Relationships between cerebrospinal fluid characteristics, injury severity, and functional outcome in dogs with and without intervertebral disk herniation

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Abstract

Background: Cerebrospinal fluid (CSF) is commonly acquired in dogs with intervertebral disk herniation (IVDH) and is a common method to assess inflammatory responses following spinal cord injury (SCI).

Objectives: The purpose of the study was to describe relationships between cisternal CSF characteristics, behavioral measures of SCI, T2 weighted (T2W) hyperintensity on magnetic

resonance imaging (MRI), and long-term outcome in dogs with IVDH. Diagnostic accuracy of CSF for differentiating IVDH from other myelopathies was also assessed.

Methods: The retrospective case series included 727 dogs, 443 with thoracolumbar IVDH, 103 with cervical IVDH, and 181 with other spinal cord diseases. Signalment, initial neurologic function, ambulatory function at long-term follow-up, T2W MRI, and CSF variables were recorded for dogs with IVDH. Signalment, etiology, and CSF data were retrieved for dogs with other myelopathies. Associations between CSF predictors, diagnosis, and outcomes were assessed.

Results: CSF total nucleated cell count (TNCC) increased with SCI severity (rho -0.256, P<.001) in dogs with IVDH, TNCC was significantly higher in the presence of T2W hyperintensity (P = .001) in dogs with thoracolumbar IVDH, but TNCC, RBC count, microprotein, and percent neutrophils decreased with increasing injury duration (rho -0.253, P<.001; rho -0.269, P<.001; rho -0.141, P=.004, and rho -0.356, P <.001, respectively). CSF characteristics were not accurate for differentiating IVDH from other spinal cord diseases.

Conclusions: In dogs with IVDH, CSF TNCC, RBC count, microprotein, and percent neutrophils are correlated to clinical aspects of SCI such as injury severity and duration, but cannot differentiate IVDH from other etiologies.

Intervertebral disk herniation (IVDH) occurs in both the cervical and thoracolumbar vertebral column and is a common cause of spinal cord injury (SCI) in dogs.[1] In IVDH, compressive/contusive SCI is believed to result in a complex cascade of secondary mechanisms, including vascular disturbance, oxidative stress, excitotoxicity, and inflammation.[2-5] The inflammatory cell infiltrate in SCI has been well characterized in several species.[6] Blood-spinal cord barrier disruption and associated innate inflammatory events occur within hours of SCI and cause neutrophilic diapedesis and microglial activation.[6] In the days and weeks after SCI, adaptive immune responses are initiated due to exposure of self antigens, and may cause lymphocytic and mononuclear cell infiltration within injured parenchyma.[6]

In people and veterinary species, cerebrospinal fluid (CSF) analysis has been proposed as a minimally invasive method to assess inflammatory responses following SCI.[7-9] Small studies with populations of 35-72 dogs have suggested that following thoracolumbar IVDH, 12.5-54% of cisternal CSF samples have an increased total nucleated cell count (TNCC), and often neutrophils are identified as the predominant cell type.[9-12] In a large retrospective study of dogs with thoracolumbar IVDH (n=312), lumbar CSF pleocytosis was identified in 61% of samples, and lymphocytes were the most frequently reported predominant cell type.[13] The presence of CSF pleocytosis in dogs with thoracolumbar IVDH is associated with behavioral measures of SCI severity at the time of sampling.[12-14] Two previous studies have examined relationships between cisternal CSF pleocytosis and functional outcome in IVDH, with one report indicating that long-term ambulatory status was related to CSF TNCC at the time of SCI.[12,14] Magnetic resonance imaging (MRI) T2 weighted (T2W) sequences are increasingly being utilized as an alternative means to assess SCI severity, as hyperintensity has been associated with histologic features of SCI, impaired sensory and motor function, and poor motor outcomes in dogs with

thoracolumbar IVDH; to date, relationships between CSF inflammatory responses and MRI T2W spinal cord signal have not been assessed in dogs with IVDH.[15,16] Finally, it remains unclear as to whether cisternal CSF microprotein and TNCC in IVDH-associated SCI differs from other diseases of the spinal cord and could be used to differentiate etiologies.[17]

The retrospective study reported here was designed to describe cisternal CSF variables in a large population of dogs with IVDH-associated SCI, and to estimate relationships between CSF characteristics and initial behavioral SCI measures, spinal cord T2W hyperintensity at the time of injury, and long-term functional outcome. Cisternal CSF variables have not been previously assessed in a robust population of dogs with IVDH, and correlations with motor outcome have been attempted only in much smaller scale studies and have not utilized logistic regression to adjust for confounding variables. A second objective was to compare cisternal CSF data in dogs with IVDH to dogs with divergent etiologies that affect the spinal cord. We hypothesized that 1) CSF TNCC would increase with injury severity; 2) that CSF TNCC would decrease with longer SCI duration; 3) that CSF variables would be related to functional outcome; and 4) that CSF would not permit the reliable differentiation of IVDH from other etiologies causing spinal cord dysfunction.

