Comparison of cerebellomedullary and lumbar cerebrospinal fluid analysis in dogs with neurological disease

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Abstract

Background: Cerebrospinal fluid (CSF) analysis aids in categorizing underlying disease processes in patients with neurologic disease. Convention suggests that CSF should be collected caudal to the lesion. However, little evidence exists to justify this assertion.

Hypothesis/Objectives: Evaluate the clinicopathologic differences between CSF collected from the cerebellomedullary (CM) and lumbar cisterns in dogs presented for evaluation of neurologic disease.

Animals: Fifty-one client-owned dogs undergoing magnetic resonance imaging (MRI) and CSF collection for investigation of neurologic disease.

Methods: Cerebrospinal fluid was prospectively collected from the CM and lumbar cisterns in all patients. The total protein (TP) concentration, red blood cell (RBC) count, and total nucleated cell count (TNCC) were analyzed within 30 minutes of collection. Results and cytology findings were interpreted by a single pathologist.

Results: Fifty-one paired samples were collected. The TNCC \( P < .001 \), RBC \( P < .001 \), and TP \( P < .001 \) were different between collection sites. When grouped by neurolocalization, TP (intracranial, \( P < .001 \); cervical, \( P < .001 \); thoracolumbar, \( P < .001 \)) and RBC (intracranial, \( P < .001 \); cervical, \( P < .002 \); thoracolumbar, \( P = .006 \)) counts were significantly different. The TNCC was significantly different in the cervical (\( P = .04 \)) and thoracolumbar localizations (\( P = .004 \)) but not for intracranial (\( P = .30 \)) localizations. The pathologist's interpretation differed between sites in 66.7% of the cases (34/51).

Conclusions: In dogs with lesions that neurolocalized to the brain or cervical spinal cord, there may be clinical benefit in collecting fluid from both the CM and lumbar cisterns. In dogs with thoracolumbar myelopathy, CSF collected from the CM cistern may not be representative of the underlying disease process.

KEYWORDS

canine, CSF, neurolocalization, neurology

Abbreviations: CM, cerebellomedullary; CNS, central nervous system; CSF, cerebrospinal fluid; RBC, red blood cell; TNCC, total nucleated cell count; TP, total protein.

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1 | INTRODUCTION

Cerebrospinal fluid (CSF) collection and analysis are routinely performed to categorize the type of central nervous system (CNS) disease present in patients with neurologic disease. In dogs, CSF is collected from either the cerebellomedullary (CM) or the lumbar cisterns. Because of the caudal flow of CSF, it is common practice to collect CSF caudal to and in close proximity to the lesion.1 However, some investigators suggest that the CM cistern is more reliable regardless of the lesion location2,3 whereas others suggest collection from the lumbar cistern is more likely to disclose abnormal results.4,6,7 In clinical practice, the choice of CSF collection location also is influenced by other factors including clinician preference and experience, patient anatomy, and patient safety.

To our knowledge, no large studies have compared paired CM and lumbar CSF samples in the same patient with neurologic disease. One previous study compared lumbar and CM CSF samples in 31 healthy dogs and found that total protein (TP) concentration was higher in lumbar samples whereas white blood cell count was higher in CM samples.5 Another frequently cited study compared CSF analysis between CM and lumbar collection in dogs with neurologic disease, but only 13 dogs had paired samples, and the remainder were compared between different patients.7 Therefore, correlations between CM and lumbar samples could not be made because these patients likely had different underlying diseases, different disease severity, and different lesion localizations.

To provide the best treatment recommendations and accurately establish a prognosis for patients, selecting the collection site most likely to yield a diagnosis is critical. Our objective was to evaluate differences between paired CM and lumbar CSF samples obtained in dogs with neurologic disease. We aimed to provide guidance as to which site is more likely to aid in diagnosis and the likelihood of false negative results based on lesion localization. We hypothesized that differences in the total nucleated cell count (TNCC) and TP concentration would exist between CM and lumbar samples collected from the same patient. Our second hypothesis was that the most representative collection would be obtained caudal to the lesion and that increasing distance from the lesion would result in decreased TP concentration and TNCC.

