Correlation Between Histopathologic, Arthroscopic and Magnetic Resonance Imaging
Findings in Dogs with Medial Coronoid Disease

A Thesis

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Abstract

Medial coronoid disease (MCD), a component of canine elbow dysplasia, is the most common cause of thoracic limb lameness in juvenile medium- to large-breed dogs. The goal of this study is to describe the magnetic resonance imaging (MRI), arthroscopic and histopathological changes in dogs with medial coronoid disease and to identify potential relationships between these findings. Twenty-five diseased medial coronoid processes (MCP) were collected from 19 dogs with a confirmed diagnosis of medial coronoid disease that underwent surgical management with subtotal coronoid ostectomy. A reference group of normal MCPs was harvested from 9 dogs that were euthanatized for reasons unrelated to elbow disease. MCP specimens were evaluated by magnetic resonance imaging (MRI) using a novel grading scheme (all dogs), arthroscopy using a modified Outerbridge scheme (affected dogs only) and histopathology (all dogs). The common histopathologic findings were subchondral microfractures, subchondral microfractures continuous with cartilaginous fissures, moderate to severe hypercellularity of the marrow space, trabecular bone necrosis and articular cartilage degeneration. The severity of cartilage disease in the MCP was moderate to severe in most of the specimens, even in cases with minimal arthroscopic pathology. Three distinct patterns of bone marrow lesion (BML) were identified adjacent to the MCP, but there was no correlation between BML pattern and either histopathological or arthroscopic findings. There was moderate correlation between modified Outerbridge scores and MRI scores. No correlation was identified between the histopathological changes and either MRI or arthroscopic scores. There was no

significant correlation between the clinical scores and histopathological changes. Ongoing improvements in the resolution of noninvasive imaging techniques will likely improve description and understanding of the MCP disease in dogs.

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Chapter I: Introduction

Medial coronoid process (MCP) disease, a component of canine elbow dysplasia, is the most common cause of forelimb lameness in juvenile medium- to large-breed dogs.¹⁻³ The etiopathogenesis of this disease remains unproven, although various presentations are described: isolated fragmentation or fissuring of the MCP is recognized commonly, but symptomatic cartilage erosion within the medial compartment of the elbow joint can also occur. Numerous different hypotheses have been proposed: radioulnar joint incongruity,⁴⁻⁶ trauma,^{7,8} genetics,⁹⁻¹¹ growth rate,¹¹ nutrition,¹¹ ischaemia¹¹ and osteochondrosis.^{1,12} Several of these factors may simultaneously contribute to the disease. Radio-ulnar disparity and humero-ulnar mismatch are the signs of incongruency most commonly described. However, radio-ulnar incongruency in the transverse plane has also been implicated in the development of MCP disease. Aberrant forces between the radial head and the radial incisure of the MCP, through the strong bicipital/brachialis tendon, is also one of the theories of MCP disease etiopathogenesis.¹³

Radiography,¹⁴⁻¹⁶ computed tomography (CT)¹⁶ and magnetic resonance imaging (MRI)¹⁶⁻¹⁹ are diagnostic imaging techniques used for detection of MCP disease. Widely available, efficient, cost effective, radiographic assessment is a valuable component of the diagnosis of MCP disease. However, the reliability of radiography is limited by problems with resolving details on structures that are superimposed.^{20,21} In addition, a recent study by Punke et al.²² highlighted the lack of correlation between radiographic and arthroscopic findings in the same animal.

Radiography also lacks sensitivity as a means of assessing articular cartilage pathology²¹ making it unreliable as a stand-alone diagnostic or prognostic tool in dogs with elbow dysplasia.

CT allows for detailed assessment of articular subchondral bone but not articular cartilage. CT changes in MCD commonly include sclerosis, fissures, cysts, and fragmentation. Although the CT findings of MCD can be variable, CT is considered a reliable diagnostic procedure in MCD.²¹ CT has been also the most widely used diagnostic modality to assess incongruity of the elbow joint.¹⁶

MRI is widely used for the diagnosis of musculoskeletal disease in people because it provides information on both intra- and extra-articular structures, with high soft tissue contrast, high-resolution and the capability for multi-planar imaging (transverse, dorsal, sagittal). As compared with CT, MRI has greater sensitivity for detecting subtle changes in bone architecture and in the adjacent bone marrow.¹⁷ Tissue differentiation at the bone-cartilage interface is also better with MRI. A recent study used delayed gadolinium-enhanced MRI to evaluate articular cartilage in normal canine elbow.²² Accuracy of MRI for detection of free fragment of medial coronoid process was 95.5% compared with 77% for radiography.¹⁸ Sensitivity and negative predictive value were also higher for MRI than radiography.¹⁸ CT showed intermediate sensitivity and specificity with 71-88% and 84-85% respectively.²¹

Few studies have provided descriptive or quantitative data on the histologic changes in MCP disease. A histomorphometric study²⁴ revealed that accumulation of subchondral fatigue microdamage is associated with fragmentation of the MCP. Fissuring occurs in the subchondral bone before gross fibrillation of articular cartilage develops. In another study, Goldhammer et al.,²⁵ did not identify any correlation

between histopathologic findings, modified Outerbridge score²⁶ and the international elbow working group (IEWG) score.²⁷