Materials and Methods

Animal Selection

Medical records from August 2008 to December 2010 for all dogs admitted to Texas A&M University Veterinary Medical Teaching Hospital which had CSF analysis performed were retrospectively reviewed. Dogs were included in this study if they had neurologic signs localized to the spinal cord based on physical examination, acquisition of CSF from the

cerebellomedullary cistern, and a diagnosis of IVDH or other neurologic disease. A diagnosis of IVDH was determined by biopsy of herniated disk if surgical therapy was elected, or by advanced imaging (MRI, computed tomography (CT), or myelography) when medical management was performed. The diagnosis of non-IVDH diseases was based on necropsy, surgical biopsy, or a combination of MRI findings and history. Medical record data from subsequent admissions for IVDH or non-IVDH diseases were excluded.

Medical Records Data

Age, sex, weight, breed, duration of clinical signs, and duration of hospitalization were extracted from records for all dogs. Dogs were classified as chondrodystrophoid when the recorded breed was Dachshund (miniature and standard), Pekingese, West Highland White Terrier, Corgi, Japanese Chin, Bassett Hound, Shih Tzu, Cocker Spaniel, Lhasa Apso, Bichon Frise, or Beagle.[18-20] In dogs with IVDH, lesions were classified as cervical when affecting disk spaces between C2 and T1 and thoracolumbar when affecting disk spaces between T1 and L7.

Neurologic dysfunction was classified at admission, discharge from the hospital, and 42 day post-admission during an in-hospital recheck using the modified Frankel score (MFS). The MFS grades dysfunction as: paraplegia/tetraplegia with no deep nociception (grade 0), paraplegia/tetraplegia with no superficial nociception (grade 1), paraplegia/tetraplegia with intact nociception (grade 2), non-ambulatory paraparesis/tetraparesis and ataxia (grade 3), ambulatory paraparesis/tetraparesis and ataxia (grade 4), spinal hyperesthesia only (grade 5), or no dysfunction (grade 6).[21] Dogs were considered ambulatory if they walked without assistance at either discharge or 42 day recheck evaluation.

CSF Collection and Analysis

Cerebellomedullary CSF samples were collected with 1.5 in., 22 Gauge needles into 2 sterile glass red top tubes and were analyzed within 1 h of collection. TNCC and RBC counts were determined with a hemocytometer, by counting all cells of each type within 10 large squares of the grid.[22] CSF differential counts were performed on cytospin preparations made with 100 µl of CSF and stained with a modified Wright's stain or a quick Romanowsky stain. CSF microprotein concentration was determined using a commercial automated chemistry analyzer (Vitros 250, Ortho-Clinical Diagnostics Inc, Rochester, NY). The Pandy test was performed by adding 2 drops of CSF to 1mL of Pandy's reagent (10gm phenol in 100mLs distilled water), and evaluating the solution for turbidity.

A pleocytosis was diagnosed if the TNCC was ≥ 5 cells/µl, and classified according to the cell type that was >60% of the nucleated cell population. If no cell type was >60% of the TNCC, the sample was categorized as a mixed cell pleocytosis. A CSF was determined to have eosinophilic pleocytosis when >10% of cells were eosinophils, even if another cell type was predominant. Samples without a pleocytosis but whose microprotein concentration was ≥30 mg/dl were interpreted as albuminocytologic dissociation. Blood contamination was defined as >13,200 RBC/µl[23] and these samples were excluded from all statistical analyses beyond those characterizing the signalment of the population.

