inspection of the joint. The mean age of the control dogs was 17 months (±19.5 months, range 9 months–6 years) and the mean weight of the control dogs was 22.7 kg (±7.8 kg, range 13.2–40.1 kg). Tissue samples were washed in sterile Hartmann's solution (Isolec, Ivex Pharmaceuticals) and immediately stored in 10% neutral buffered formalin until processing.

The MCP samples were decalcified for up to 48 h in Decalcifier I (Surgipath). All samples were then paraffin wax embedded and sectioned at 5 μm thickness. Sections of the MCP were cut perpendicular to the weight bearing surface of the coronoid process, through the apex of the process to the midpoint of the ostectomy surface. If loose fragmentation was present, the MCP fragment was evaluated, and sectioned to the midpoint of its base. Two serial sections were mounted onto glass slides and stained with haematoxylin and eosin (HE) and Safranin-O (SO). All synovial membrane samples were stained with HE only.

Radiographic scoring

The orthogonal views of the affected elbows were evaluated for the presence of osteophytosis and were scored accordingly by a single experienced observer (NF) using the International Elbow Working Group Method (IEWG) (Grade 0, no osteophytes; Grade 1, osteophytes <2 mm; Grade 2, osteophytes 2–5 mm; Grade 3, osteophytes >5 mm) (Lang et al., 1998).

Arthroscopic scoring

The visual appearance of the articular cartilage across the surface of the MCP (but not including the fragmentation line) identified during arthroscopy was scored using the modified Outerbridge cartilage scoring system (Schulz, 2003): Grade I, signs of chondromalacia, namely, softening and swelling; Grade II, partial thickness fibrillation and fissuring of the cartilage; Grade III, full-thickness cartilage fissuring; Grade IV, full-thickness cartilage erosion with exposure of the subchondral bone.

Histopathological evaluation

The sectioned coronoid processes were evaluated microscopically (Olympus BX 41), and digital images (Olympus, CAMEDIA) were obtained concurrently for further analyses.

Images of each sample were digitally captured at 200× magnification and analysed using ImageProPlus software (Media Cybernetics). Cartilage thickness was measured at three different sites perpendicular to the weight bearing surface and a mean of the three measurements was calculated (performed by MG). In Outerbridge Grade IV cases, where full-thickness cartilage erosion had been identified, the three measurements were taken adjacent to the denuded bone.

Each section was evaluated by using the Mankin histological and histochemical scoring system (Mankin et al., 1971; Bobinac et al., 2003) more recently referred to as the Histologic Histochemical Grading System (HHGS) (Ostergaard et al., 1997, 1999). This enabled semi-quantitative evaluation of structural changes in all layers of the uncalcified cartilage, the tidemark integrity and the level of SO stain uptake. Reduced SO uptake generally equates to a reduction in proteoglycan content in the cartilage matrix (Jubb and Eggert, 1981).

The sections were evaluated at different magnifications for the individual parts of the scoring procedure (performed by MG and SS). The Mankin cartilage score was calculated for all of the samples. According to the Mankin grading system, cartilage degeneration was categorised into three stages: Stage I, Mankin score 0–6 points – indicates mild degenerative changes; Stage II, Mankin score 7–9 points – indicates moderate degenerative changes; Stage III, Mankin score 10–14 points – indicates severe degenerative changes (Mankin et al., 1971).

The synovial samples were assessed histologically using a scoring system described by (Krenn et al., 2006). Sections were assessed microscopically for thickening (i.e. hyperplasia) of the synovial lining cell layer, the density of resident cells and for the presence of infiltrating inflammatory cells (Table 1). A score of 0–1 point was considered normal, while a score of 2–4 points was classified as low-grade synovitis and 5–9 points as high-grade synovitis (Krenn et al., 2006).

Three digital images at different sites of the section were obtained at 400× magnification. The images were imported into ImageProPlus software, and an area of interest (AOI,) was defined by choosing an area immediately subjacent to the tidemark boundary between articular cartilage and subchondral bone without overlap between the three areas. The number of osteocyte nuclei was counted in the AOI and the osteocyte density (number of osteocytes per 80,000 μm^2) was calculated.

Using ImageProPlus software, the AOI was measured and its bone surface area calculated automatically by outlining the bony trabeculae with a digital marker, which determined the bone area in μm^2. Three measurements were taken and the mean was calculated and expressed in per cent compared to the whole area (80,000 μm^2) on which the measurements were based.

During the scoring process, additional microscopic abnormalities not encompassed by the semi-quantitative scoring systems or by the image analysis were recorded qualitatively for the synovium, articular cartilage and subchondral bone.

Statistical analyses

The age and weight of the dogs in the diseased and the control group were statistically compared using Student's t test. Statistical analyses to compare the IEWG elbow dysplasia score (Lang et al., 1998), the Outerbridge score (Schulz, 2003) with the mean articular cartilage thickness, mean osteocyte density, mean bone area, the Mankin cartilage pathology score, and the synovial scores were performed using one-way analysis of variance with a post hoc Tukey's pair-wise comparison. Differences were considered significant at $P < 0.05$. Correlation between the severity of the IEWG elbow dysplasia score, or the Outerbridge gross pathologic findings of the diseased samples when compared to the mean articular cartilage thickness, mean osteocyte density, mean bone area, Mankin cartilage pathology score, and the synovial scores was performed by calculating the Pearson correlation coefficient.

Results

When compared to the diseased population by t test, the control dogs were significantly lighter ($P = 0.04$) but not significantly older ($P = 0.14$).

Radiographic evaluation of the elbows

The evaluation of the radiographs revealed a distribution of the IEWG grades in the evaluated elbows of: Grade 0 ($n = 15$), Grade 1 ($n = 11$), Grade 2 ($n = 14$), Grade 3 ($n = 13$).

Arthoscopic evaluation of the articular cartilage

The distribution of the Outerbridge cartilage scores of the evaluated elbows were: Grade I ($n = 9$), Grade II ($n = 26$), Grade III ($n = 6$) and Grade IV ($n = 12$).

Cartilage thickness

The mean articular cartilage thickness of the control group (372.4 μm ± 23.4 μm) was significantly less than samples from IEWG Grades 2 (858 μm ± 109 μm) and 3 (1075 μm ± 193 μm), and the mean articular thickness of samples from dogs with IEWG Grade 0 (655.4 μm ± 58.8 μm) was significantly less than samples from IEWG Grade 3 (Fig. 1A). The mean articular thickness of samples from IEWG Grade 1 (684.6 μm ± 87.7 μm) were no different