Recent work has highlighted the potential role of subchondral bone changes in canine stifle pathologies²⁸ and it has been suggested that MRI may be useful in assessing subchondral bone changes in MCP disease.¹⁶ Early experience with MRI in humans highlighted the existence of focal regions of hyper-intense signals, now commonly termed “bone marrow lesions” (BMLs). In humans, BMLs have been shown to be predictive of knee pain and subsequent cartilage degradation in patients with osteoarthritis.^{29,30} The significance of BMLs in dogs in general, and in MCP disease in particular, has not been evaluated. We hypothesized that BML are more common in elbows with MCP disease and that the severity of these BML correlates with the degree of cartilage damage (Outerbridge score) and histopathological findings. The specific aim of this study was therefore to describe the pathologic changes observed on MRI, arthroscopy and histopathology and to determine the extent of any correlation between these different parameters in a series of dogs with a range of Outerbridge scores indicative of the early stages of MCP disease.

Chapter 2: Materials and Methods

Inclusion Criteria

Medical records and MRI findings were retrieved from dogs that had thoracic limb lameness attributed solely to MCP disease and that were examined between May 2009 and December 2009. Dogs with MCP disease that also had any other disease of the thoracic limb (including osteochondrosis of the medial aspect of the humeral condyle or caudal humeral head, or ununited anconeal process) identified by any diagnostic technique modality were excluded. Only cases with focal disease of the medial coronoid process were included; cases with a modified Outerbridge score²⁶ of 1 or greater affecting the articular surface of the medial aspect of the humeral condyle were excluded. Incongruity was subjectively assessed and any cases with radio-ulnar step or humero-ulnar incongruity on MRI were also excluded.

MRI was performed based on the score of the radiographic modified IEW grading system:³¹ only cases with a low modified IEW score (equal or less than 1) were included.

For comparison with the diseased MCP samples, normal MCP samples were harvested from nine dogs that were euthanatized for reasons unrelated to elbow disease. MCP were evaluated by both MRI and histopathology, and only samples without evidence of abnormality in either evaluation were included in the final reference population. For both the diseased or the reference group, samples were

excluded from the study if histopathological evaluation was deemed unreliable as a result of sampling or tissue processing artifacts.

MRI Evaluation

A dorsal 3-dimensional T2-weighted gradient echo (GRE) sequence (TR 17ms, TE 10ms) was selected using a 1.5-Tesla system (Magnetom Symphony; Siemens Medical Systems, Erlangen, Germany) in all cases. Slice thickness was 0.6mm with no inter-slice gap in all studies. Two observers (WTD and VW) reviewed the MRI studies and graded the MCP disease using a standardized scoring scheme (Table 1) according to the extent of synovitis (volume, signal of synovial tissue and volume of joint fluid), the shape of the MCP (abnormal, fissured, or fragmented) and the presence/absence and severity of the extension of any BML associated with MCP. BMLs were scored using a semi-quantitative scheme: grade 0 indicates absence of BML, grade 1 indicates increased signal involving less than 50% of the MCP and grade 2 indicates increased signal involving more than 50% of the MCP. The intra and inter-observer reproducibility for the MRI score was calculated.

Parameter	Description	Score
<i>Bone marrow lesion extension</i>	Absent	0
	< 50%	1
	>50%	2
<i>Synovitis</i>	Absent	0
	Minimal	1
	Moderate	2
	Severe	3
<i>Medial coronoid process</i>	Normal	0
	Abnormal shape	1
	Fissured	2
	Fragmented	3

Table 1: MRI scoring scheme used to score MRI findings in dogs with medial coronoid disease.

Arthroscopic Scoring

The 19 elbows with a diagnosis of MCP were evaluated arthroscopically using a 2.7 mm 30° oblique arthroscope (Hopkins, Karl Storz, Tuttlingen, Germany). 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 system²⁶ (Table 2).

Grade	Description
I	Signs of chondromalacia, namely, softening and swelling
II	Partial thickness fibrillation and fissuring of the cartilage
III	Full-thickness fibrillation and fissuring of the cartilage
IV	Full-thickness cartilage loss
V	Full thickness loss with subchondral bone eburnation.

Table 2: Modified Outerbridge classification scheme used to score arthroscopic findings in dogs with medial coronoid disease.²⁶

Tissue Sampling and Processing

Tissue samples were obtained at the time of sub-total coronoid ostectomy performed either with arthroscopic assistance or via mini-arthrotomy (Figure 1). The MCP specimens were fixed in 10% neutral buffered formalin and decalcified in ethylenediaminetetraacetic acid (EDTA) for a period of approximately 14 days until complete decalcification was confirmed by microradiography. Specimens were then embedded in paraffin wax and sectioned at right angles to the weight-bearing surface of the coronoid process. Three consecutive 5-µm sections were obtained from each specimen and these were stained with haematoxylin and eosin (HE), Safranin-O (SO) and Masson's Trichrome. As controls, samples of unaffected MCP were harvested from dogs that were euthanized for reasons other than fore limb lameness. None showed clinical or MRI evidence of elbow disease, and all of the elbow joints were considered normal on visual inspection at the time of tissue collection. Tissue samples from control joints were collected, processed, embedded, sectioned and stained using the same protocol as was used for the affected specimens.

