Micromorphometry and Cellular Characteristics of the Canine Cervical Intervertebral Discs

J.A. Johnson, R.C. da Costa, and M.J. Allen

**Background:** Dogs have a high prevalence of disc-associated cervical spinal cord disorders. Despite this, there are no descriptions of the micromorphometry or cellular characteristics of canine cervical intervertebral discs.

**Hypothesis/Objectives:** To compare micromorphometric and cellular characteristics at 4 disc regions (outer annulus [OA], inner annulus [IA], transitional zone [TZ], nucleus pulposus [NP]). We hypothesized that measurements would differ between chondrodystrophic (CH) and nonchondrodystrophic (NCH) dogs.

**Animals:** Six CH dogs and 6 NCH dogs, under 3 years old.

**Methods:** Histologic sections of all cervical discs from each dog were examined. Micromorphometric variables included relative ratio of ventral to dorsal annulus fibrosus (AF), number and mean width of AF fibers, and relative percentage of NP. Cellular variables included cell density, morphology, and grouping.

**Results:** The NP from CH dogs was smaller, more rounded, and more dorsally located compared with that from NCH dogs. The NP occupied a greater proportion of the disc in NCH dogs compared with CH dogs (27.7 versus 15.8%; \(P<.001\)). The ratio of ventral to dorsal AF was approximately 3:1 in the CH group and 2:1 in the NCH group. Cellular variables were region dependent. Cell density was 2.4-fold higher in the OA compared with the NP. Approximately 50% of NP cells and 4.5% of OA cells occurred in groups.

**Conclusions and Clinical Importance:** Micromorphometric variables differed by CH status. Cellular variables differed by disc region. Our findings potentially can explain the high incidence of intervertebral disc extrusions in CH dogs compared with NCH dogs.

**Key words:** Dog; Neck; Spine.

The incidence of cervical spinal cord lesions in the dog ranges from 13.9 to 25.4%.\(^1\) Clinical signs associated with cervical intervertebral disc disease (IVDD) include severe neck pain, low head carriage, thoracic limb lameness or paresis, proprioceptive ataxia, and varying degrees of tetraparesis.\(^4\) The C2-C3 intervertebral disc is the most frequently affected. Incidence decreases caudally, but disc lesions at C6-C7 are seen commonly in large breed dogs with cervical spondylomyelopathy (CSM).\(^5\)-\(^7\)

Dog breeds can be categorized into 1 of 2 groups: chondrodystrophic (CH) and nonchondrodystrophic (NCH). Chondrodystrophy is characterized by a disturbance in endochondral ossification, and affected breeds include the Dachshund, Bassett Hound, Pekingese, Beagle, and Corgi.\(^8\) Dachshunds have a 19% prevalence of IVDD\(^9\) and account for 53–70% of all cases of canine disc disease.\(^10,11\) In these dogs, the notochordal cells found in the nucleus are replaced by chondrocyte-like cells, usually within the 1st year of life, forming a more fibrocartilaginous-type tissue.\(^10,11\) NCH dogs maintain a more mucoid nucleus, with notochordal cells persisting into adulthood.\(^11,12\) In humans, the notochordal cells of the disc are senescent in the fetus and disappear within the first 10–20 years.\(^13-15\) As such, the developmental stages of the human disc resemble those of CH breeds of dog.\(^14,16\)

Anatomic descriptions of the canine intervertebral disc frequently cite the lumbar disc as a model for all discs in the spine. This practice also was common in the human medical literature until it was recognized that the structure of human cervical discs differs substantially from that of discs at other locations.\(^17\) The annulus of human cervical discs is not constructed of concentric lamellae as thought previously. Instead, the annulus is thickened anteriorly, tapered laterally, and deficient posteriorly except for a small paramedian area of longitudinal fibers.

