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Intervertebral disc disease in dogs – Part 1: A new histological grading scheme for classification of intervertebral disc degeneration in dogs

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ABSTRACT

Intervertebral disc (IVD) degeneration is common in dogs and can lead to serious disorders. Current treatments can relieve clinical signs of disease, but do not restore IVD function. The development of regenerative strategies for IVD dysfunction requires detailed knowledge of the pathogenesis of IVD degeneration and its underlying mechanisms. Histological examination of IVDs at different stages of degeneration might provide this knowledge, but as there is currently no histological grading scheme for canine IVD degeneration, the aim of this study, which is the first of a two-part series, was to design and validate an appropriate scheme.

Three independent observers evaluated 35 IVDs at different stages of degeneration using the scheme. Glycosaminoglycan contents of the nucleus pulposus and macroscopic grading according to Thompson, which are considered 'gold standards' for IVD degeneration, were used to validate the scheme. Reproducibility was assessed by analysing the inter-observer reliability of all individual variables of the grading scheme, using a weighted κ analysis. Significant correlations were found between Thompson grading and total histological score ($r = 0.94$; $P < 0.01$) and between glycosaminoglycan content and total histological score ($r = -0.72$; $P < 0.01$). Most individual histological variables showed 'moderate' to 'almost perfect' inter-observer reliability. The high correlation with the gold standards in combination with the high reproducibility indicates that the proposed histological grading scheme is reliable and objective for classification of IVD degeneration in both chondrodystrophic and non-chondrodystrophic dog breeds.

Introduction

Intervertebral disc (IVD) degeneration is common in dogs. Although it often occurs without causing clinical signs of disease, IVD degeneration may lead to cervical and thoracolumbar IVD herniation, degenerative lumbosacral stenosis, and cervical spondylomyelopathy (Bergknut, 2010). Different aetiological factors for IVD degeneration have been reported, such as genetic origin, trauma, inadequate nutrition, physiological ageing ('senile remodelling') and loading history (Hansen, 1952; Bray and Burbidge, 1998; Adams and Roughley, 2006). Although several studies have described the histological features of canine IVD degeneration (Hansen, 1951, 1952; Braund et al., 1975; Gillett et al., 1988; Bray and Burbidge, 1998; Johnson et al., 2010), the pathogenesis is still not clear.

Current treatment regimens for IVD degenerative diseases in dogs include conservative treatment (rest and anti-inflammatory/analgesic medication) and surgery with decompression of neuronal tissue combined with stabilization of the spinal segment as needed. As none of these approaches restores the biomechanical function of the affected spinal segment, the degeneration of the spinal segment is likely to continue which may lead to a stiffer spinal segment and arguably an increased risk for adjacent segment disease. Regenerative treatments restoring normal biomechanical function to the spinal segment would eliminate the risk of adjacent segment disease and would therefore be a preferred treatment method for IVD degenerative disorders.

The development of regenerative treatments for IVD degenerative diseases in dogs requires knowledge of the pathways involved in the pathogenesis. Histological changes in the IVD are frequently referred to as the 'gold standard' for IVD degenerative research in both dogs and humans (Seiler et al., 2003; Ganey et al., 2009; Saar

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et al., 2010), and recent studies have highlighted similarities in the degenerative process occurring in human and canine IVDs (Bergknut et al., 2011c). While human grading schemes for degenerative changes in IVDs, such as Pfirrmann's grading of magnetic resonance (MR) images (Pfirrmann et al., 2001) and the Thompson method for grading gross pathological changes (Thompson et al., 1990), have been validated for use in dogs (Bergknut et al., 2011a–c), there are some important differences between human and canine IVDs, the most notable being that the cartilaginous endplates are thicker in human IVDs than in canine IVDs (Bergknut et al., 2011c). These morphological differences mean that the histological grading scheme most commonly used to classify degenerative changes in human lumbar IVDs (Boos et al., 2002) is unsuitable for grading degeneration in canine IVDs. The main reason for this is that a substantial part of the grading scheme according to Boos et al. (2002) is focused at endplate pathology, which is difficult to accurately assess in the thin canine endplates.

This study is the first part of a two-part series. The aim of this present report was to develop and validate an objective histological grading scheme for the classification of IVD degeneration that is applicable in both chondrodystrophic and non-chondrodystrophic dog breeds. We wished to improve the applicability of histopathology as the gold standard for canine IVD research and to enable objective comparison of degenerated IVDs in dogs. Part 2 compares the clinical severity of IVD disease with the degree of IVD degeneration using this histological grading system (Kranenburg et al., 2012). Such a grading scheme will not only be useful in the development of new therapies for IVD degenerative disease, but will also be a valuable tool for evaluating and improving the accuracy of diagnostic techniques, thereby potentially facilitating the early identification of IVDs susceptible to degenerative disease, which in turn could make early initiation of preventive treatment possible.