Figure 1: Schematic representation of tissue sampling and processing: clinically affected dogs were evaluated by MRI and then an arthroscopic examination was performed. Samples were collected via sub-total coronoid osteotomy (green line). The red line represents the typical location of radial incisure fragmentation. After fixation and decalcification, 3 consecutive sections were obtained from each specimen and these were stained with haematoxylin and eosin, Safranin-O and Masson's trichrome.

Histopathological Evaluation

The MCP sections were evaluated microscopically. A board-certified veterinary pathologist (DR) provided a descriptive analysis for each sample, and the sections were then evaluated by using a modified Histologic Histochemical Grading System (HHGS) by two observers (DR and VW).^{32, 33} The HHGS grading system allows a semi-quantitative evaluation of (1) structural changes in all layers of the uncalcified cartilage, (2) cellularity, (3) tidemark integrity and (4) the degree of SO uptake. Reduced SO uptake generally reflects a reduction in proteoglycan content in the cartilage matrix.³⁴ For this study, the HHGS was adapted to include evaluation of the presence, location and extension of microfracture using a 3-point grading scheme (Table 3). The length of microfractures was assessed on a 20x magnification by direct comparison with the length of the field. Images of each sample were digitally captured and analyzed using “Preview” (Version 6.0.1, Copyright © 2002–2012 Apple Inc.). The intra and inter-observer reproducibility for the modified HHGS was calculated.

Parameter		Description	Score
HHGS as described by Mankin et al. ³²	<i>Structure</i>	Normal	0
		Surface irregularities	1
		Pannus and surface irregularities	2
		Clefts to transitional zone	3
		Clefts to radial zone	4
		Clefts to calcified zone	5
		Complete disorganization	6
	<i>Cells</i>	Normal	0
		Diffuse hypercellularity	1
		Cloning	2
		Hypocellularity	3
	<i>Safranin O staining</i>	Normal	0
		Slight reduction	1
		Moderate reduction	2
		Severe reduction	3
		No Dye noted	4
	<i>Tidemark integrity</i>	Intact	0
		Crossed by blood vessels	1
<i>Microfracture</i>	No microfracture evident		0
	Microfracture in subchondral bone only		1
	Subchondral microfracture associated with cartilage fissure		2

Table 3: Histological scoring scheme. The standard HHG score was supplemented by a three-point scoring scheme for microfracture. Total scores were then used to classify the lesions into three stages of degeneration: Stage I (mild) = 0 to 8 points; Stage II (moderate) = 9–11 points; Stage III (severe) = 12–16 points.^{32, 33, 34}

The bone volume/tissue volume (BV/TV) ratio (%) was obtained using a direct manual count of points on an acetate grid overlaid on the digital images: the number of points overlapping with bony structures and points overlapping with soft tissue structures were calculated manually. A direct ratio was calculated using these two numbers (bone or soft tissue).

Statistical Analysis

Statistical software (GraphPad Prism version 5.00 for Mac OS X, GraphPad Software, San Diego California USA, www.graphpad.com) was used to generate descriptive statistics and to investigate potential correlations between MRI, histopathological, and arthroscopic variables. Weight of dogs in the diseased and the control group were statistically compared using Student's t test. Correlation was assessed by calculating the Pearson correlation coefficient. The D'Agostino & Pearson normality test was performed to confirm the normal distribution of the data. Statistical analyses to compare the MRI score, the Outerbridge score²⁶ and the BML extension with the HHGS cartilage pathology score,^{32,33} the BV/TV ratio and the presence and type (subchondral only versus subchondral and cartilage) of microfractures were performed using one-way analysis of variance (ANOVA) with a post hoc Tukey's pair-wise comparison. A post-test was performed to identify a linear trend. Differences were considered significant at $P < 0.05$. The intra-observer and inter-observer reproducibility for the modified HHGS score and MRI score was investigated using the intraclass correlation coefficient (ICC). This was calculated using a two-way random-effects ANOVA, type consistency, and single/average measures.

Chapter 3: Results

Operative reports, MRI scans and resection specimens were obtained for 25 elbows from 19 dogs with MCP disease. Control tissues were harvested from 18 elbows from 9 skeletally mature dogs (see below), but six of these elbows were subsequently excluded because of mild abnormalities identified on MRI or histopathology. The final control series therefore included 12 unaffected elbows.

Five breeds of dog were represented in the diseased group, with Labrador Retrievers being the most common (n=16, 64% of elbows). Other breeds were Rottweiler (n=3 elbows), mixed breed dog (n=3 elbows), Staffordshire Bull Terrier (n=2 elbows) and Gordon Setter (n=1 elbow). Male dogs represented 53% of all dogs, with 60% being castrated. Twenty-six percent of dogs were spayed females. The mean age of the diseased group was 36 months (SD $\pm$ 31 months). The mean weight was 31 kg (SD $\pm$ 5.7 kg). Accurate ages were not available for the control dogs, but all were subjectively younger than 4-5 years old. The controls weighed less than the affected dogs ($p = 0.0091$) with a mean weight of 25.3 kg (SD $\pm$ 6.4 kg). Mixed breed dog was the most common breed in the control group (N=6 dogs). The control group included also two German Shepherd Dogs and one Beagle.