Several histologic descriptions of the canine intervertebral disc have been published.\(^10,18,19\) However, these studies have focused on the thoracic, lumbar, and lumbosacral discs, or have extrapolated data from studies in humans. Currently, no published accounts describe the histologic appearance of the cervical disc in the dog. The goal of this study was to describe the micromorphometry and regional cellular characteristics of the canine cervical
intervertebral disc, and to compare the disc features between CH and NCH breeds. We hypothesized that the micromorphometry and cellular characteristics of the cervical discs of CH and NCH dogs would be different.

**Materials and Methods**

**Slide Preparation**

The investigation was conducted in accordance with the guidelines and approval of the Institutional Animal Care and Use Committee of The Ohio State University. The cervical spine (C1-T1) was harvested en bloc from non-client–owned dogs euthanized for reasons unrelated to this study. Dogs were divided into 2 groups: CH and NCH. Skeletally mature dogs estimated on the basis of body and dental condition to be <3 years of age were included in this study. All spines were radiographed to confirm skeletal maturity, to exclude preexisting skeletal pathology (eg, vertebral malformation, disc space narrowing, or calcification) and to evaluate morphological integrity. Four spines were excluded for skeletal immaturity. The final study sample consisted of 12 spines: 6 from CH breeds and 6 from NCH breeds.

After radiography, each spine was stripped of all musculature and connective tissue. Each of the 5 cervical discs (C2-C3, C3-C4, C4-C5, C5-C6, and C6-C7) was removed with the endplates intact with a diamond band saw. discs were fixed in 10% neutral buffered formalin for at least 48 hours, and then decalcified in 10% ethylenediaminetetraacetic acid (pH 7.4) for up to 6 weeks. Progress was monitored by weekly radiography of the specimens. After decalcification, the discs were processed into paraffin. Two 5-μm-thick transverse slices were made from the cranial aspect of each disc. The sections then were deparaffinized, rehydrated, and stained with a standard hematoxylin and eosin protocol.

**Micromorphometric Analysis**

Slides were scanned up to 40× magnification with an Aperio Scanscope and analyzed with Aperio ImageScope Viewer software (v. 10.2.1.2314). The margins of the annulus fibrosus (AF), transitional zone (TZ), and nucleus pulposus (NP) were determined by hand tracing. The annulus was divided into outer (OA) and inner (IA) sections by dividing the total AF in half (Fig 1). The TZ was defined as the area between the AF and NP where distinct annular fibers could no longer be distinguished. Morphometric measurements were made with the distance and area features of the ImageScope software. Morphometric measurements included:

1. **Width of the AF, TZ, and NP**—Direct measurement of the width of each element was made. The ratio of ventral to dorsal AF was calculated for each disc.
2. **AF fibers**—A vertical line was drawn from dorsal to ventral bisecting the disc, and the numbers of distinct fibers in the dorsal and ventral annulus crossing this line were counted. Average dorsal and ventral fiber thickness was determined by taking the mean thickness of 5 randomly selected fibers from both the dorsal and ventral annulus.
3. **Cross-sectional area of the NP and total disc**—Cross-sectional area of NP and the entire intervertebral disc were determined, and a ratio of nuclear to total disc area calculated.

**Regional Cellular Characteristics**

Cellular measurements were made within a standardized 1.00 ± 0.01 mm² box within each of the 4 areas of interest (OA, IA, TZ, NP). The box was placed randomly within each region. To avoid bias, the areas of measurement were taken in different locations of the disc on the 2 slides, and the areas were selected from areas with no distortion of disc architecture. The magnification for counting was 20×. Cellular measurements were made as described previously, and included:

1. **Cell density**—The number of cells counted divided by the area of analysis provided a measurement of cellular density.
2. **Cell morphology** (Fig 2)—Cell morphology was recorded with cells defined as rounded or elongated (a cell with nuclear length equal to or greater than twice its width unless surrounded by a clearly defined round pericellular matrix).
3. **Cell grouping** (Fig 3)—Cells were classified as being present singly or as one of a pair (2 cells sharing a common pericellular or intracellular matrix) or cluster. The number of cells in each group was recorded.
Statistical Analysis

The pair of measurements obtained from 2 slides of each disc was averaged to create a set of mean values for each measurement. Data were compared across the 12 spines to identify any effect of disc level, region of interest (OA, IA, TZ, NP), or CH status. Micromorphometry data were analyzed by ANOVA with Tukey’s posthoc testing as appropriate. Because of heteroscedasticity in the data, the regional cellularity measurements were analyzed between CH and NCH groups on a site-by-site basis by an independent t-test. A significance level of \( P < .05 \) was used for all analyses.