2 | MATERIALS AND METHODS

Fifty dogs were prospectively recruited from patients presented to the University of Illinois Veterinary Teaching Hospital for neurodiagnostic evaluation in which CSF analysis was clinically recommended. The study was approved by and conducted in accordance with the University of Illinois Institutional Animal Care and Use Committee. To be enrolled in the study, dogs were required to have magnetic resonance imaging (MRI) before CSF collection. The initial CSF collection site was chosen by the supervising clinician. If a sample could not be obtained from 1 of the locations after 3 attempts, the patient was not enrolled in the study.

Samples were collected into sterile glass tubes with no additives and into EDTA tubes. Analysis was performed on the EDTA sample within 30 minutes of collection, including TNCC, red blood cell (RBC) count, TP concentration, and preparation of a cytocentrifuge slide. Total nucleated cell and RBC counts were performed manually, using the mean of counts from both sides of the hemocytometer chamber. The manual cell count was performed by certified medical technologists trained in the University of Illinois Veterinary Diagnostic Laboratory Clinical Pathology Laboratory. The TP concentration was measured on a Beckman Coulter AU680. Cytospin preparations were prepared for cytolysis analysis using 100 μL of fluid at 1000 rpm for 3 minutes using disposable cytofunnels and glass slides, with subsequent Wright-Giemsa staining. A clinical pathologist performed cytological analysis of each of the cytocentrifuge slides to provide a cell differential and description. A single clinical pathologist (A.M.B.), blinded to patient history, provided an interpretation based on the collection site, TNCC, TP concentration, RBC count, and cytological description.

3 | STATISTICAL ANALYSIS

Before enrolling patients, a power analysis was performed and identified a minimum sample size of 44 to detect a 5 cell difference with 80% power. The power calculation was performed using an expected mean from a previous study comparing CSF in normal dogs,5 but the SD was increased because we suspected dogs with CNS lesions to have a wider variety of results and differences between collection sites. Data were recorded in an Excel spreadsheet (Microsoft Excel for Mac 2011; version 14.5.3), and statistical analysis and sample size calculation were performed using an open-source statistical software program (R Core Team 2019, R foundation for Statistical Computing, Vienna, Austria). Data were assessed for normality graphically and using the Shapiro-Wilk’s test. Data did not meet the criteria for normality, and consequently was analyzed nonparametrically.

Differences among TNCC, RBC count, and TP concentration were assessed for significance using a Wilcoxon signed rank test. The data were analyzed overall, and then separated by neurolocalization (infracranial, cervical, thoracolumbar, multifocal). The presence of hemodilution (RBC > 500 cell/mm3) was compared among groups using a McNemar’s Chi-squared test. Significance for all statistical tests was set at P < .05. All samples, regardless of RBC count, were included in statistical analysis comparing quantitative results between collection sites. Samples then were categorized and classified as normal or abnormal. Abnormal CSF was defined as having a TNCC > 5 cells/mm³ or a TP concentration > 25 mg/dL (CM) or > 40 mg/dL (lumbar) or both. For this categorical analysis, samples with RBC count > 13 200 cell/mm³ were excluded.

4 | RESULTS

Sixty-two dogs qualified for enrollment in the study and informed client consent was obtained before sample collection. Cerebrospinal fluid was collected and analyzed for TNCC, TP concentration, RBC count, and cytological description. The results showed that CM and lumbar CSF samples were statistically different for TNCC, TP concentration, and RBC count. The most likely site to yield a diagnosis was determined by the location of the lesion and the presence of abnormal results.
fluid could not be collected from 11 dogs. Collection of CSF was unsuccessful from the lumbar cistern in all 11 patients, and unsuccessful from both sites in a single patient. Fifty-one paired samples were collected. Enrolled patients were subdivided into 1 of 4 neurolocalizations: intracranial, cervical, thoracolumbar, and multifocal. The sample population consisted of 23 dogs with intracranial localization, 13 dogs with cervical myelopathy, 13 with thoracolumbar myelopathy, and 2 with multifocal neurolocalization. The most common presumptive diagnosis was meningoencephalitis or myelitis of unknown origin (MUO; n = 13), intervertebral disc disease (IVDD; n = 9), idiopathic epilepsy (n = 5), neoplasia (n = 4), cognitive dysfunction (n = 2), and discospondylitis (n = 2). All presumptive or confirmed diagnoses along with CSF results are included in Supplemental Table 1. In samples from the CM cistern, the median TNCC was 2 (0-1955) cells/mm³, the median RBC count 27 (0-55 044) cells/mm³, and the median TP concentration 31.3 (14.1-709.8) mg/dL. In the CSF collected from the lumbar cisterna, the median TNCC was 9 (0-2772) cells/mm³, the median RBC count was 1052 (7-271 000) cells/mm³, and the median TP concentration was 100.6 (28.7-3724) mg/dL (Figures 1 and 2).

