

Cerebrospinal Fluid from a Dog with Neurologic Collapse

**What Is Your
Diagnosis?**

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Case Presentation

A 3-year-old intact male Staffordshire Terrier was presented to the referring veterinarian with an approximately 1-week history of anorexia, ataxia, and weakness, progressing to collapse. The owners had recently detected extreme weight loss. At presentation, the dog was nonambulatory but able to urinate voluntarily. Vaccinations were current. The dog had been receiving oral prednisone (0.43 mg/kg twice daily for 3 days), prescribed by the primary veterinarian, without improvement. The dog lived in a controlled outdoor environment with access to a small fishpond. One month prior to presentation, another dog from the same household was diagnosed and treated for lead intoxication. Lead levels were not evaluated in this animal. Because of declining neurologic status despite treatment, the dog was referred to the Texas Veterinary Medical Center (TVMC) on an emergency basis.

Physical examination at the TVMC revealed temperature, pulse, and respiration within normal limits. The dog was emaciated, nonambulatory, tetraparetic, and mentally obtunded. Empirical therapy was initiated, consisting of intravenous crystalloid fluid, dexamethasone, methocarbamol, and doxycycline. The following day, a complete neurologic examination was performed, as were a CBC, serum chemistry profile, and coagulation profile. The neurologic status of the animal had worsened considerably since presentation, with the dog now stuporous and having little to no voluntary motor activity. The neurologic assessment revealed an absent menace response, dilated pupils in room light, unilaterally absent pupillary light response, spontaneous rotary nystagmus, decreased flexor reflexes in all four limbs, bilaterally absent patellar reflexes, absent proprioception in all limbs, decreased facial sensation, and decreased or absent spinal reflexes at all levels of the spinal cord. These findings were consistent with multifocal to diffuse disease involving the forebrain, spinal cord, and brainstem.

Results of the hemogram were unremarkable, except for rare schistocytes and microfilaria compatible with *Dirofilaria immitis*. Serum chemistry profile abnor-

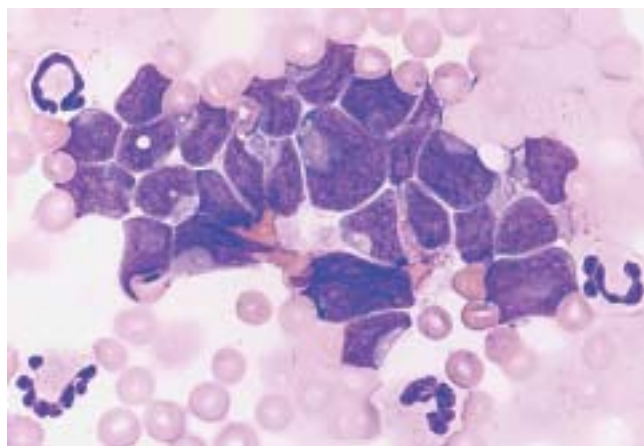


Figure 1. Cytocentrifuged preparation of cerebrospinal fluid from a dog with severe neurologic abnormalities. Diff-Quik, $\times 100$ objective.

malities included hyperproteinemia (8.8 g/dL, reference interval 5.7-7.8 g/dL) and hyperglobulinemia (5.6 g/dL, reference interval 1.7-3.8 g/dL). The schistocytes and protein abnormalities may have been caused by RBC fragmentation and chronic inflammation associated with the dog's positive heartworm status. Results of the coagulation profile included slightly shortened partial thromboplastin time (10.0 seconds, reference interval 10.3-16.7 seconds) and slightly decreased antithrombin III concentration (81% of control value, reference interval $>85\%$ of control value). These findings may have represented early changes associated with subclinical disseminated intravascular coagulation; however, the magnitude of the change was slight. The dog was anesthetized and cerebrospinal fluid (CSF) was obtained via cerebello-medullary cisternal puncture. A cytocentrifuged specimen of CSF was prepared (Shandon Cytospin II, Shandon Lipshaw, Pittsburgh, PA, USA; 500 rpm for 10 min) and stained with Diff-Quik (Dade Behring Inc, Newark, DE) for cytologic evaluation (Figure 1). The quantity of CSF was insufficient for full analysis (ie, microprotein, total nucleated cell count, RBC count, Pandy test). An aliquot of the CSF was submitted for aerobic bacterial culture, which yielded no growth after 72 hours.

(Continued on next page)

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Cytologic Interpretation

The cytocentrifuged preparation of CSF was highly cellular and consisted of numerous nucleated cells randomly arranged in a moderately heavy background of erythrocytes. Based on a 100-cell differential count, nucleated cells were composed of 88% medium to large atypical round cells, 9% nondegenerate neutrophils, and 3% large mononuclear cells (Figure 1). Rare eosinophils and small lymphocytes also were noted. Many neutrophil nuclei were hypersegmented, indicating senescence. The large mononuclear cells were morphologically consistent with lightly vacuolated macrophages. The atypical round cells were approximately 15-25 μm in diameter. They were usually round, but occasionally mildly pleomorphic, with polygonal, ovoid, and oblong shapes observed. The cells contained a single nucleus that was usually round, but occasionally was notched, segmented, reniform, or cloverleaf in shape. Nuclei had a finely granular chromatin pattern. N:C ratios were uniformly high. Frequent bizarre mitotic figures were seen. Several microfilaria consistent with *D. immitis* were observed.