MRI Evaluation

MRI scores of diseased MCP were significantly higher than those of the normal control samples (4.7 ± 1.4 versus 0 ± 0 , respectively) ($p < 0.05$). A moderate but

statistically significant positive correlation was identified between MRI score and Outerbridge score ($r=0.47$, $p=0.0071$) (Figure 2). Specimens with an Outerbridge score of IV had higher MRI scores than specimens with Outerbridge scores of II ($p=0.0098$) or III ($p=0.0255$). There was no difference in MRI scores from specimens with Outerbridge scores of II versus III ($p=0.7241$). Only one of the elbows had an Outerbridge score of I, precluding further analysis of this sub-group. There was no significant difference in MRI grade between specimens with different micro-fracture grades (0, 1 or 2) ($p=0.6433$) and no correlation between the MRI score and either the modified HHGS score or the BV/TV ratio. BML were identified in 25 of 25 elbows in the affected group and 0 of 12 dogs in the control group.

Figure 2: Graph indicating Outerbridge score versus MRI score. A moderate positive correlation was identified between MRI score and Outerbridge score ($r=0.47$, $p=0.0071$).

Three different patterns of BML were observed on MRI (Figure 3): *focal/linear* BML within the subchondral region (Figure 3a); *focal* BML involving only the tip of the MCP (Figure 3b); and *diffuse* BML involving the whole MCP (Figure 3c). The intra- and inter-observer variabilities were low. The ICC intra-observer value was good (0.67) and the overall ICC inter-observer value for the two observers was excellent (0.70).

Figure 3: Sagittal reconstructed T2 GRE sequence magnetic resonance imaging (MRI) image of the 3 different dogs. (a) focal BML involving only the tip of the MCP (arrows); (b) focal/linear BML within the subchondral region (arrows); and (c) diffuse BML involving the whole MCP (arrows) : U, ulna ; H, humerus

Arthroscopic Evaluation

The distribution of the Outerbridge cartilage scores of the affected elbows was: Grade I (N = 1), Grade II (N = 11), Grade III (N= 9) and Grade IV (N=4). The mean modified Outerbridge score was 2.6 (SD $\pm$ 0.8).

Descriptive Histopathology

A similar spectrum of histopathological findings was observed in the subchondral bone across specimens with different Outerbridge and MRI scores. The marrow space in 84% of samples was moderately to severely hypercellular compared to the control specimens (Figure 4) but the precise identification of the cellular component was

made challenging due to sectioning artifact. Staining with Masson's Trichrome revealed only a small amount of collagen within the marrow spaces (Figure 5). Trabecular bone necrosis, evidenced by empty osteocyte lacunae, was present in 19 of 25 affected samples (76%) and was associated with osteoclast resorption and woven bone formation (Figure 6). These areas were frequently adjacent to the micro-fractures. Micro-fractures were also present in specimens where the surface cartilage was relatively intact (surfaces irregularities or clefts to transitional zone), as well as in severe cases (clefts to radial or calcified zone).

Degenerate articular cartilage was hypocellular and fibrillated, with decreased Safranin-O staining (Figure 7). Only two of 25 cases (8%) had normal cartilage cellularity. Two cases presented cartilage thickening, and one of these was associated with subchondral bone necrosis.

Figure 4: **A.** Photomicrograph of articular cartilage surface showing complete eburnation (arrows) and hypercellularity in the bone marrow spaces (stars) HE, x100 magnification. **B.** Photomicrograph of normal articular cartilage surface showing a relative hypocellularity in the bone marrow spaces (stars). HE, x100 magnification.

Figure 5: Photomicrograph of subchondral bone showing a small amount of collagen within the marrow spaces (*). A subchondral microfracture is also present (arrows). Masson's trichrome, x100 magnification

Figure 6: Photomicrograph of subchondral bone showing trabecular bone necrosis characterized by empty osteocyte lacunae (arrows). HE, x200 magnification

Figure 7: **A.** Photomicrograph of normal articular cartilage surface showing normal staining intensity in the cartilage (*). Safranin-O, x20 magnification. **B.** Photomicrograph of articular cartilage surface showing superficial fibrillation. There is decreased staining intensity in the cartilage (*) directly adjacent to a microfracture (arrows), indicating reduced proteoglycan content. Safranin-O, x20 magnification.

Quantitative Scoring

The mean modified HHGS score for affected samples was 10 ($SD \pm 2$), which was significantly higher than that of the control specimens ($p < 0.0001$). According to the HHGS scheme, the 25 affected joints were classified into stage I ($N=4$), stage II ($N=10$) and stage III ($N=11$). When broken down by modified Outerbridge scores, there was no significant difference between modified HHGS scores for Outerbridge

scores of II, III and IV ($p=0.7390$). There was a moderate and statistically significant correlation between standard HHGS scores (i.e. not including assessment of microfracture) and Outerbridge score ($r=0.404$, $p=0.0453$). No correlation was identified between modified HHGS score and BV/TV ratio. The ICC intra-observer value was excellent (0.81). The overall ICC inter-observer value for the two observers was also excellent (0.85).