Magnetic Resonance Imaging

All dogs with IVDH that had vertebral column T2W MRI performed at the time of initial admission were identified. A 1.0T magnet (Magnetom Expert, Siemens Medical USA, Malvern, PA) was used to acquire images in all cases (generally, for sagittal images repetition time (TR) = 3500 ms, echo time (TE) = 90 ms, slice thickness = 2 mm; for transverse images TR = 4600 ms,

TE 99 ms, slice thickness = 3mm). Images were evaluated by a board certified neurologist (JML) using a computer workstation and commercially available software (eFILM 3.2 Veterinary, Merge Healthcare, Cleveland, OH); the neurologist was blinded to clinical information during image review. The reviewer evaluated T2W sagittal and transverse images to determine whether spinal cord hyperintensity was present at or immediately adjacent to the site of IVDH.

Statistical Analysis

Data were presented as median and interquartile ranges (IQR) because the normality assumption was violated for some variables. Categorical data were reported as proportions with 95% mid-p adjusted exact confidence intervals (CI).[24] Frequencies were compared among treatment groups using chi-square tests. Quantitative data were compared using Kruskal-Wallis tests when the number of groups was 3, and Mann-Whitney U tests for 2 groups. Bonferroni adjustment of P values was employed for post-hoc pairwise comparisons. Correlations between quantitative variables were estimated using Spearman's rho. Receiver-operating characteristics (ROC) analysis was performed to identify cutoffs for quantitative variables that best differentiated IVDH from non-IVDH dogs. Sensitivity (Se), specificity (Sp), and their 95% CI were reported for the cutoffs that maximized the Youden index (Se + Sp -1). Multivariable logistic regression was used to identify factors significantly associated with functional recovery (ambulatory status) and differentiation of IVDH from non-IVDH dogs. An initial bivariable screening of variables was performed and all factors with $P < 0.2$ were included in a backwards stepwise procedure. Initial modified Frankel score (MFS) was included in all models predicting functional recovery. Other confounders and possible effect measure modification were not

assessed due to limited availability of literature suggesting that signalment affects the accuracy of CSF analysis. Statistical analysis was performed in commercially available software (SPSS version 18.0, SPSS Inc., Chicago, Ill, USA) and results interpreted at the 5% level of significance.

Results

In total of 443 dogs with thoracolumbar IVDH, 103 dogs with cervical IVDH, and 181 dogs with other diseases of the spinal cord were identified (Table 1). Dogs with other spinal cord disease were diagnosed with myelitis (n=34), cervical spondylomyelopathy (34), ischemic myelopathy (28), unknown (26), neoplasia (17), degenerative myelopathy (12), lumbosacral disease (10), and 5 other disease categories with <10 individuals per category (20). Of the identified dogs, 10 with thoracolumbar IVDH and 7 with other diagnoses were excluded from analysis concerning relationships between CSF, injury severity, functional outcome, and disease classification (IVDH versus other spinal cord diagnosis) due to CSF blood contamination. The majority dogs with thoracolumbar (401/443) and cervical (86/103) IVDH were treated surgically. Euthanasia was performed by the 42 day re-check in 27/443 dogs with thoracolumbar IVDH, 3/103 dogs with cervical IVDH, and 10/181 dogs with other spinal cord diseases.

The median TNCC was 3/µL (IQR 1, 8 cells/µL; range 0-448 cells/µL) in dogs with thoracolumbar IVDH, 3/µL (IQR 1, 6 cells/µL; range 0-172 cells/µL) in dogs with cervical IVDH, and 1/µL (IQR 1, 4 cells/µL; range 0-6230 cells/µL) in dogs with other spinal cord disease (Table 2). In dogs with thoracolumbar IVDH, 134 (30.9%) individuals had CSF pleocytosis with 58 dogs with neutrophilic pleocytosis (range 5-448 cells/µL), 30 dogs with large mononuclear pleocytosis (range 5-245 cells/µL), 23 dogs with mixed cell pleocytosis (range 6-43

Table 1. Descriptive statistics and comparisons for signalment in 727 dogs with neurological disease examined between August 2008 and December 2010 at a single referral hospital.