A cytologic interpretation of lymphoma was made, based on the morphology of the discrete round cells, with secondary consideration given to a reactive or inflammatory process. Reactive lymphoid pleocytosis seemed less likely because of the paucity of small lymphocytes, lack of plasma cells, large number of bizarre mitotic figures, and marked nuclear pleomorphism. Because of a grave prognosis, the animal was euthanized and a necropsy was performed.

Gross and Histologic Findings

At necropsy, two pulmonary arterial thrombi and several pulmonary filarids were identified grossly. The right ventricle and pulmonary artery also contained numerous filarid worms consistent with *D. immitis*. The tracheobronchial lymph nodes were enlarged grossly and found histologically to be diffusely hyperplastic. No grossly identifiable lesions were found within the central nervous system.

Histologic sections from many parts of the central nervous system were stained with hematoxylin and eosin and examined. Multiple sections of cerebellum, medulla, and spinal cord contained a diffuse neoplastic infiltrate that was predominantly meningeal and rarely invading into the superficial neuropil. The infiltrate was composed of small, round to ovoid cells that appeared to

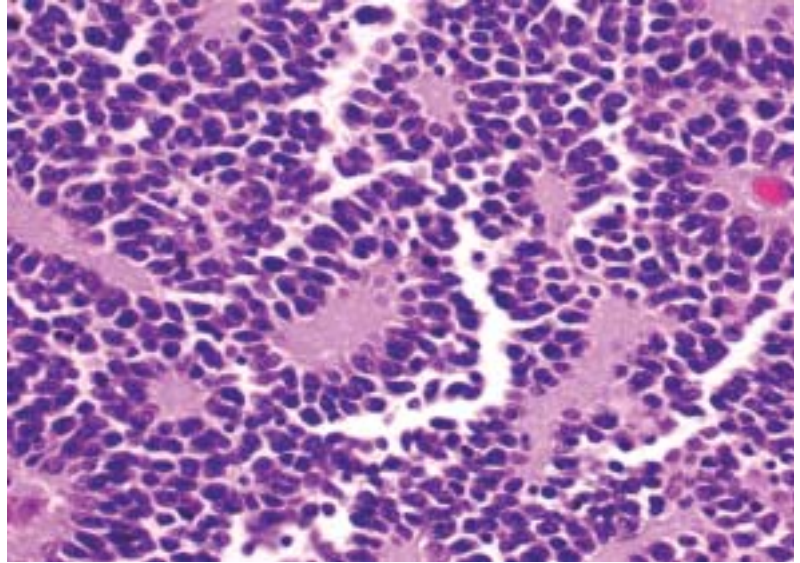


Figure 2. Histologic section of cervical spinal cord showing the architecture of the neoplastic infiltrate within the CNS. Sheets, cords, and rosettes are noted. The neoplastic cells are small, round, and discrete, with scant amphophilic cytoplasm. Hematoxylin and eosin, $\times 40$ objective.

be discrete (Figure 2). The cells had single, round to slightly pleomorphic nuclei with slightly clumped chromatin. A fine fibrovascular stroma was observed throughout the infiltrate. The neoplastic cells were arranged in sheets, cords, and rosettes (Homer-Wright in appearance), which had pale eosinophilic fibrillary material in their "lumina." The mitotic index was 4-6 per high-power field. The preliminary histologic diagnosis was medulloblastoma.

The neoplastic cells stained negative with immunohistochemical stains for CD3, CD79a, S-100, pancytokeratin, neuron-specific enolase (NSE), synaptophysin, and vimentin. The arrangement of cells in cords and rosettes as well as the negative staining for CD3 and CD79a ruled out lymphoma as a possible diagnosis. CD3 and CD79a are markers for T and B lymphocytes, respectively.¹ Weak positive staining for glial fibrillary acidic protein (GFAP) was noted within the lumen of the rosettes. It was unclear whether the staining was within the cytoplasm of the cells forming the rosette or was an artifactual finding caused by heavy background staining so its significance remained uncertain. A diagnosis of medulloblastoma was made on the basis of the signalment, history, location of the tumor, histologic finding of Homer-Wright rosettes, and negative staining for lymphoid immunohistochemical markers.