Micro-fracture Evaluation

The median microfracture grade was 1 (IQR= 0-2). Six samples (24%) had only subchondral microfractures. Microfractures in subchondral bone were contiguous with cartilaginous fissures in 12 samples (48%). Isolated cartilaginous microfractures were not observed. The mean microfracture length was 3.3 times a 20x magnification field ($\approx 1\text{mm}$). Microfracture scores differed across specimen groups with different Outerbridge scores, but these differences just failed to reach statistical significance ($p=0.054$). A negative linear relationship was identified between microfracture score and Outerbridge score ($r=0.46$; $p=0.0245$).

BV/TV values for samples with a BML score of 1 or 2 were not significantly different ($p=0.1841$). When broken down according to microfracture classification, there were no statistically significant differences between the three scores (0, 1 and 2) ($p=0.2626$). BV/TV values from diseased samples were however significantly lower than those from control specimens ($p< 0.0001$) (Figures 8 and 9).

Figure 8: Box-and-whisker plots of BV/TV score of the diseased and normal samples. For each box, the horizontal line represents the median value and lower and upper boundaries of boxes represent 25th and 75th percentiles, respectively. Whiskers represent the range of values. BV/TV values were significantly lower for diseased samples than control samples ($p < 0.0001$).

Figure 9: Photograph of 2 medial coronoid process specimens. (A) Diseased specimen characterized by increased porosity and a large microfracture (arrows), (B) normal specimen.

Chapter 4: Discussion

The rationale behind the current study was to determine whether the use of MRI would allow for the identification of previously unidentified relationships between imaging parameters and disease at either a gross or microscopic level. We expected to find that the increased sensitivity of MRI would allow for detection of earlier (i.e., less severe) lesions, as well as for a more complete description of lesion location, signal characteristics and sizing. Support for this hypothesis would have opened up opportunities for using MRI as a noninvasive tool for identifying and staging osteochondral changes in MCP disease, as well as for tracking response to medical or surgical therapies. Although the data from the current study are insufficient to validate the utility of MRI as a stand-alone staging tool, a number of intriguing observations were made with regards the assumptions that we currently make about MCP disease, especially as they relate to the true extent of disease and to its etiopathogenesis.

Histopathology confirmed microscopic pathology in the subchondral bone and articular cartilage of all of the diseased specimens, irrespective of the extent of the arthroscopic and MRI findings. The severity of histologic change in the subchondral bone did not correlate with modified Outerbridge and MRI scores, suggesting that current clinical assessments of osteoarticular pathology do not provide a very accurate estimate of the true extent of disease. Moderate to severe changes were present in almost all the specimens, even those with a low modified Outerbridge score (12 cases had a modified Outerbridge score of I or II). A previous study²⁵ failed to identify any

strong correlation between a radiographic measure of elbow disease (the IEWG), modified Outerbridge score and histopathological findings. Since MRI is generally considered a more sensitive and accurate diagnostic tool than plain radiography,¹⁸ we anticipated that the current study might demonstrate a relationship between MRI, arthroscopic and histopathologic findings in dogs with fragmented MCP. However, this expectation was not realized.

Qualitatively, the histopathology from these animals provided insight into several important issues relating to the etiopathogenesis of fragmented MCP. First, although implicated by Olsson as a factor in the development of MCP disease,² osteochondrosis was identified in only 1 out of the 25 specimens in this series (4%). Lesions indicating impaired endochondral ossification were not observed in the other samples. Our findings in this series are consistent with those from other studies²⁵ and tend to argue against a strong role for osteochondrosis, at least in fragmented MCP. Second, we were unable to identify the “cartilage islands” reported by Goldhammer et al.²⁵ as being the result of either non-physiological loading or the response of subchondral bone to repetitive microtrauma.

The third interesting observation is that our data lend support to a previous hypothesis that the fragmentation seen in fragmented MCP is initiated in the trabecular bone.²⁴ Six of the 25 specimens (24%) had isolated subchondral microfractures in the absence of an identifiable cartilaginous microfracture. Interestingly, a trend was observed between the microfracture score and the modified Outerbridge score: a lower microfracture score was associated with a higher modified Outerbridge score. This association may reflect the nature of the disease process. As described by Danielson

et al.,²⁴ fatigue of the subchondral trabecular bone appears to be important to the pathogenesis of MCP disease. Microfracture formation may be the first stage of the disease, explaining a higher microfracture score in the specimens with lower modified Outerbridge scores. Adaptive bone remodeling then occurs, leading to a reduction in microfracture score but an increase in overall histologic score due to secondary degenerative changes in and around the lesion.