Overall, differences among the TNCC (P < .001), RBC count (P < .001), and TP concentration (P < .001) between the CM and lumbar cisterns were significant (Table 1). Data then were grouped and analyzed by neurolocalization (intracranial, cervical, or thoracolumbar). The TP concentration (intracranial, P ≤ .001; cervical, P < .001; thoracolumbar, P < .001) and RBC count (intracranial, P < .001; cervical, P = .002; thoracolumbar, P = .006) were significantly different among the 3 neurolocalizations. The TNCC was significantly different in the thoracolumbar (P = .004), and cervical (P = .04) cases, but not for intracranial (P = .30) localizations (Table 1). Hemodilution (RBC > 500 cells/mm³) was present in samples taken from the CM cistern in 3 cases, from the lumbar cistern in 22 cases, and from both sites in 5 cases. Hemodilution was more likely to occur in samples obtained from the lumbar cistern compared to the CM cistern (P < .001).

The pathologist interpretation, with regard to TNCC, TP concentration, and cytological description (cell types present) differed between collection sites in 66.7% of cases (34/51). Five of these cases had pleocytosis in which the cell population differed between

### TABLE 1  Median difference of CSF analysis between CM and lumbar collection

|                | TNCC       | RBC          | TP          |
|----------------|------------|--------------|-------------|
|                | Diff (range) | P value | Diff (range) | P value | Diff (range) | P value |
| Overall        | 5 (-94 to 1956) | <.001 | 1045 (-54 930 to 271 000) | <.001 | 81.1 (-21.9 to 3014) | <.001 |
| Intracranial   | 2 (-94 to 277) | .30 | 615 (-54 934 to 75 990) | <.001 | 56.45 (-21.9 to 473.2) | <.001 |
| Cervical       | 6 (-13 to 1596) | .04 | 2316 (-413 to 99 470) | .002 | 106.4 (27.4 to 3014.2) | <.001 |
| TL             | 6 (0 to 1250) | .004 | 1045 (-3393 to 271 000) | .006 | 104 (22.4 to 1385) | <.001 |

Note: Median difference of CSF analysis between two collection sites, evaluating TNCC, RBC, TP. Data are organized by patient neurolocalization. Difference is calculated by subtracting the CM from the lumbar values. Abbreviations: CM, cerebellomedullary; CSF, cerebrospinal fluid; TL, thoracolumbar; TNCC, total nucleated cell count; RBC, red blood cell count.
TABLE 2  Number and percent of samples that were abnormal from 1 or both collection sites organized by neurolocalization. Pleocytosis defined as TNCC >5 cells/mm³. Total protein (TP) concentration defined as increased if >25 mg/dL from the CM or >40 mg/dL from the lumbar cistern. Nine paired samples with hemodilution (RBC > 13 200 cells/mm³) were not included.

| Localization | Pleocytosis | Increased TP |
|--------------|-------------|--------------|
|              | CM only     | LM only      | Both | Total | CM only | LM only | Both | Total |
| Intracranial | 2 (16.7%)   | 4 (33.3%)    | 6 (50%) | 12      | 0       | 2 (11.8%) | 15 (88%) | 17   |
| Cervical     | 1 (10%)     | 5 (62.5%)    | 3 (30%) | 10      | 0       | 4 (33.3%) | 8 (66.7%) | 12   |
| Thoracolumbar| 0           | 4 (80%)      | 1 (20%) | 5       | 0       | 5 (55.5%) | 4 (44.4%) | 9    |
| Total        | 3 (11%)     | 14 (52%)     | 10 (37%)| 27      | 0       | 11 (28.9%) | 27 (71.1%) | 38   |

Abbreviations: CM, cerebellomedullary; TNCC, total nucleated cell count.