Materials and methods

Study population

The thoracolumbar and lumbosacral vertebral columns (T11-S1) from 15 randomly selected fresh (<12 h of death) canine cadavers (150 IVDs in total) were used for this study. The dogs were of various breeds (both chondrodystrophic and non-chondrodystrophic), ages, and gender. There were five Beagles (five chondrodystrophic dogs), three Foxhounds, three Kerry Beagles, one Welsh Terrier, and three mixed-breed dogs (10 non-chondrodystrophic dogs). The dogs (10 females, 5 males) ranged from 1 to 16 years of age (median age, 5 years) and weighed between 9 and 44 kg.

All dogs were research dogs that had been euthanased in an unrelated study or were client-owned dogs (permission to use the spine was granted by the owners) that were submitted for necropsy to the Department of Pathobiology at the Faculty of Veterinary Medicine, Utrecht University. None of the dogs had a reported history of back problems.

Collection and processing of the spines

After dissection, the spines were transected in the midsagittal plane using a belt saw. High-resolution photographs were taken of the midsagittal surface of each spinal unit (endplate-intervertebral disc-endplate) and were used for grading according to the Thompson scheme (Thompson et al., 1990; Bergknut et al., 2011b).

Fresh nucleus pulposus (NP or nucleus pulposus) tissue from one half of each IVD was snap frozen for glycosaminoglycan (GAG) analysis (see below). Due to the cutting procedure there was only enough NP material to perform a GAG assay on 118/150 IVDs and these were the only IVDs used in this study. The remaining half of each IVD was retained for histological examination. Midsagittal slices (3–4 mm thick) were cut and the 118 intervertebral segments were fixed in 4% neutral buffered formaldehyde solution and subsequently decalcified in EDTA. After decalcification, all samples were embedded in paraffin, sliced with a microtome, and stained with haematoxylin and eosin (H&E) and Alcian blue/Picrosirius red (Gruber et al., 2002). The latter stain was used to evaluate changes in the composition of the extracellular and intercellular matrix, where Alcian blue stains mostly GAG and Picrosirius red stains collagen, with a higher affinity for collagen type I.

Thompson grading

The Thompson grading scheme (Thompson et al., 1990) is a five-category scheme for assessing the gross morphology of midsagittal sections of human lumbar IVDs and has been validated for use in dogs (Bergknut et al., 2011b). Pathological changes of the NP, the annulus fibrosus (AF), the endplates, and of the periphery of the vertebral body were assessed and all 118 IVDs were grouped by Thompson grade (i.e., I, II, III, IV or V), where grade I is healthy and grade V represents end-stage degeneration (Fig. 1). Grading was performed by: (1) a PhD student (NB), (2) a board-certified veterinary surgeon (BM), and (3) a board-certified veterinary pathologist (GG). All grading of the photographs was performed individually and blinded.

Glycosaminoglycan assay

The Farndale (dimethylmethylene blue) assay was used to measure the sulfated GAG content of the NP of all 118 IVDs (Farndale et al., 1986). Tissue samples were weighed in pre-weighed 1.5-mL Eppendorf cups. Protein digestion was performed overnight at 56 °C in a Proteinase K digestion buffer. The reaction buffer contained 50 mM TRIS (pH 7.6) dissolved in 100 mL milliQ water, 1 mM EDTA, 1 mM iodoacetamide, 10 μg/mL pepstatin A, and 1 mg/mL proteinase. After incubation, the samples were heated to 100 °C for 10 min to inactivate the proteinases K and then six dilutions in PBS/EDTA, ranging from 1:500 to 1:2000, were prepared from each sample. The composition of the PBS/EDTA (pH 6.5) was 0.1 M Na_2HPO_4 and 0.01 M EDTA. Each dilution (100 μL) was pipetted into a 96-well, flat-bottom, microtitre plate for spectrophotometric analysis (Greiner bio-one). Just prior to the spectrophotometric analysis, 200 μL of filtered dimethylmethylene blue solution were added to each well (standard and sample dilutions). The dimethylmethylene blue solution