Bone marrow hypercellularity was a very consistent histologic finding, seen in 23 of 25 specimens (92%). In an experimental model of stifle osteoarthritis in dogs, Martig et al.³⁵ described hematopoiesis and myxomatous transformation of the bone marrow in the areas corresponding to regions of MR signal changes. Previously described as bone marrow edema due to capillary leakage caused by local change in the capillary wall (trauma, tumor) or by increased intravascular pressure, bone marrow lesions are in fact characterized by fibrosis, lymphocytic infiltrates and increased vascularization.³⁶ In this study, definitive identification of the cells within these lesions was precluded because of tissue processing artifacts, but the microstructural observations were consistent with those reported previously in human BML.³⁶

BML characteristics have not been defined in veterinary medicine, despite a number of recent studies.^{28, 35} To our knowledge, this is the first description of the different morphologies of BML in the canine elbow. Three distinct BML morphologies were identified, but at this time the clinical significance of the different lesions is unclear. We were unable to identify a significant correlation between the type of lesion and any other parameter (MR or arthroscopic), but this may reflect a lack of statistical power due to the limited sample size. In humans with acute tears of the anterior

cruciate ligament, BML are identifiable by MRI in characteristic locations in the femur and tibia in more than 80% of patients.³⁷ In a clinical canine study assessing stifle pathology with MRI,²⁸ BML of varying size and signal intensity were present in stifles that had a known, acute injury as well as in stifles with chronic lameness and no known acute injury. In the preclinical model cited earlier,³⁵ BML were seen but they varied in size and intensity, without any recognizable trend throughout the whole study. The challenge resides in the fact that the high sensitivity of MRI allows for detection of subtle changes that may or may not be associated with pathology. For example, BML were seen in the knee, ankle and foot of clinically healthy marathon runners after competition, but similar lesions were also seen in the control group.^{38,39} BML with no evidence of osteoarthritis were seen in 14 of 34 knees of clinically asymptomatic basketball players.⁴⁰ BML may reflect a normal physiologic response to microtrauma^{35,36} and, if so, the location, shape and extent of the BML will likely be influenced by the patient's level of activity at presentation, age, and duration of clinical signs. One of the clear conclusions from the current study is that additional work is now needed to more completely understand the pathophysiology and evolution of BML in the context of MCP disease. Changes in limb use are also likely to lead to adaptive bone remodeling, and it was interesting to note that there were significant reductions in BV/TV (indicative of increased porosity) in the tissues from affected dogs. These results are consistent with previous reports of increased porosity in disease samples^{24, 25} and may be an indicator of active bone modeling in response to changes in loading.^{41,42}

As is often the case with studies based on clinical samples, this work suffers from a number of important potential limitations that merit further discussion. Some of these

are inherent limitations of any clinical study or of studies in which cadaveric specimens serve as controls; others are unique to the way in which this specific study was conducted.

Any attempt to identify correlations between large numbers of parameters is dependent on sample size and the magnitude of the treatment (or disease) effect. Ideally, for a study of this type, comparisons would be based on equal (or near-equal) numbers of specimens in each sub-group (Outerbridge score, HHGS score etc.). However, since dogs were only included in this study if they underwent surgical management of their disease, there is an inherent bias towards dogs with more severe disease, thereby limiting the sensitivity of our analyses in identifying differences between mildly affected dogs and either severely affected dogs or normal dogs. The use of normal dogs as controls is very reasonable and appropriate, but we were not able to control the age, breed or gender of these animals, opening up the potential for greater variability in the data. Although all of these dogs were skeletally mature, they were slightly smaller in size and likely younger than the affected dogs. In order to control the age, the breed and the gender of the control group, a longitudinal study could be performed but this approach leads to some obvious ethical and practical difficulties. Nevertheless, the current study may help to define the design of such a prospective study.

The other major potential limitations with this study relate to the specific procedures used to prepare and analyze the specimens. It has been suggested that to accurately assess the size and extent of BMLs, a combination of different MRI sequences should be used.⁴³ However, for practical and financial reasons we elected to focus on a single

clinically relevant, standardized MRI scan protocol. Additional information might have been obtained through the use of contrast-enhanced MRI protocols, but these were considered beyond the scope of the current study. Micro-MRI analysis of the retrieved fragmented MCP specimens could have been very informative, but the costs associated with this, coupled with the fact that it is not yet technically feasible to do micro-MRI in the live dog, limited our enthusiasm for incorporating this into the current study design. Finally, the histological analysis focused on a limited number of sections that may not have been entirely representative of the whole tissue specimen. Our data may therefore over- or under-represent the true extent of pathology. Nevertheless, we were careful to ensure that specimens were harvested from a consistent location in each animal, so we remain confident that the histopathology data are representative of the two sample populations. Finally, tissue artifacts occasionally complicated the interpretation of the cellular components of the bone marrow in these samples. There are a number of potential causes of tissue artifacts in decalcified bone samples: thermal necrosis due to the use of an oscillating saw for the subtotal coronoidectomy procedure; understaining or fissuring of the sample due to incomplete fixation, decalcification or embedding of the bone sample; or shrinkage of bone marrow during tissue processing.⁴⁴ While artifacts are always frustrating, with the exception of a more complete description of the cellular composition of BML, they did not ultimately limit our ability to describe the changes in these tissues. All of the specimens (affected and control) were handled the same way. The analytical methods used to describe the histopathology were also validated through the documentation of good to excellent inter- and intra-examiner reliability.