Results

The NCH group consisted of 6 intact female dogs ranging from 18 to 22.5 kg in body weight. Breeds included were 2 Husky crosses, 3 German Shepherd crosses, and 1 Pit Bull cross. The CH groups consisted of 6 Beagles, ranging from 8 to 14 kg, including 4 intact females and 2 intact males.

Micromorphometry

A summary of the micromorphometry measurements is found in Table 1. The C2-C3 disc was consistently dorsally flattened, whereas the caudal discs were more rounded (Fig 4). The NP of discs from CH dogs had a smaller, more rounded shape that was more dorsally located when compared with the NP from the NCH dogs. The NP of the NCH dogs consisted of a centrally distributed syncytium of notochordal cells contained within a fibrillar matrix with peripheral invasion of more fibrous tissue. The NP of CH dogs had minimal to no residual notochordal tissue and replacement by chondrocyte-like cells surrounded by a denser, more fibrocartilaginous matrix (Fig 5).

Disc level did not have a significant effect on any measurement with the exception of disc area. C2-C3 was found to have a significantly smaller area than C5-C6 and C6-C7 (\( P = .042 \) and \( .02 \), respectively), and C3-C4 was significantly smaller than C6-C7 (\( P = .026 \)). A number of measurements were found to be significantly dependent on CH status, including the ratio of the ventral AF to the dorsal AF, number of ventral AF fibers, width of dorsal AF fibers, and relative percentage of NP. The NP of NCH dogs accounted for nearly 28% of the total disc surface area, whereas the NP of the CH dogs accounted for <16%. The ratio of ventral to dorsal annulus was nearly 3:1 in the CH group compared with the 2:1 ratio in the NCH group.

Regional Cellularity

A summary of the regional cellularity measurements is found in Table 2. Within individual discs, statistically significant differences were identified for all of the cellular variables according to disc region (OA, IA, TZ, NP). No significant differences were noted among discs at the 5 disc levels.

In the NCH group, cell density in the OA was approximately 2.4 times that in the NP, whereas in the CH group this difference was decreased to 1.65 times (Fig 6). Cell density differed significantly between the NCH and CH groups in the outer AF and NP (\( P = .007 \) and \( .022 \), respectively). Cells were predominately of an elongated morphology in the AF, and transitioned to a more rounded morphology in the NP.

Cell grouping increased in frequency from the outer to inner regions of the disc (Fig 7). This difference between groups was statistically significant in the IA and TZ (\( P = .004 \) and \( P < .001 \)). The mean number of cells per group increased from the OA to the NP, but this increase was not statistically significant for all regions.

Table 1. Summary of micromorphometry results for chondrodystrophic and nonchondrodystrophic dogs across all cervical disc levels.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Chondrodystrophic Mean ± SD</th>
<th>Nonchondrodystrophic Mean ± SD</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventral AF: dorsal AF ratio</td>
<td>2.78 ± 0.94</td>
<td>1.94 ± 0.83</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Number of ventral AF fibers</td>
<td>29.22 ± 5.35</td>
<td>23.34 ± 4.18</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Number of dorsal AF fibers</td>
<td>21.55 ± 3.20</td>
<td>21.32 ± 4.09</td>
<td>.805</td>
</tr>
<tr>
<td>Mean width of ventral AF fibers (mm)</td>
<td>0.16 ± 0.04</td>
<td>0.17 ± 0.04</td>
<td>.352</td>
</tr>
<tr>
<td>Mean width of dorsal AF fibers (mm)</td>
<td>0.08 ± 0.02</td>
<td>0.11 ± 0.40</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Total disc area (mm²)</td>
<td>126.36 ± 14.80</td>
<td>169.86 ± 13.86</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Relative % of NP (%)</td>
<td>15.82 ± 4.91</td>
<td>27.75 ± 7.40</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

*AF, annulus fibrosus; NP, nucleus pulposus.