Variable	Thoracolumbar IVDH (n=443)		Cervical IVDH (n=103)		Other diagnosis (n=181)	
	Proportion/ median (n)*	95% CI/ IQR*	Proportion/ median (n)*	95% CI/ IQR*	Proportion/ median (n)*	95% CI/ IQR*
Dachshund†	0.62[a] (443)	0.57, 0.66	0.34[b] (103)	0.25, 0.44	0.03[c] (181)	0.01, 0.06
Labrador retriever†	0.02[a] (443)	0.01, 0.03	0.04[a] (103)	0.01, 0.09)	0.17[b] (181)	0.12, 0.23
Mixed breed dog	0.06 (443)	0.04, 0.08	0.11 (103)	0.06, 0.18	0.07 (181)	0.04, 0.12
Chondrodystrophoid breed†	0.76[a] (443)	0.71, 0.79	0.51[b] (103)	0.42, 0.61	0.09[c] (181)	0.06, 0.14
Intact female	0.07 (443)	0.05, 0.09	0.02 (103)	0.00, 0.06	0.06 (181)	0.03, 0.10
Spayed female†	0.40[a] (443)	0.35, 0.44	0.59[b] (103)	0.50, 0.68	0.40[a] (181)	0.33, 0.47
Intact male	0.14 (443)	0.11, 0.17	0.15 (103)	0.09, 0.22	0.14 (181)	0.09, 0.19
Neutered male†	0.40[a] (443)	0.35, 0.44	0.24[b] (103)	0.17, 0.33	0.40[a] (181)	0.33, 0.48
Age (years)†	6.0[a] (443)	4.0, 8.0	8.0[b] (103)	6.0, 10.0	6.0[a] (181)	3.0, 9.0
Weight (kg)†	7.1[a] (442)	5.6, 9.8	7.1[a] (103)	4.8, 12.5	26.9[b] (179)	11.2, 37.7
Initial MFS†	3.0[a] (443)	2.0, 3.0	4.0[b] (103)	3.0, 5.0	4.0[b] (181)	3.0, 4.0
Duration of signs (days)†	2.0[a] (443)	1.0, 7.0	7.0[b] (103)	4.0, 24.0	14.0[b] (181)	3.0, 90.0

IVDH indicates Intervertebral disk herniation; MFS, modified Frankel score

*Proportion and 95% confidence intervals (CI) presented for categorical and median and interquartile range (IQR) presented for quantitative variables.

†P < .05 for Kruskal Wallis comparison of differences among medians or chi-square tests for proportions. Medians and proportions without superscripts in common are statistically different based on Mann-Whitney U or chi-square tests with Bonferroni adjustment for multiple comparisons.

Table 2. Descriptive statistics and comparisons of cerebral spinal fluid analysis from 710 dogs with neurological disease and valid results examined between August 2008 and December 2010 at a single referral hospital.

	Thoracolumbar IVDH (n=433)		Cervical IVDH (n=103)		Other diagnosis (n=174)	
Variable	**Median (n)**	**IQR**	**Median (n)**	**IQR**	**Median (n)**	**IQR**
TNCC (/µL)†	3[a] (433)	1, 8	3[a] (103)	1, 6	1[b] (174)	1, 4
RBC (/µL)†	14[a] (433)	1, 181	6[a,b] (103)	0, 123	4[b] (174)	1, 118
Microprotein (mg/dL)†	23[a] (427)	17, 30	24[a,b] (100)	19, 34	27[b] (172)	20, 37
Pandy test (ordinal)†	Neg[a] (419)	Neg, Neg	Neg[a,b] (96)	Neg, Neg	Neg[b] (172)	Neg, Neg
Neutrophils (%)†	8[a] (433)	0, 48	1[b] (103)	0, 14	0[b] (174)	0, 21
Large mononuclear cells (%)†	32[a] (433)	14, 56	55[b] (103)	33, 73	38[a] (174)	13, 67
Lymphocytes (%)	20 (433)	6, 46	25 (103)	7, 43	27 (174)	5, 50
Eosinophils (%)	0 (433)	0, 0	0 (103)	0, 0	0 (174)	0, 0

IVDH indicates Intervertebral disk herniation; IQR , interquartile range.

*P < .05 for Kruskal Wallis comparison of differences among medians. Medians without superscripts in common are statistically different based on Mann-Whitney U tests and Bonferroni adjustment for multiple comparisons.

cells/µL), 13 dogs with lymphocytic pleocytosis (range 7-110 cells/µL), and 4 dogs with eosinophilic pleocytosis (range 8-15 cells/µL); 6 samples were of insufficient volume to allow a differential cell count to be performed. The cervical IVDH population had 26 (25.2%) dogs with CSF pleocytosis, with 9 dogs having neutrophilic pleocytosis (range 9-172 cells/µL), 9 dogs having large mononuclear pleocytosis (range 6-26 cells/µL), 5 dogs having mixed cell pleocytosis (range 7-37 cells/µL), and 3 dogs having lymphocytic pleocytosis (range 6-25 cells/µL). The median RBC count was greater in dogs with thoracolumbar IVDH than other groups, and the median microprotein concentration was higher in dogs with IVDH compared to those with other spinal cord diseases. Albuminocytologic dissociation was diagnosed in 30 dogs with thoracolumbar IVDH, 14 dogs with cervical IVDH, and 45 dogs with other diagnoses. Pandy was negative in 382/419 dogs with thoracolumbar IVDH, 81/96 dogs with cervical IVDH, and 139/172 dogs with other diseases.