Conclusions

MRI scoring system and the modified Outerbridge score fail to provide a complete picture of the nature or extent of histopathology in diseased MCP. Microscopic evidence of severe cartilage degeneration was evident in cases with minimal arthroscopic pathology. Additional studies are needed to define and validate improved strategies for diagnosing, staging and assessing treatment response in fragmented MCP. The identification of early, subclinical lesions is especially problematic and currently limits our ability to better define the etiopathogenesis of these lesions, but osteochondrosis did not appear to play a significant role in the development of the lesions in this series of dogs. Ongoing and future improvements in the resolution of noninvasive imaging techniques will likely hold the key to better describing and understanding the initiation and evolution of the subchondral and articular pathology in dogs with MCP disease.

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Appendix A: Data

Case	Id	Side	Arthro Assessment	Outerbridge	Age (month)	Gender	Weight (kg)	Breed
1	17468	L	Fissured	I	9	ME	34.4	Rottweiler
2	17468	R	Fragmented	II	9	ME	34.4	Rottweiler
3	17497	L	Fissured	II	104	FS	26.5	Labrador
4	17463	L	Fissured	II	21	ME	50.6	Rottweiler
5	17498	L	Fissured	II	74	FS	35.5	Labrador
6	17536	L	Fissured	II	15	FE	29.3	Setter Gordon
7	17673	L	Fissured	II	15	MC	31.4	SBT
8	17673	R	Fissured	II	15	MC	31.4	SBT
9	17703	R	Fissured	II	15	ME	27	Labrador
10	17743	R	Fragmented	II	13	FE	27.9	Labrador
11	17996	R	Fragmented	II	11	FE	26.1	Lab x German Shepherd
12	18534	R	Fissured	II	12	MC	31.9	Labrador x
13	17497	R	Fissured	III	104	FS	26.5	Labrador
14	17484	L	Fragmented	III	58	MC	30.5	Labrador
15	17484	R	Fragmented	III	58	MC	30.5	Labrador
16	17487	L	Fragmented	III	20	MC	30	Labrador
17	17487	R	Fragmented	III	20	MC	30	Labrador

Continued

Table 4: Case number, identity number, arthroscopic assessment, Outerbridge score, age, gender, weight and breed of the diseased cases.

Table 4: Continued

18	17498	R	Fissured	III	74	FS	35.5	Labrador
19	17644	L	Fissured	III	63	MC	36.8	Labrador
20	17644	R	Fissured	III	63	MC	36.8	Labrador
21	18534	L	Fragmented	III	12	MC	31.9	Labrador x
22	17723	L	Fragmented	IV	54	FE	25.4	Labrador
23	17535	L	Fragmented	IV	51	FS	28.2	Labrador
24	17690	L	Fragmented	IV	10	MC	26.6	Labrador
25	17979	R	Fragmented	IV	5	ME	21.2	Labrador

Case	Side	HHGS				Microfracture Description	Total
		Structure	Cells	Safranin O staining	Tidemark integrity		
17536	L	3	3	3	0	2	11
17644	R	4	3	3	0	2	12
18534	R	4	2	4	0	2	12
17498	R	3	3	3	0	2	11
17487	R	6	3	2	1	0	12
17497	R	5	3	4	1	2	15
17979	R	5	3	3	1	0	12
17996	R	1	3	3	1	1	9
17673	R	3	3	3	1	2	12
18534	L	4	3	4	0	1	12
17723	L	5	3	3	1	1	13
17484	R	4	3	2	0	0	9
17644	L	3	3	3	0	2	11
17487	L	3	3	2	0	0	8
17743	R	6	3	3	1	2	15
17673	L	1	3	3	0	0	7

Continued

Table 5: Modified Histological Histochemical Grading System scores (grading performed by DR)

Table 5: Continued

17468	L	1	2	1	0	1	5
17463	L	2	3	2	0	2	9
17498	L	5	3	3	0	1	12
17690	L	4	3	2	0	1	10
17497	L	4	3	3	1	2	13
17484	L	4	3	3	0	0	10
17535	L	3	3	2	0	0	8
17703	R	2	3	4	0	2	11
17468	R	2	3	2	0	2	9

Case	Side	Structure	Cells	HHGS		Microfracture Description	Total
				Safranin O staining	Tidemark integrity		
17536	L	3	3	3	0	1	10
17644	R	4	3	3	1	2	13
18534	R	3	2	3	0	1	9
17498	R	3	3	3	0	2	11
17487	R	4	3	2	0	0	9
17497	R	4	3	3	0	2	12
17979	R	5	3	4	1	0	13
17996	R	3	2	1	0	1	7
17673	R	3	2	3	0	1	9
18534	L	3	2	3	0	1	9
17723	L	5	3	3	0	1	12
17484	R	2	3	3	0	0	8
17644	L	2	3	3	0	0	8
17487	L	3	3	2	0	0	8
17743	R	4	3	3	0	2	12
17673	L	1	2	2	0	1	6

Continued

Table 6: Modified Histological Histochemical Grading System scores (grading performed by VW)

Table 6: Continued

17468	L	1	1	1	0	1	4
17463	L	4	2	2	0	2	10
17498	L	5	3	3	1	2	14
17690	L	4	3	3	1	1	12
17497	L	4	3	3	0	2	12
17484	L	3	3	3	1	2	12
17535	L	3	3	3	1	0	10
17703	R	3	3	3	0	2	11
17468	R	2	3	2	0	1	8