Results are presented as mean ± standard deviation.
difference was not significant, nor were any differences between CH and NCH groups.

Both the percentage of cells occurring in pairs and the percentage occurring in clusters were found to be strongly correlated to disc region (both $P < .001$). The CH group had a higher percentage of cells in pairs than did the NCH groups in all regions, but these differences were not statistically significant. The CH group also had a higher percentage of cells in clusters across all regions, but this difference was significant only in the TZ region ($P = .030$). The mean number of cells per cluster tended to increase from the outer to inner regions, but this difference was not statistically significant. No difference between CH status was found for this measurement.

Pair density (pairs/mm$^2$) and cluster density (cluster/mm$^2$) also were strongly correlated with disc region (both $P < .001$), with both increasing from the outer to inner regions. Significant differences in pair density were found between the CH and NCH groups in the IA, TZ, and NP ($P = .021$, .013, and .004, respectively). Significant differences in cluster density were found only in the NP ($P = .013$).

**Discussion**

We identified significant differences in the micromorphometry and cellular characteristics of canine cervical intervertebral discs both across the 4 regions of the disc (OA, IA, TZ, and NP), and between CH and NCH dogs. The NP of CH dogs was smaller, more rounded, and more dorsally located than that of NCH dogs, accounting for 16% of total disc surface area compared with 28% in the NCH group. The ventral AF was 3 times the height of the dorsal AF in the CH group and

Fig 4. Discs C₂-C₃ and C₆-C₇ from a nonchondrodystrophic (A and C) and chondrodystrophic (B and D) dog, demonstrating dorsal flattening of C₂-C₃.

Fig 5. Photomicrographs of nuclear tissue from a chondrodystrophic dog (A) demonstrating rounded cells in a fibrocartilaginous matrix, and a nonchondrodystrophic dog (B) showing persistent notochordal syncytium embedded in a more fibrillar matrix (12×).
twice the height of the dorsal AF in the NCH group, a significant difference. The CH group also showed significantly more fibers in the ventral AF, and a narrower average width of fibers in the dorsal AF. This combination may play a role in Type I disc disease of CH breeds. The eccentrically located, smaller NP of CH dogs may be less able to withstand pressure than the NP of NCH dogs. In combination with a proportionally more

Table 2. Summary of cellularity results for chondrodystrophic and nonchondrodystrophic dogs across all cervical disc levels. 