the 2 collection sites. Of these 5 cases, 2 had a presumptive diagnosis of MUO (case 21 and 35), and 1 of each of the following: IVDD (case 28), vertebral subluxation (case 48), and idiopathic epilepsy (case 19; Supplemental Table 1). When grouped by neurolocalization, 56% (13/23) of patients with intracranial localization, 85% (11/13) with cervical myelopathies, and 77% (10/13) with thoracolumbar myelopathies resulted in different pathologist interpretations between the sites. Data then were evaluated categorically as abnormal if the CSF analysis had pleocytosis (TNCC >5 cells/mm³ with RBC count <13 200 cells/mm³) or increased TP concentration (>25 mg/dL [CM] or >40 mg/dL [lumbar]). Nine samples were excluded from analysis because of hemodilution (RBC count >13 200 cell/mm³). In dogs with intracranial or cervical neurolocalization, the pleocytosis analysis identified 3/24 potential false negatives if samples were collected only from the lumbar cistern, and 10/24 potential false negatives is samples were collected only from the CM site. In dogs with thoracolumbar localization, the pleocytosis results identified 4/5 potential false negatives if samples were collected only from the CM site. Of cases with increased TP concentration, the risk of a false negative if CSF was collected only from the CM cistern was 2/17 with intracranial localization, 4/12 with cervical myelopathy, and 5/9 with thoracolumbar myelopathy (Table 2).

5 | DISCUSSION

We found that CSF results differed significantly between the CM and lumbar cisterns in dogs with neurologic disease. To our knowledge, ours is the first study comparing paired CSF samples collected from both sites in a large number of dogs with neurologic disease. In dogs with thoracolumbar localization, CSF collected from the CM cistern was likely to cause a false negative result. Dogs with intracranial or cervical neurolocalization however had more inconsistent results, indicating CSF collection from both sites may be beneficial.

In this population of dogs, lesions in the thoracolumbar spinal cord were unlikely to cause abnormal results when samples were collected from the CM cistern. This finding is in agreement with a previous study evaluating creatine kinase and lactate dehydrogenase activities in paired CSF samples in dogs with thoracolumbar intervertebral disc disease, which found that these 2 enzyme activities were less likely to be abnormal in samples from the CM site.6 Another study evaluating CSF in dogs with neurologic disease also concluded that CSF was more likely to be abnormal when collected from a site caudal to the lesion, but the majority of samples in this study were not paired.7 This finding most likely is consistent with the caudal flow of CSF. Only 1 dog in our study with thoracolumbar myelopathy had pleocytosis in the fluid collected from the CM cistern. This patient was diagnosed with MUO. Although the dog's neurologic examination was consistent with lumbar myelopathy, MUO generally is a more diffuse disease process affecting the meninges, which could explain the pleocytosis cranial to the lesion.

In cases with an intracranial or cervical localization, the results were not as consistent. Several cases with 1 of these localizations had pleocytosis at only 1 collection site; 10/30 cases had pleocytosis only at the lumbar collection site, whereas 3/30 cases had pleocytosis only at the CM collection site. In these cases, collecting CSF from only 1 location would have yielded false negative results. These findings indicate that lesion location and proximity should not be the only factors guiding collection site choice.

Cerebrospinal fluid mainly is produced by the choroid plexus in the brain and travels caudally in the CNS.3,8,9 It is absorbed back into the peripheral circulation through 1-directional valves in the arachnoid villi in the subarachnoid space.5,9 Thus, increased TP concentration or TNCC or both can be present because of injury anywhere along the CSF pathway as a result of changes in production of CSF, breakdown in protective barriers, or disrupted resorption.4,9 We suspect that, in these particular cases, obstruction or resorption of CSF may be the predominant cause of the abnormalities, resulting in different CSF findings in different parts of the CNS. Studies in normal dogs have reported higher TNCC in CSF collected from the CM compared to the lumbar cistern,5 whereas other studies have reported higher TNCC from the lumbar cistern.6 Possible explanations for this difference could be related to variability in the permeability of the subarachnoid space or rates of cell lysis throughout the CNS.4

Cerebrospinal fluid analysis generally includes quantitative determination of TNCC, RBC count, and TP concentration, as well as interpretation of these results and cytology findings by a clinical pathologist. This interpretation is a succinct summary of the clinicopathologic characteristics of the CSF. In our study, the pathologist's interpretation varied between collection sites in the majority of cases in patients with all
3 neurolocalizations. The majority of this data is reflected in the quantitative analysis already discussed, indicating that TNCC and TP concentration often were different between collection sites. Evaluating the pathologist’s interpretation also indicated that 5 cases had a shift in the cell population causing the pleocytosis. Two of these cases neurolocalized intracranially, 2 to the cervical spine, and 1 to the thoracolumbar spine. The presumptive diagnosis in these cases also varied, with 2 having focal lesions (IVDD, vertebral subluxation), and 3 having more diffuse disease (MUO, idiopathic epilepsy with or without MUO). These findings suggest that cell permeability and lysis in the subarachnoid space throughout the CNS may vary by cell type, allowing a different population of cells at different locations in the CNS. Considering the small number of cases with differences in cell population, along with the variety of both focal and diffuse disease, further investigation is warranted to better understand this phenomenon.