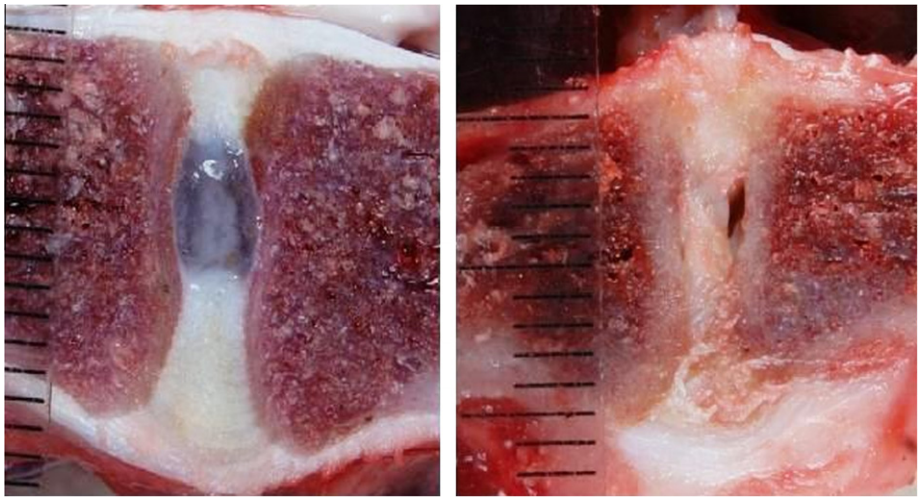

Fig. 1. Midsagittal sections of canine intervertebral discs as used for Thompson grading. A healthy intervertebral disc (Thompson grade I) is depicted on the left and a severely degenerated intervertebral disc (Thompson grade V) on the right.

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was prepared by dissolving 2.37 g NaCl and 3.04 g glycine in 1 L of milliQ water, and then 16 mg of dimethylmethylene blue (Polysciences) dissolved in 5 mL ethanol was added. The plate was read at wavelengths of 530 nm and 590 nm. A standard curve made up of different amounts of chondroitin sulfate (shark cartilage sodium salt, Sigma–Aldrich) was used.

Development of a histological grading scheme

The grading scheme of Boos et al. (2002) characterizes the histomorphology of the IVD (including AF and NP, cartilaginous endplate, and subchondral bone). Cellular changes (e.g., presence of notochordal cells, presence and proliferation of chondrocytes) and changes in the intercellular matrix and structural abnormalities (e.g., tearing) are also scored. As already mentioned, there are anatomical differences between canine and human IVDs rendering the Boos grading scheme less suitable for grading of histological changes of canine IVDs. A number of pilot studies using this human histological grading scheme were performed in order to identify and select suitable variables for a histological grading scheme for canine IVDs (data not shown).

Table 1
Histological grading scheme for canine intervertebral disc degeneration.

Morphology of annulus fibrosus (AF)	
0	Well-organized, half ring-shaped, collagen lamellae
1	Mild disorganized; some loss of half ring-shaped structure, most lamellar layer, still distinguishable (<25%)
2	Moderately disorganized; partly ruptured AF, loss of half ring-shaped structure (25–75%)
3	Completely ruptured AF; no or few distinguishable half ring-shaped collagen lamellae (>75%)
Chondrocyte metaplasia of AF	
0	No chondrocyte morphology, just spindle-shaped fibroblasts
1	Mild chondrocyte proliferation (i.e. limited to inner most AF layers)
2	Moderate chondrocyte proliferation (i.e. chondroid cells in up to half of the AF)
3	Marked chondrocyte proliferation (i.e. chondroid cells up to outer layers of the AF)
Tears and cleft formation	
0	Absent
1	Rarely present
2	Present in intermediate amounts
3	Abundantly present
4	Scar/tissue defects
Chondrocyte proliferation of nucleus pulposus	
0	No proliferation
1	Increased chondrocyte-like cell density
2	Connection of two chondrocytes
3	Small size clones (i.e., several chondrocytes group together, i.e. 2–7 cells)
4	Moderate size clones (i.e. >8 cells)
5	Huge clones (i.e. >15 cells)
6	Scar/tissue defects
Presence of notochordal cells in nucleus pulposus	
0	Abundantly present (>50%)
1	Present (1–50%)
2	Absent
Matrix staining of the nucleus pulposus with Alcian blue/Picrosirius red staining	
0	Blue stain dominates
1	Mixture of blue and red staining
2	Red stain dominates
Endplate morphology	
0	Regular thickness; homogeneous structure
1	Slightly irregular thickness
2	Moderately irregular thickness
3	Severely irregular thickness with interruption of the endplate
New bone formation	
0	Absent
1	Minor new bone formation
2	Moderate amounts of new bone formation
3	Abundant new bone formation; tendency towards bridging/complete bridging
Subchondral bone sclerosis	
0	No sclerosis (<2 × the thickness of the dorsal vertebral cortex)
1	Mild sclerosis (2–4 × the thickness of the dorsal vertebral cortex)
2	Moderate sclerosis (>4 × the thickness of the dorsal vertebral cortex)
3	Severe subchondral bone irregularities