CSF measurements were significantly correlated to initial MFS and duration of signs at admission in dogs with both thoracolumbar and cervical IVDH. In dogs with thoracolumbar IVDH, CSF TNCC, RBC count, microprotein concentration, and percent neutrophils were negatively correlated to MFS (rho -0.256, P<.001; rho -0.282, P<.001; rho – 0.133, P<.006; and rho -0.325, P<.001, respectively). Similarly, CSF TNCC, RBC count, microprotein concentration, percent neutrophils, and percent eosinophils were negatively correlated with SCI duration (rho -0.253, P<.001; rho -0.269, P<.001; rho -0.141, P=.004, rho -0.356, P <.001; and rho -0.134, P=.005, respectively), whereas percent large mononuclear cells was positively correlated with duration (rho 0.225, P<.001). In dogs with cervical IVDH similar relationships were identified. Both CSF TNCC and microprotein concentration were negatively correlated to initial MFS (rho -0.374, P<.001 and rho -0.277, P=.005). The CSF TNCC, RBC count, and

percent neutrophils were negatively correlated with duration of SCI (rho -0.269, P=.006; rho -0.227, P=.021; and rho -0.223, P=.024 respectively) whereas the percent lymphocytes was positively associated with duration of SCI (rho 0.271, P=.006).

Table 3. Cerebrospinal fluid analysis for 281 dogs with thoracolumbar intervertebral disk herniation examined between July 2008 and December 2010 at a single referral hospital compared based on the presence or absence of spinal cord T2-weighted hyperintensity on magnetic resonance imaging.

	T2W hyperintensity present (n=101)		T2W hyperintensity absent (n=180)		
Variable	**Median**	**IQR**	**Median**	**IQR**	**P value***
TNCC (/µL)	5	1, 11	2	1, 5	0.001
RBC (/µL)	117	7, 709	4	1, 34	<0.001
Microprotein (mg/dL)	27	19, 34	21	16, 26	<0.001
Pandy test (ordinal)	Neg	Neg, Neg	Neg	Neg, Neg	0.214
Neutrophils (%)	36	0, 62	2	0, 27	<0.001
Large mononuclear cells (%)	28	13, 50	35	16, 63	0.187
Lymphocytes (%)	16	6, 33	27	7, 53	0.023
Eosinophils (%)	0	0, 1	0	0, 0	0.001

*P value based on Mann-Whitney U tests.

IQR indicates interquartile range.

Thirty-six percent (101/281) of dogs with thoracolumbar IVDH and 38% (36/94) of dogs with cervical IVDH had T2W hyperintensity after MRI review. Dogs with thoracolumbar IVDH that had spinal cord T2W hyperintensity had significantly higher median TNCC, RBC count, microprotein concentration, percent neutrophils, and percent eosinophils than dogs lacking T2W signal change (Table 3). In dogs with cervical IVDH, median CSF TNCC was higher in dogs with spinal cord T2W hyperintensity (median 4, IQR 2, 19) compared to those lacking signal change (median 2, IQR 1, 4) (P=.005). The percent lymphocytes were significantly higher in dogs without T2W spinal cord hyperintensity (median 32, IQR 18, 50) compared to those with signal change (median 15, IQR 2, 37) (P=.007).

In total 344 with thoracolumbar IVDH and 27 dogs with cervical IVDH were non-ambulatory at initial evaluation (MFS 0-3), were surgically treated for IVDH, and had data available concerning functional motor outcome (Table 4). The multivariable logistic regression model suggested that no CSF variables were significant predictors of functional recovery independent of initial MFS, however CSF TNCC (odds ratio 0.987, 95% CI 0.974-1.000, P=.056) was close to the significance threshold. Dogs with cervical IVDH that recovered ambulatory function had higher median CSF microprotein (35 mg/dL, IQR 23, 46) than those that did not ambulate (21 mg/dL, IQR 15, 32) (P=.049). It was not possible to perform multivariable logistic regression in dogs with cervical IVDH due to small sample size.