<table>
<thead>
<tr>
<th>Measurement</th>
<th>CH/NCH</th>
<th>OA</th>
<th>IA</th>
<th>TZ</th>
<th>NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell density (cells/mm²)</td>
<td>NCH 219.03 ± 35.37</td>
<td>178.80 ± 44.58</td>
<td>131.37 ± 32.53</td>
<td>89.50 ± 46.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CH 191.83 ± 39.28</td>
<td>160.80 ± 47.36</td>
<td>120.83 ± 30.58</td>
<td>116.13 ± 41.22*</td>
<td></td>
</tr>
<tr>
<td>% of rounded cells</td>
<td>NCH 18.67 ± 6.26</td>
<td>20.49 ± 7.72</td>
<td>49.00 ± 15.60</td>
<td>60.89 ± 7.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CH 17.53 ± 6.17</td>
<td>22.87 ± 7.02</td>
<td>50.71 ± 9.92</td>
<td>66.33 ± 8.93*</td>
<td></td>
</tr>
<tr>
<td>% of elongated cells</td>
<td>NCH 81.45 ± 6.20</td>
<td>79.05 ± 9.52</td>
<td>52.86 ± 15.85</td>
<td>38.93 ± 7.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CH 82.51 ± 6.16</td>
<td>77.34 ± 6.94</td>
<td>49.51 ± 9.84</td>
<td>34.68 ± 8.56*</td>
<td></td>
</tr>
<tr>
<td>% of total cells in groups</td>
<td>NCH 3.71 ± 3.26</td>
<td>5.37 ± 4.95</td>
<td>17.89 ± 10.15</td>
<td>47.97 ± 14.56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CH 5.25 ± 2.86</td>
<td>9.67 ± 6.22*</td>
<td>27.82 ± 9.70***</td>
<td>53.95 ± 13.81</td>
<td></td>
</tr>
<tr>
<td>Mean number of cells per group</td>
<td>NCH 2.16 ± 0.42</td>
<td>2.08 ± 0.11</td>
<td>2.19 ± 0.19</td>
<td>2.66 ± 0.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CH 2.09 ± 0.33</td>
<td>2.09 ± 0.09</td>
<td>2.16 ± 0.09</td>
<td>2.65 ± 0.28</td>
<td></td>
</tr>
<tr>
<td>% of total cells in pairs</td>
<td>NCH 3.25 ± 2.83</td>
<td>4.65 ± 3.83</td>
<td>13.77 ± 6.92</td>
<td>23.27 ± 9.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CH 4.73 ± 2.95</td>
<td>6.48 ± 5.76</td>
<td>15.31 ± 8.36</td>
<td>28.07 ± 9.32</td>
<td></td>
</tr>
<tr>
<td>% of total cells in clusters</td>
<td>NCH 0.44 ± 0.90</td>
<td>0.63 ± 1.37</td>
<td>4.00 ± 3.85</td>
<td>24.88 ± 10.92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CH 0.50 ± 0.95</td>
<td>1.49 ± 1.60*</td>
<td>5.79 ± 3.56</td>
<td>28.07 ± 12.79</td>
<td></td>
</tr>
<tr>
<td>Mean number of cells per cluster</td>
<td>NCH 3.15 ± 0.22</td>
<td>3.20 ± 0.84</td>
<td>3.80 ± 0.45</td>
<td>3.30 ± 0.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CH 3.13 ± 0.93</td>
<td>3.04 ± 0.95</td>
<td>3.16 ± 0.18</td>
<td>3.89 ± 0.49</td>
<td></td>
</tr>
<tr>
<td>Pair density (pairs/mm²)</td>
<td>NCH 3.70 ± 3.67</td>
<td>4.15 ± 3.46</td>
<td>9.40 ± 6.47</td>
<td>10.10 ± 6.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CH 4.58 ± 3.44</td>
<td>6.38 ± 3.82*</td>
<td>13.45 ± 5.73*</td>
<td>15.65 ± 7.99**</td>
<td></td>
</tr>
<tr>
<td>Cluster density (clusters/mm²)</td>
<td>NCH 0.35 ± 0.66</td>
<td>0.38 ± 0.74</td>
<td>1.73 ± 0.63</td>
<td>5.80 ± 3.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CH 0.32 ± 0.56</td>
<td>0.67 ± 0.72</td>
<td>2.23 ± 1.61</td>
<td>8.63 ± 4.72*</td>
<td></td>
</tr>
</tbody>
</table>

NCH, nonchondrodystrophic; CH, chondrodystrophic; OA, outer annulus; IA, inner annulus; TZ, transitional zone; NP, nucleus pulposus.

*Results are presented as mean ± standard deviation.
**P < .05.
***P < .01.
****P < .001 (P-value of CH in relation to NCH).

Fig 6. Mean cell density results for the 4 regions of the cervical intervertebral discs of chondrodystrophic and nonchondrodystrophic dogs. Cell density differed significantly between the 2 groups of dogs in the outer annulus and the nucleus pulposus (*P < .05). Bars represent mean ± standard deviation.

Fig 7. Percentage of cells occurring in groups for the 4 regions of the cervical intervertebral disc of chondrodystrophic and nonchondrodystrophic dogs. The percentage of grouped cells differed significantly between the 2 groups of dogs in the inner annulus and transitional zone (*P < .05). Bars represent mean ± standard deviation.
narrow dorsal AF and more narrow dorsal AF fibers, this difference may predispose CH dogs to traumatic NP extrusion rather than protrusion.