Variables were included if they fulfilled at least two of three criteria: applicability, validity, and reproducibility. A variable was considered applicable if it could easily be identified in canine IVDs and if all stipulated grades of the specific criteria could be identified in IVDs of different stages of degeneration. A variable was considered valid if it showed a substantial, significant correlation of $r > 0.7$ with gross pathological changes graded according to the Thompson scheme (Thompson et al., 1990) and/or with the GAG content of the NP. Both the Thompson grading system and quantification of GAG content in the NP are well recognized and accepted 'gold standards' for IVD degeneration and are therefore used as such in this study. Finally, a variable was considered reproducible if inter- and intra-observer reliability of the variable had weighted κ values that were 'substantial' or higher (Koch et al., 1977; Landis and Koch, 1977a). Care was taken to include variables ensuring that the grading scheme would evaluate all different parts of the intervertebral segment (AF, NP, the cartilaginous endplates, and the subchondral bone of the adjacent vertebrae). A grading scheme composed of nine histological variables was developed (Table 1; Figs. 2a and b).

Validation of the histological grading scheme

All 118 IVD samples were graded according to Thompson et al. (1990) and grouped by Thompson grade. Seven histological samples per each Thompson grade (grades I, II, III, IV, and V), i.e. 35 samples in total, were randomly selected and histologically evaluated (Olympus BX41 microscope) in a blinded fashion by the three independent observers.

The reproducibility of grading of the nine individual histological variables was evaluated through a weighted kappa analysis of inter-observer reliability. During the pilot studies, the intra-observer reliability was consistently much higher than the inter-observer reliability, hence only the inter-observer reliability was evaluated in the current study (intra-observer reliability data of one of the observers from the final pilot study are shown in Table 2). The total histological score (the sum of all individual histological variables) for each IVD was then averaged between the scores of the three observers, and this averaged total histological score was then used for correlation with the Thompson score and the GAG content of the NP for the same IVD.

Statistical analysis

Inter- and intra-observer agreement was analysed using Cohen's weighted κ analysis. The interpretation of the κ values is as follows: poor (<0.00), slight (0.00–0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80), and almost perfect (0.81–1.00) (Landis and Koch, 1977a,b). A Pearson's correlation test was performed using SPSS 16.0 (IBM) to investigate the correlation between the total histological score and the individual histological variables and the two reference standards (i.e. Thompson grade and GAG content). Results were considered statistically significant if $P \leqslant 0.05$.

Results

This new histological grading scheme allowed for evaluation of all different parts of the canine IVD (AF, NP and endplate) as well as the subchondral bone, and all morphological characteristics typically seen in maturation and degeneration of the IVD were represented in the scheme.

Total histological scores

The total histological scores showed some inter-observer variation. Scores ranged from 4 to 11 (mean, 7.6) for Thompson grade I IVD degeneration, from 4 to 15 (mean, 10.3) for Thompson grade II, from 11 to 22 (mean, 17.0) for Thompson grade III, from 14 to 23 (mean, 18.9) for Thompson grade IV, and from 19 to 29 (mean, 24.8) for Thompson grade V.

Inter-observer agreement for the individual histological variables

The inter-observer agreement for the individual histological variables was generally good. The variables 'morphology of AF', 'presence of notochordal cells in NP' and 'new bone formation' showed the highest inter-observer ('almost perfect') agreement. The only variable to show 'slight' inter-observer agreement was 'Subchondral bone sclerosis'. The inter-observer agreement of the remaining five individual histological variables was 'moderate' or 'substantial'.