Case	Side	Structure	Cells	HHGS		Microfracture Description	Total
				Safranin O staining	Tidemark integrity		
17536	L	2	3	3	1	1	10
17644	R	4	3	3	0	2	12
18534	R	3	3	3	0	1	10
17498	R	2	3	3	0	2	10
17487	R	3	3	2	0	0	8
17497	R	2	3	3	1	2	11
17979	R	4	2	4	1	0	11
17996	R	1	3	3	0	1	8
17673	R	2	3	3	0	1	9
18534	L	3	3	3	1	0	10
17723	L	4	3	3	0	0	10
17484	R	4	3	2	1	0	10
17644	L	2	3	3	0	1	9
17487	L	1	3	2	0	0	6
17743	R	4	3	3	0	1	11
17673	L	2	3	2	0	0	7

Continued

Table 7: Modified Histological Histochemical Grading System scores (grading performed by VW, second set of measurements)

Table 7: Continued

17468	L	1	2	1	0	1	5
17463	L	3	2	2	1	2	10
17498	L	3	3	3	1	1	11
17690	L	3	3	3	1	1	11
17497	L	3	3	3	1	2	12
17484	L	4	3	3	1	0	11
17535	L	3	3	3	0	0	9
17703	R	1	3	3	0	1	8
17468	R	2	2	2	0	2	8

Case	Side	Synovitis	MCP	BML extension	Total
17463	L	2	1	1	3
17468	L	1	1	1	2
17468	R	2	3	1	5
17484	L	2	3	2	5
17484	R	2	3	2	5
17487	L	1	0	0	1
17497	L	0	3	2	3
17497	R	1	0	1	1
17498	L	2	2	2	4
17498	R	1	2	1	3
17535	L	2	3	2	5
17536	L	3	3	2	6
17644	L	1	1	1	2
17644	R	2	3	2	5
17673	L	0	0	1	0
17673	R	1	1	1	2
17690	L	2	3	2	5
17703	R	2	2	2	4
17723	L	2	1	1	3
17743	R	0	0	1	0
17979	R	3	3	1	6
17996	R	2	1	2	3
18534	L	1	3	2	4
18534	R	1	1	2	2
17487	R	2	3	2	5

Table 8: MRI scores of diseased samples (grading performed by TD)

Case	Side	Synovitis	MCP	BML extension	Total
17673	L	1	1	1	3
17498	R	0	3	2	5
17536	L	2	2	2	6
17468	R	1	3	1	5
17703	R	1	1	1	3
17498	L	1	1	2	4
17484	L	1	3	1	5
17484	R	1	1	1	3
17644	L	1	3	2	6
17487	L	1	1	1	3
17487	R	1	3	1	5
17468	L	2	1	2	5
18534	L	1	1	1	3
17723	L	2	3	1	6
17535	L	2	3	2	7
18534	R	1	1	1	3
17497	R	1	1	1	3
17690	L	2	3	2	7
17644	R	1	1	2	4
17743	R	1	1	1	3
17979	R	3	2	1	6
17996	R	2	2	1	5
17497	L	1	2	1	4
17463	L	3	1	1	5
17673	R	2	1	1	4

Table 9: MRI scores of diseased samples (grading performed by VW)

Case	Side	Synovitis	MCP	BML extension	Total
17536	L	2	1	2	5
17644	R	2	2	1	5
18534	R	2	1	1	4
17498	R	1	2	1	4
17487	R	2	2	2	6
17497	R	1	1	1	3
17979	R	3	2	2	7
17996	R	1	2	1	4
17673	R	2	2	1	5
18534	L	2	1	1	4
17723	L	2	1	2	5
17484	R	2	1	2	5
17644	L	1	3	1	5
17487	L	1	1	1	3
17743	R	1	0	1	2
17673	L	1	1	1	3
17468	L	2	1	1	4
17463	L	2	1	1	4
17498	L	1	1	2	4
17690	L	3	3	2	8
17497	L	2	2	2	6
17484	L	1	3	1	5
17535	L	1	3	2	6
17703	R	2	2	1	5
17468	R	1	3	2	6

Table 10: MRI scores of diseased samples (grading performed by VW, second set of measurements)

BV/TV Diseased	BV/TV Control
1,91	7,06
2,54	2,68
5,56	5,11
1,07	6,67
1,97	9,40
1,64	10,50
3,14	5,16
1,26	3,38
3,10	5,10
3,22	3,96
1,21	3,46
2,52	10,92
1,07	
7,25	
1,07	
2,17	
1,61	
1,89	
4,19	
0,69	
0,80	
0,70	
1,32	
1,18	
0,89	

Table 11: BV/TV ratio of diseased and control samples

Case	Side	Weight (kg)	Gender	Breed
1	L	31	FE	German shepherd dog
2	L	23	FE	Pitbull x
3	L	18	MN	Beagle
4	R	18	MN	Beagle
5	L	17	ME	Pitbull x
6	R	39	ME	German shepherd dog
7	L	24	ME	Pitbull x
8	R	24	ME	Pitbull x
9	L	29	ME	Pitbull x
10	R	29	ME	Pitbull x
11	R	23	ME	Pitbull x
12	L	29	ME	Pitbull x

Table 12: Case number, side, weight, gender, and breed of the control cases.