We did not identify any significant differences across the 5 disc levels for any measurement with the exception of disc area. This result was expected, because it was evident grossly that the discs increased in size from cranial to caudal levels. C2–C3 was observed consistently to be dorsally flattened compared with the remaining cervical discs.

The AF of the canine cervical discs had 13–32 distinct laminae, similar to the 15–30 laminae reported in human lumbar discs.21,22 The gross structure of the canine cervical disc is similar to earlier general descriptions of the thoracolumbar intervertebral discs,18,23,24 whereas reports in medical literature indicate that the cervical discs of humans are significantly different in structure from discs of the lumbar spine, with the AF having a crescentic shape that tapers posterolaterally rather than concentric rings.17 We identified concentric annular fibers encircling the disc with no evidence of posterolateral tapering.

The structure of the NP was markedly different between the CH and NCH groups. The NP of the NCH dogs consisted of a centrally distributed syncytium of notochordal cells contained in a fibrillar matrix with peripheral invasion of more fibrous tissue. The NP of the CH dogs demonstrated rounded chondrocyte-like cells surrounded by a denser, more fibrocartilaginous matrix with minimal residual notochordal syncytium. This observation is consistent with reports describing the structure of the thoracolumbar discs.10,18

Cell density consistently decreased from the outer to inner regions of the disc. Cells in the outer and inner AF tended to be elongated and were frequently aligned with the direction of each fiber. The fibers became indistinguishable in the TZ, and cell morphology became approximately 50% elongated and 50% rounded and disorganized in both groups. The percentage of rounded cells reached a maximum of 60–66% in the NP. These findings are comparable with similar reports in both humans and rats.20,25 This information has not been described previously in dogs.

Cell grouping increased when transitioning from the outer to inner regions of the intervertebral disc. This change included both the percentage of cells occurring in groups, and the number of pairs and clusters per square millimeter. Cellular grouping is well described in the intervertebral discs of humans, as are similar regional differences as those identified in this study.20,26,27 Intervertebral disc cell clustering has been well reported in both the human and rat, and although the exact cause of grouping has yet to be established, it is possibly linked with disc degeneration.15–17 This possibility should be further investigated in dogs, because changes in cell density and clustering may be useful to distinguish normal aging changes from degenerative changes in the intervertebral discs.

Intervertebral disc tissue is subject to some distortion during slide preparation, and all slides showed a slight degree of folding artifact. This artifact is a limitation to this study and may have resulted in underestimation of the area measurements. To minimize error, measurements were taken from areas of the disc with little to no folding artifact. The intervertebral disc is also subject to both changes in water content that may occur after death and to swelling that occurs during formalin fixation.30–32 The majority of this swelling occurs in the NP and is related to the high glycosaminoglycan content of this region. Glycosaminoglycans also may be leached from the tissues during formalin fixation.33 These limitations are well recognized in histologic studies of human intervertebral discs. Disc fixation, decalcification, and slide preparation in this study followed guidelines published for previous intervertebral disc studies.20,34–36

All of the dogs in this study were young, and future studies should include comparisons between young and old dogs to identify changes in both micromorphometry and cellularity that occur with aging and degeneration. Comparisons between clinically affected and normal breed-matched dogs with IVDD and disc-associated CSM also should be performed to further our understanding of the pathogenesis of these diseases.

In conclusion, our study identified significant differences in micromorphometry of the cervical intervertebral disc between CH and NCH dogs. Particularly, we found evidence of characteristics that may make clinical IVDD more common in CH canine breeds.

---

**Footnotes**

*Marmed Bone Saw; Marmed Inc, Cleveland, OH
*a Scanscope XT, Aperio, Vista, CA

---

**Acknowledgment**

Funding for this study was provided by the Canine Health Fund of the College of Veterinary Medicine, The Ohio State University.

**References**