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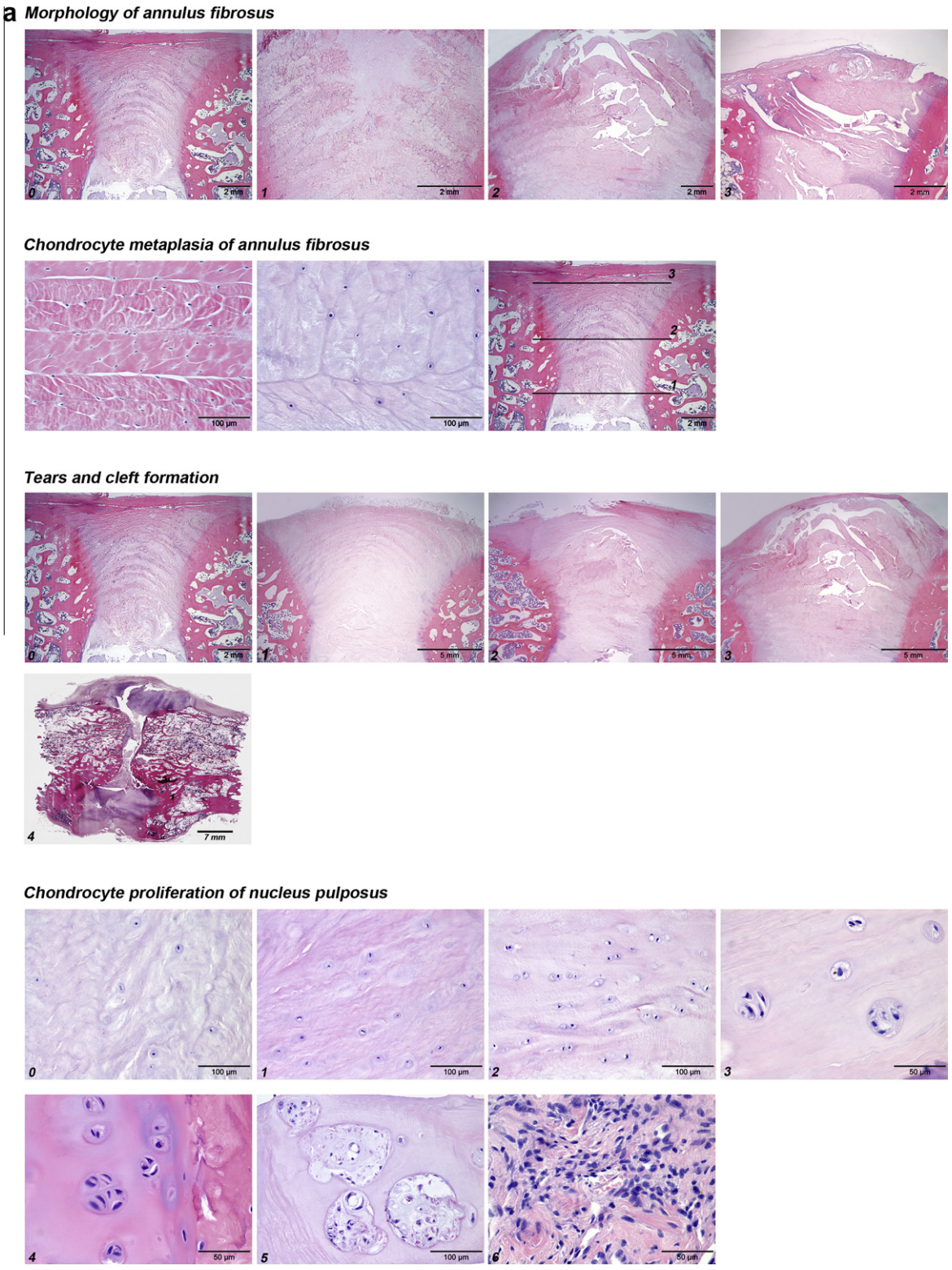

Fig. 2. (a and b) Representative histological images (H&E or Alcian blue/Picrosirius red stains) depicting increasing degree of degeneration from left to right, of the nine different variables included in the histological grading scheme. Chondrocyte metaplasia of the annulus fibrosus: the left image depicts fibrocyte morphology of stromal cells in the annulus fibrosus while the middle image shows chondroid morphology. The right image indicates the extent of the presence of chondroid cells in the annulus fibrosus and the accompanying grades.

Correlation with Thompson grades

Increasing severity (Thompson grade) of gross pathological degeneration was significantly correlated with increasing total histological score ($r = 0.94$, $P < 0.01$) (Fig. 3). All individual histological variables were also significantly correlated with increasing Thompson grade. The correlation was weakest for the variable 'new bone formation' ($r = 0.62$, $P < 0.01$) and strongest for the variable 'morphology of AF' ($r = 0.92$, $P < 0.01$) (Table 2).