CSF measurements compared between dogs with thoracolumbar or cervical IVDH and other diseases did not identify an accurate method to differentiate groups (Table 5). The multivariable logistic regression model estimated that TNCC > 0 (odds ratio 2.49, 95% CI 1.65-3.76, P<.001), negative Pandy test (odds ratio 3.37, 95% CI 2.00-5.71, P<.001), and percent neutrophils ≥19 (odds ratio 1.69, 95% CI 1.10-2.60, P = .016) were significant predictors of

IVDH. The combination of these three CSF variables had a sensitivity of 26% (95% CI 22-30%), specificity of 92% (95% CI 87-95%), and Youden index of 0.18 for the diagnosis of IVDH.

Table 4. Cerebrospinal fluid analysis for 344 non-ambulatory dogs at admission diagnosed with thoracolumbar intervertebral disk herniation between July 2008 and December 2010 at a single referral hospital compared based on post-surgical ambulatory status.

	Ambulatory post-surgery (n=226)		Non-ambulatory / died / euthanized (n=118)		
Variable	**Median (n)**	**IQR**	**Median (n)**	**IQR**	**P value***
TNCC (/µL)	3 (226)	1, 8	4 (118)	1, 10	0.090
RBC (/µL)	14 (226)	1, 165	35 (118)	4, 439	0.006
Microprotein (mg/dL)	22 (223)	17, 28	25 (115)	20, 34	0.006
Pandy test (ordinal)	Neg (222)	Neg, Neg	Neg (114)	Neg, Neg	0.445
Neutrophils (%)	14 (226)	0, 47	30 (118)	0, 66	0.025
Large mononuclear cells (%)	33 (226)	19, 56	24 (118)	9, 51	0.027
Lymphocytes (%)	20 (226)	6, 46	17 (118)	5, 41	0.378
Eosinophils (%)	0 (226)	0, 0	0 (118)	0, 0	0.669

*P value based on Mann-Whitney U tests.

IQR indicates interquartile range.

Table 5. Diagnostic accuracy using cerebrospinal fluid (CSF) analysis for 536 intervertebral disk herniation (IVDH) affected dogs and 174 dogs with non-IVDH neurological disease examined between July 2008 and December 2010 at a single referral hospital.

CSF parameter	Optimal cutoff	Sensitivity (95% CI)	Specificity (95% CI)	Youden index
TNCC (/μL)	≥1	0.55 (0.51, 0.59)	0.65 (0.58, 0.72)	0.20
RBC (/μL)	≥3	0.65 (0.61, 0.69)	0.49 (0.41, 0.56)	0.14
Microprotein (mg/dL)	>0	1.0 (0.99, 1.0)	0.00 (0.00, 0.02)	0.00
Pandy test (ordinal)	Negative	0.90 (0.87, 0.92)	0.19 (0.14, 0.26)	0.09
Neutrophils (%)	≥19	0.41 (0.37, 0.45)	0.74 (0.67, 0.80)	0.15
Large mononuclear cells (%)	≥5	0.87 (0.84, 0.90)	0.20 (0.15, 0.27)	0.07
Lymphocytes (%)	≥2	0.82 (0.79, 0.86)	0.19 (0.14, 0.25)	0.01
Eosinophils (%)	>0	0.13 (0.10, 0.16)	0.91 (0.86, 0.94)	0.04
Neutrophilic pleocytosis	Present	0.13 (0.11, 0.17)	0.90 (0.85, 0.94)	0.03
Mononuclear pleocytosis	Present	0.08 (0.05, 0.10)	0.98 (0.94, 0.99)	0.06
Lymphocytic pleocytosis	Present	0.03 (0.02, 0.05)	0.95 (0.91, 0.98)	-0.02
Lymphocytic + mononuclear pleocytosis	Present	0.11 (0.08, 0.13)	0.93 (0.88, 0.96)	0.04
Eosinophilic pleocytosis	Present	0.01 (0.00, 0.02)	0.99 (0.96, 1.0)	0.00
Mixed cell pleocytosis	Present	0.06 (0.04, 0.08)	0.98 (0.94, 0.99)	0.04
Any pleocytosis	Present	0.31 (0.27, 0.35)	0.80 (0.73, 0.85)	0.11
Myelin (presence/absence)	Present	0.03 (0.02, 0.05)	0.98 (0.95, 0.99)	0.01

CI indicates confidence interval.