One of our goals was to provide evidence-based guidance as to which CSF collection site would be most likely to facilitate diagnosis based on lesion location. Our results indicate that other factors, in addition to lesion location, should be considered when choosing a CSF collection site. Some other variables that were not assessed in our study and that may alter CSF resorption and flow include lesion type, chronicity, and corticosteroid administration. We did not evaluate these other factors because of sample size and variability of our patient population. Future prospective studies comparing paired CSF samples in a less variable population (eg, patients with MUO) may be able to identify other factors that could guide CSF collection site. Based on our current findings, we recommend collecting both CM and lumbar CSF samples in dogs with an intracranial lesion or cervical myelopathy.

One possible limitation in our study is the inclusion of samples with hemodilution. Iatrogenic blood contamination is a frequent problem when collecting and analyzing CSF, especially when collected from the lumbar cistern. Peripheral circulating blood contains protein as well as white and red blood cells, thus blood contamination likely affects CSF analysis and interpretation. Multiple studies have evaluated the effect of hemodilution on CSF analysis and yielded conflicting results. Some have suggested formulas to correct the TP concentration and TNCC based on the RBC count, whereas others have suggested that blood contamination with up to 8280 or 13 200 RBC/μL did not affect CSF TNCC or TP concentration, respectively. Because of the lack of consensus, as well as lack of direct correlation between RBC count and other CSF variables, we elected to include all samples in our initial statistical analysis. The goal of this analysis was to detect the extent of difference between the 2 samples, thus the study included all samples acquired so as to be more reflective of a clinical setting. However, when analyzing samples for pleocytosis and increased TP concentration, those samples with RBC count >13 200 cell/mm³ were excluded. This cutoff was based on a commonly cited report that indicated that the presence of up to 13 200 cells/mm³ did not affect CSF TP concentration or TNCC in patients with neurologic disease. For our data analysis, an objective quantitative cutoff was used to categorize these samples (ie, pleocytosis is defined as TNCC >5 cells/mm³), and thus even a subtle increase in TNCC because of hemodilution would affect the results.

Another limitation of our study was that the entire CNS was not imaged in any patient. Some of the patients with abnormal results at only 1 CSF collection site could have had a lesion in a location that was not imaged, and thus missed. Performing MRI of the entire CNS in each patient is not routine in clinical practice, and is not practical from a patient health or financial perspective. In a clinical setting, the imaging location is chosen based on neurolocalization after a thorough neurological examination. Our study was designed to make recommendations about CSF collection site based on a clinical neurodiagnostic evaluation and to be more representative of clinical practice. As such, it is possible that additional lesions were missed, which could have affected CSF interpretation. Another potential limitation is that the first CSF collection site was chosen by the supervising clinician in each case. An alternative option would have been to randomly choose which site was collected first. However, a previous study evaluating paired CSF samples in healthy dogs determined that the order of collection did not affect the CSF results. This previous study has not been repeated in patients with neurologic disease, and thus the order of collection may have affected our results.

Cerebrospinal fluid collection and analysis are important parts of the neurodiagnostic evaluation, often guiding treatment plans and prognosis. Ideally, the clinician should choose the collection site most likely to yield abnormal results. Based on our results, in patients with thoracolumbar myelopathy, collection of CSF caudal to the lesion was more consistently abnormal compared to CSF from the CM cistern. In these patients, CSF collected from the CM cistern was likely to be normal, providing a false negative result. In patients with intracranial localization or cervical myelopathy, neurolocalization did not predict which site was more likely to be abnormal. In these patients, it may be beneficial to collect and analyze CSF from both the CM and lumbar cisterns.

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CONFLICT OF INTEREST DECLARATION
Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION
Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION
This study was approved by and conducted in accordance with the University of Illinois IACUC.

HUMAN ETHICS APPROVAL DECLARATION
Authors declare human ethics approval was not needed for this study.
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