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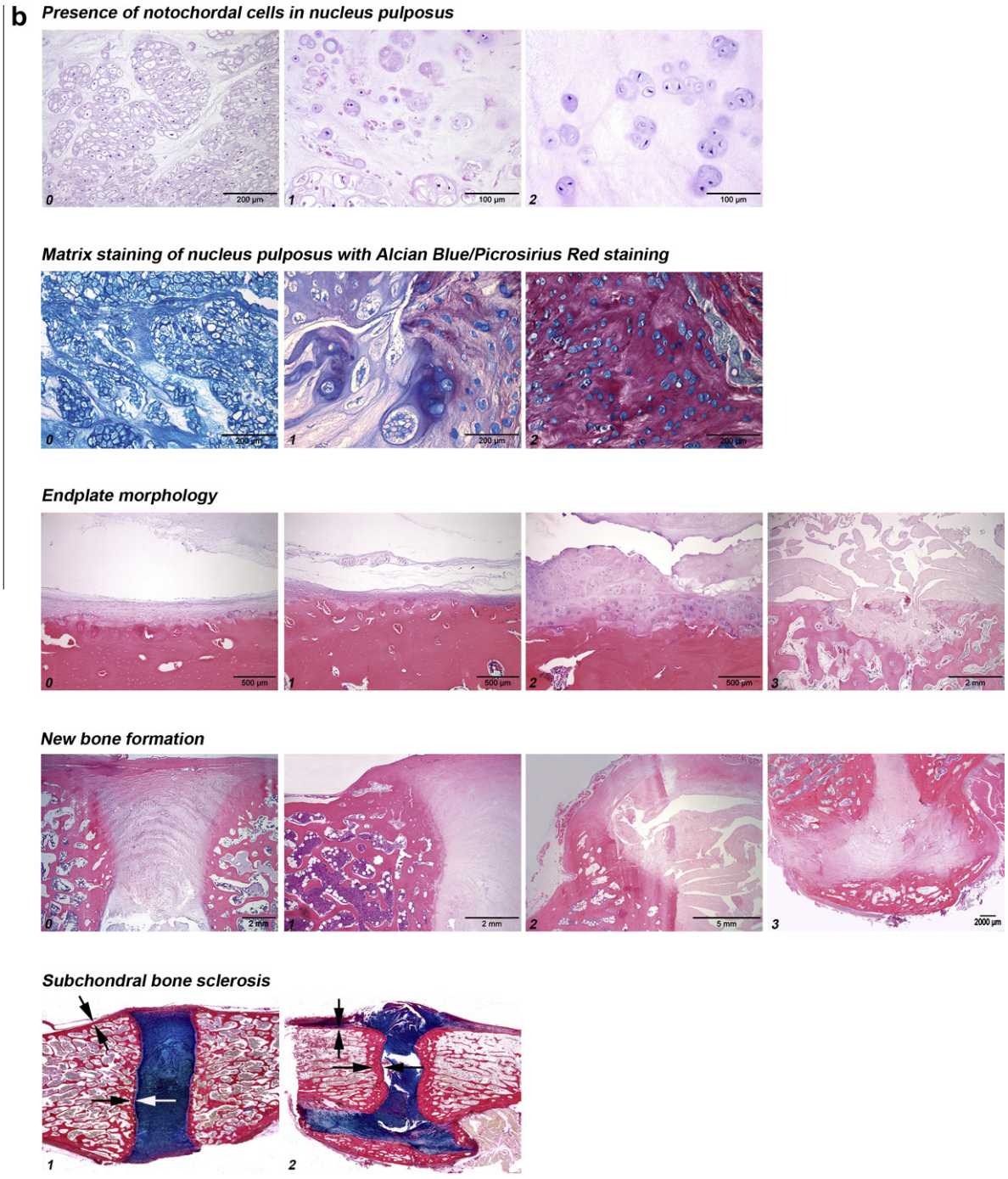

Fig. 2 (*continued*)

Correlation with GAG content

A higher concentration of GAG in the NP of IVDs was significantly and negatively correlated with a higher total histological score ($r = -0.72$, $P < 0.01$) (Fig. 4). The correlations between individual histological variables and GAG content of the NP were weaker but still significant (Table 2).

Discussion

The proposed histological grading scheme for classification of IVD degeneration in dogs was highly reproducible and strongly correlated with the two gold standards, indicating that it is an objective and reliable scheme for classification of IVD degeneration in dogs. The nine histological variables that comprise the scheme allow for evaluation of cytological changes, alterations in matrix composition, and structural changes that are often seen in spinal segments of dogs suffering from IVD degenerative disease.

The initial aim of this study was to evaluate and validate the commonly used histological classification scheme for IVD degeneration in humans according to Boos et al. (2002), for the use in dogs. However, the pilot study showed that the Boos grading scheme was not applicable for grading of degenerative changes seen in canine IVDs. The main reason for this is that a substantial part of the Boos grading scheme is focused on pathological changes of the cartilaginous endplates, which are much thicker in humans than in

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Table 2
Inter- and intra-observer reliability for the individual histological variables.

	Inter-observer reliability (κ)			Intra-observer reliability (κ)[a]	Correlation with macroscopic Thompson grading	Correlation with glycosaminoglycan content in NP
	Observers NB–GG	Observers JR–GG	Observers NB–JR	Observer NB		
Morphology of AF	0.83	0.70	0.76	0.84	0.92**	0.71**
Chondrocyte metaplasia of AF	0.42	0.58	0.41	0.72	0.75**	0.61**
Tears and cleft formations	0.80	0.51	0.59	0.80	0.86**	0.50**
Chondrocyte proliferation of NP	0.45	0.48	0.46	0.91	0.76**	0.44**
Presence of notochordal cells in NP	1.00	0.98	0.98	1.00	0.72**	0.59**
Matrix staining of NP with Alcian Blue/ Picrosirius Red staining	0.51	0.69	0.54	0.79	0.78**	0.55**
Endplate morphology	0.58	0.56	0.76	0.80	0.84**	0.62**
New bone formation	0.83	0.74	0.81	0.88	0.62**	0.55**
Subchondral bone sclerosis	0.35	0.19	0.60	0.71	0.75**	0.58**

Observer reproducibility was established using weighted kappa (κ) analyses. The correlation between the histological grade and the two gold standards (macroscopic degeneration according to Thompson and glycosaminoglycan content of the NP) was evaluated using Pearson correlation analyses. AF, annulus fibrosus; NP, nucleus pulposus.