Discussion

Cisternal CSF characteristics in dogs with thoracolumbar and cervical IVDH in the present study were similar to smaller populations that have been previously described.[10-12,14] In general, TNCC was low, RBC count was only subtly elevated, CSF microprotein concentrations approximated reference range concentrations, and Pandy's reaction was usually negative. In dogs with thoracolumbar IVDH, the proportion of animals with pleocytosis was lower than has been previously described in dogs when CSF was obtained from the lumbar cistern (30.9% versus 61%).[13] Likewise, in contrast to a previous study[13] of lumbar CSF where lymphocytic pleocytosis was most frequently identified in dogs with thoracolumbar (41%) and cervical (42%) IVDH, only 9.7% (13/134) of dogs with thoracolumbar IVDH and 11.5% (3/26) of dogs with cervical IVDH in the study reported here had lymphocytic pleocytosis. The reasons for this discrepancy could include the site of CSF acquisition, chronicity of SCI, or divergence in classification systems between reports.

TNCC and microprotein concentration increased with the severity of SCI (decreasing MFS) in the study population. In dogs with thoracolumbar IVDH, high RBC count and percent neutrophils were likewise correlated with injury severity. Previous studies of dogs with thoracolumbar IVDH have associated TNCC and microprotein concentration with behavioral measures of SCI at the time of sampling.[12-14] In a study of 27 people with traumatic SCI, CSF RBC count and TNCC were correlated with SCI severity independent of injury duration.[7] SCI severity is likely related to these CSF variables as they may reflect pathomechanisms associated with neurotrauma. For example, CSF RBC count in people with SCI is speculated to suggest injury-related subarachnoid hemorrhage.[7,25] Likewise, elevations in CSF microprotein

concentration may reflect disruption of the blood-spinal cord barrier, which is critical to exacerbation of early secondary SCI in rodent models.[26,27]

Duration of SCI at the time of CSF acquisition was negatively correlated to TNCC, RBC count, and percent neutrophils in dogs with cervical and thoracolumbar IVDH. In people with traumatic SCI, CSF TNCC and RBC count decline with time independent of injury severity.[7] While the exact mechanisms for this shift in cell concentrations are unknown, in neurotrauma models parenchymal RBC are phagocytized within days of SCI and secondary inflammatory mechanisms abate in a time-dependent manner.[7,25,28,29] In the present study, percent neutrophils in dogs with thoracolumbar and cervical IVDH decreased with increasing duration of SCI. In the CSF and parenchyma of people with traumatic SCI, neutrophils are the predominant inflammatory cells for the first several days following injury.[7,29] The predominance of neutrophils early after SCI may be due to innate immune responses to injury including microglial activation, IL-8 release, and blood-spinal cord barrier disruption.[7,29] The duration of SCI was positively related to the percentage CSF large mononuclear cells in dogs with thoracolumbar IVDH and the percentage of CSF lymphocytes in dogs with cervical IVDH. In a previous study of dogs with IVDH, lymphocytic pleocytosis was associated with longer SCI duration.[13] People with traumatic SCI have cellular infiltrates that are largely macrophages in the days to weeks after SCI, whereas CD8+ lymphocytes become more numerous weeks to months after neurotrauma.[29]

The present study is the first to show that various CSF measurements in dogs with thoracolumbar and cervical IVDH correlate with the presence of spinal cord T2W hyperintensity. This signal change is believed to reflect the presence of vasogenic or cytotoxic edema, cellular infiltrates, and/or tissue necrosis based on studies in animals with experimentally induced

SCI.[30,31] Dogs with thoracolumbar IVDH and spinal cord T2W hyperintensity had significantly higher CSF TNCC, RBC count, microprotein concentration and percent neutrophils compared to those lacking T2 signal change. This correlation further suggests that high T2 signal within the spinal cord following IVDH may reflect innate inflammatory events and barrier disruption. Lower percentages of lymphocytes in the CSF of dogs with both thoracolumbar and cervical IVDH was significantly associated with spinal cord T2W hyperintensity. Longitudinal MRI studies in rodents with experimentally induced SCI have shown that T2W hyperintensity and measures of blood-spinal cord barrier diffusability decrease with time, as lesions contain less edema and begin to evolve towards an adaptive inflammatory response where lymphocytes are more common.[31,32]