[a] Intra-observer reliability data were obtained in a pilot study and are only shown for observer (NB).

** $P < 0.01$.

dogs (relatively to the entire IVD width) (Bergknut, 2010; Bergknut et al., 2011c). This difference probably occurs because vertebral growth in dogs is primarily regulated through separate epiphyseal growth plates located within the vertebrae at both the cranial and caudal ends, whereas in humans vertebral growth mainly takes place in the interface between the cartilaginous endplates and the subchondral bone. As the grading scheme for classification of human IVD degeneration was inadequate for grading canine IVD degeneration, a new grading scheme specifically for canine IVD degeneration was developed, based largely on the human grading scheme by Boos et al. (2002).

It has often been stated that there are two types of IVD degeneration in dogs, namely, chondroid IVD degeneration in chondrodystrophic dog breeds and fibroid IVD degeneration in non-chondrodystrophic dog breeds, and that the cell populations involved are to some extent different (Hansen, 1951, 1952; Braund et al., 1975; Gillett et al., 1988; Bray and Burbidge, 1998; Johnson et al., 2010). There is substantial evidence that IVD degeneration in chondrodystrophic dogs is a hereditary disorder, whereas IVD degeneration in non-chondrodystrophic dogs is more likely to be acquired through trauma or 'wear and tear' (Hansen, 1951, 1952; Braund et al., 1975; Gillett et al., 1988; Bray and Burbidge, 1998). Although IVD degeneration in chondrodystrophic and non-chondrodystrophic dogs may have a different aetiology and appears to have (to some extent) different distribution patterns of degeneration within the IVDs, a recent publication suggested that the cellular changes during the course of degeneration in chondrodystrophic and non-chondrodystrophic IVDs are more similar than previously described (Bergknut, 2010). Although this corroborates somewhat with the present study (in which fibrocyte-like cells were not seen in the degenerating NP of any dog breed and the degenerated IVDs of both types of dog breeds had a similar cartilaginous histopathological appearance), this warrants further evaluation, especially as none of the dogs in the present study had a history of spinal disease.

It is important to note that fibrocytes, which are reported to invade the IVD after penetrating tears or after surgical trauma of the AF (Shores et al., 1985; Wagner et al., 1987), do not seem to play a primary role in the degenerative process of the NP. A recent study investigating the micromorphometry and cellular characteristics of canine cervical IVDs identified significant differences in the size and position of the NP between the IVDs of chondrodystrophic

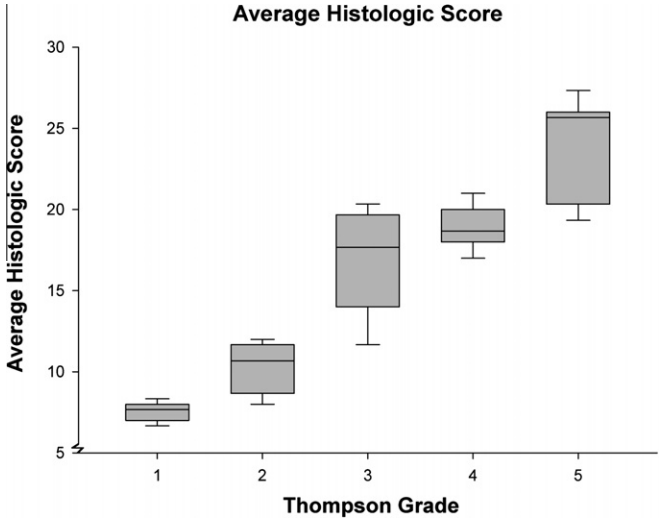

Fig. 3. Box plot showing the average total histological score per Thompson grade. A significant correlation ($r = 0.94$; $P < 0.01$) was found between increasing average total histological score of the intervertebral discs and increasing degree of degeneration as graded according to Thompson.