Functional outcome in dogs with thoracolumbar and cervical IVDH that were non-ambulatory and treated surgically was significantly correlated to several CSF measurements. For example, in dogs with thoracolumbar IVDH higher CSF TNCC, RBC count, microprotein concentration, percent neutrophils, and percent large mononuclear cells were seen in animals that were euthanized or failed to ambulate. Multivariable logistic regression was used in dogs thoracolumbar IVDH to determine whether these variables were independent of initial SCI severity as determined by MFS. Although each cell/µl increase in CSF TNCC resulted in a 0.987 lower odds of functional recovery, this finding was non-significant and no other CSF variables were retained in the model. While a recent report in a small cohort of dogs with thoracolumbar IVDH has shown that CSF routine measurements may be used to predict outcome, logistic regression techniques to adjust for confounding factors such as initial behavioral SCI severity were not attempted.[12] It appears that CSF biomarkers that are more closely associated with specific secondary injury processes may hold more promise in outcome

prediction than the routine measurements assessed here. For example, CSF myelin basic protein concentration and CK activity, when used in tandem were able to predict recovery in >95% of dogs with thoracolumbar IVDH across various injury severity groups.[14]

Although CSF acquisition is extremely common at some institutions in animals suspected of IVDH, limited data are currently available to indicate the ability of CSF analysis to differentiate IVDH from other etiologies affecting the vertebral column or spinal cord. One previous retrospective study qualitatively assessed CSF in several vertebral column diseases and suggested that there was significant overlap between etiologies in CSF measurements.[17] In the present study, we established that dogs with thoracolumbar IVDH, cervical IVDH, and other neurological diseases had significant differences in CSF TNCC, RBC count, microprotein concentration, Pandy test, and differential cell count. For example, median TNCC was significantly greater in dogs with cervical and thoracolumbar IVDH compared to dogs with other diagnoses. However, the diagnostic accuracy of various CSF variables to differentiate IVDH from other diseases was poor. The Youden index was highest for CSF TNCC, which had a sensitivity of 55% and a specificity of 65%, using a cutoff of $\geq$ 1 cell/μL to differentiate IVDH from other diseases. Multivariable logistic regression established that TNCC, negative Pandy test, and high percent neutrophils were significantly associated with IVDH, but the model only had a sensitivity of 26% and a specificity of 92% for prediction of disease group. Advanced imaging technologies such as MRI and CT remain the best means of detecting IVDH, and have the added advantage of providing data concerning the location of a lesion and degree of spinal cord compression. For example, CT has a reported accuracy of 81.8-100% for detecting IVDH in dogs with thoracolumbar lesions, and a recent report has suggested MRI is superior to CT in predicting the location of surgical IVDH lesions.[33-35]

The study reported here established in dogs with IVDH typical cisternal CSF variables; relationships between these variables and behavioral SCI severity, SCI duration, and T2W spinal cord signal; a lack of a relationship between CSF characteristics and functional outcome when controlling for initial injury severity; and limited ability of CSF to differentiate IVDH from other spinal cord diseases. The report does have several important limitations, including the retrospective design, single center population, use of cisternal samples, and a lack of differentiation between other disease processes (eg, neoplasia versus myelitis) when attempting to determine the diagnostic value of CSF analysis. A prospective design would have likely increased clinical data standardization and may have improved the availability of long-term follow-up. Beginning at the time of this study, our institution implemented standardized behavioral neurological scoring (the MFS) for all animals with spinal cord disease and began standardizing long-term follow-up; these factors may have lessened data acquisition limitations common in some veterinary retrospective studies. Single center studies may introduce bias via a number of mechanisms. For example, disease severity may be higher in a single center study originating from a tertiary care facility compared to one from an institution that functions as a general referral hospital. By acquiring a large dataset and stratifying dogs based on clinical severity, these limitations may have been mitigated. Importantly, all CSF samples included in this study were acquired at the cisterna magna. While data suggest that in dogs with neurological disease samples acquired at the lumbar cistern are more likely to have abnormalities, cisternal CSF acquisition is standard at our institution as RBC contamination is less common and sample volume in small dogs is believed to be more robust.[36] Finally, dogs with other diagnoses that varied in pathogenesis were grouped for certain aspects of analysis. As CSF characteristics are likely different in diseases that might induce inflammation (myelitis)

compared to other etiologies, it may not be appropriate to generalize these finding. However, the sample size of various other diagnoses was small, which prohibited comparisons between specific etiologies and IVDH. Additionally, inferences made concerning diagnostic accuracy when comparing IVDH to a particular etiology may not be relevant due to the possibility that any of the other diagnoses could be seen clinically; thus, an analysis assessing the diagnostic accuracy of CSF to differentiate IVDH from myelitis would likely yield inflated results.

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