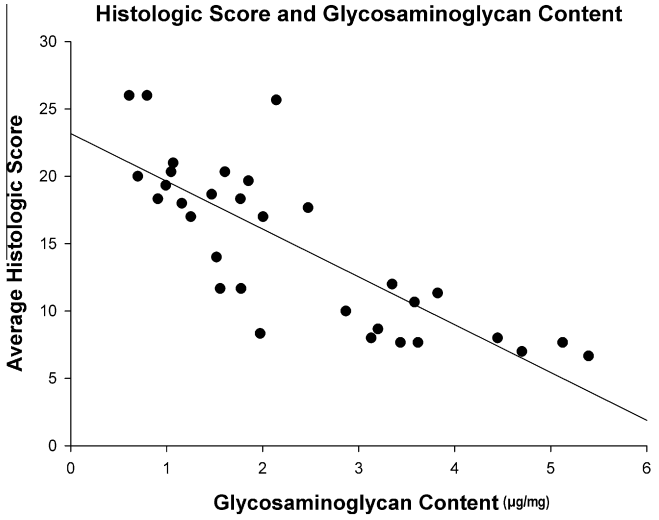

Fig. 4. Scatter plot depicting the significant negative correlation ($r = -0.72$; $P < 0.01$) between increasing average total histological score and increasing glycosaminoglycan content (µg/mg) of the nucleus pulposus.

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and non-chondrodystrophic dogs (Johnson et al., 2010). In the same study elongated cells, previously not described in canine IVDs, were identified in the NP of both chondrodystrophic and non-chondrodystrophic dogs. This may be explained by the fact that previous studies have only investigated thoracic, thoracolumbar or lumbar, and not cervical IVDs.

Although the histological score was strongly correlated with the Thompson grade, there were some discrepancies, mainly with Thompson grades III and IV. This is probably because pathological changes are often not homogenously distributed throughout the IVD. Localized, severely degenerated parts of the IVD might not be grossly visible and small focal lesions are likely to increase the histological score more than they would with the morphology-based Thompson score. The scores for some variables were highly correlated with the gold standards but showed poor reproducibility, whereas others had a lower correlation with the gold standards but a very high reproducibility. Histological variables that had a lower correlation with one of the two gold standards were included as long as they showed a high level of reproducibility and applicability, such as the variable 'new bone formation'.

Care was also taken to include histological variables that would allow for evaluation of all parts of the entire intervertebral segment and not only the IVD itself, because the surrounding bony structures can greatly influence and mirror the degeneration of the IVD. However, the two histological variables that evaluated the surrounding bony structures, namely, 'new bone formation' and 'subchondral bone sclerosis', were relatively weakly correlated with the gold standards, and the latter showed the lowest inter-observer reliability of all individual histological variables. The correlation between the GAG content of the NP and the average histological score was significant and consistent with earlier findings showing a correlation between decreasing GAG content and increasing degree of IVD degeneration (Braund et al., 1976; Gruber et al., 2002).

Although the histological variable 'tears and cleft formations' is intended for evaluation of the entire IVD, it was easier to distinguish annular tears (which are also referred to as radial tears) from histological artefacts caused by shrinkage and dehydration than clefts in the NP (also referred to as concentric tears). Thus NP clefts are more likely to be artefactual and not counted in contrast to annular tears. For this reason the variable 'tears and cleft formations' generally provides a better reflection of annular health compared to NP health.

The grading scheme proposed in this study has been developed for the post-mortem evaluation of entire intervertebral segments, and is thus not applicable for the investigation of surgical IVD biopsies. However a recent study indicated that the scheme could be adapted and used for the investigation of surgical IVD biopsies in dogs by omitting variables typically not visible in biopsies, such as endplate morphology, new bone formation, and subchondral bone sclerosis (Kranenburg et al., 2012). It is important to note that surgical samples, especially of herniated IVDs, may show inflammatory changes that should be taken into consideration (Kranenburg et al., 2012). In addition, the current grading system does not take into consideration IVD protrusion and extrusion, which are important characteristics of IVD disease. For this reason, future studies should investigate the relation between histological grading of IVD degeneration and clinical IVD disease.

The described grading scheme will be useful not only to help unravel the mechanisms underlying IVD degeneration in both chondrodystrophic and non-chondrodystrophic dog breeds, but also for developing new therapies for IVD degenerative disease in dogs. The scheme could also be valuable for evaluating and improving the accuracy of in vivo diagnostic techniques, leading to earlier diagnosis of dogs at risk of IVD degenerative diseases and opening the way for possible prophylactic and regenerative interventions.

Conclusions

The high correlation with the gold standards (macroscopic IVD grading by the Thompson grading scheme and GAG content of the NP) in combination with the high reproducibility indicates that the proposed histological grading scheme is reliable and objective for classification of IVD degeneration in both chondrodystrophic and non-chondrodystrophic dog breeds.

Conflicts of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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