Discussion

Medulloblastomas are rare malignant brain tumors that are categorized as one of the embryonal tumors of the

central nervous system. They occur most often in young humans, dogs, and cattle² and are less frequently observed in pigs, rats, and cats.^{3,4} One case has been reported in a baboon and one in a kowari.³ Medulloblastomas most often originate in the cerebellum, where they make up the largest proportion of a class of tumors known as primitive neuroectodermal tumors (PNETs).^{2,4} Other PNETs include spongioblastomas, pineoblastomas, and neuroblastomas.^{4,6} PNETs arise from germinal epithelial cells. The precise cell of origin for medulloblastomas is uncertain; however, it is suspected to be matrix cells from the external granular layer of the cerebellum.² The cells are small, round to polygonal cells that have scant cytoplasm. When exfoliated into CSF, their cytomorphology is easily confused with lymphoid cells.⁶

It was assumed that the neoplasm in this case originated in the cerebellum and spread to other portions of the CNS. This behavior is unusual for medulloblastomas in animals⁴; however, dissemination throughout the CNS in human patients is described.⁶

The immunochemical staining pattern for the tumor in this case was inconsistent with previously reported findings in the medical and veterinary literature.^{2,4,6-9} Medulloblastomas have been reported to stain positively for vimentin,^{8,9} S-100,³ triple neurofilament,² and synaptophysin,^{2,6,8} and variably express NSE² and GFAP.^{4,6-9} Positive GFAP staining may be attributed to a reactive astrocytic response to the neoplasm^{6,8} or to astrocytic differentiation of medulloblastoma cells.^{4,6,8} In light of differing reports as to the immunohistochemical staining patterns of medulloblastomas, it appears likely that staining characteristics depend on the degree of differentiation of the neoplastic cells.^{2,4,6} The negative staining by the tumor in this case for all immunohistochemical markers (with the possible exception of GFAP) may indicate that it was poorly differentiated. Weak positive GFAP staining may have indicated minimal astrocytic differentiation, may have been an artifact, or, less likely, could have indicated reactive astrocytes. The staining was more diffuse than would be expected for well-differentiated astrocytes.

The neoplastic cells in the CSF of this animal were a unique and unusual finding. It is rare to identify exfoliated neoplastic cells in CSF from dogs and cats.^{10,11} The most commonly detected neoplasm is lymphoma.¹² Frequency of detection of primary brain tumors in cats and dogs via CSF cytology ranges from 0% to 8%.^{10,11} In one study, neoplastic cells were detected in the CSF of 35% of cats with spinal lymphoma.¹² The reported frequency of finding neoplastic cells in CSF is higher in humans than in animals, and neoplastic cells were found in the CSF in 26% of cases with histologically-confirmed neoplasia of the CNS.^{13,14} The most common tumor diagnosed via CSF cytology in adult humans is metastatic

adenocarcinoma (41% of CSFs with a diagnosis of malignant neoplasia), but in children it is medulloblastoma/PNET (77%).¹⁵ Regardless of species, the chance of successfully diagnosing neoplasia from a CSF sample is likely to increase if the tumor involves the subarachnoid space or ventricles, compared with tumors that are deep in the parenchyma.¹⁰⁻¹³ It also would seem logical that the likelihood of detection of neoplastic cells in the CSF hinges on the propensity of the tumor to exfoliate, although we are unaware of studies or reports in this regard.

This case demonstrates that medulloblastoma should be considered as a differential diagnosis for atypical round cells in the CSF of young dogs. Careful evaluation of the signalment, history, neurologic examination, and physical examination coupled with thorough cytologic evaluation is necessary to diagnose this neoplasm correctly. Additionally, the use of immunocytochemical and immunohistochemical stains or even flow cytometry might prove useful in making a definitive diagnosis. ◊

Abstract

A 3-year-old Staffordshire Terrier was presented to the Texas Veterinary Medical Center with a short progressive history of anorexia, weight loss, and weakness that had progressed to ataxia and collapse with empirical treatment. The dog was tetraparetic and obtunded. Results of a complete neurologic evaluation were consistent with severe, multifocal to diffuse disease involving the forebrain, spinal cord, and brainstem. Cerebrospinal fluid, obtained via cerebellomedullary cisternal puncture, was highly cellular and contained large atypical round cells with small numbers of nondegenerate neutrophils and large mononuclear cells. Rare eosinophils and small lymphocytes were noted. The atypical round cells were approximately 15-25 µm in diameter with a single nucleus set in a small amount of cytoplasm. The nuclei were typically round to slightly ovoid; however, occasional notched, lobulated, and reniform nuclei were observed. These cells were interpreted as malignant lymphocytes. Owing to a grave prognosis, the animal was euthanized and a necropsy was performed. No gross lesions were found in the central nervous system. Multiple sections of cerebellum, medulla, and spinal cord contained a diffuse neoplastic infiltrate that was predominantly meningeal with rare superficial neuropil invasion. The neoplastic cells were arranged in sheets, cords, and rosettes. Immunohistochemical staining for vimentin, pancytokeratin, CD3, CD79a, synaptophysin, S-100, and neuron-specific enolase was negative; glial fibrillary acidic protein (GFAP) staining was equivocal. Based on histologic findings, a diagnosis of medulloblastoma was made. This case documents the rare occurrence of a canine medul-

loblastoma and illustrates the difficulty in distinguishing between some embryonal brain tumors and lymphoma. (Thompson CA, Russell KE, Levine JM, Weeks BR. Cerebrospinal fluid from a dog with neurologic collapse [medulloblastoma]. *Vet Clin Pathol*. 2003; 32:143-146)

Key Words: Cerebrospinal fluid, dog, embryonal brain tumors, medulloblastoma, neoplasia, PNET, primitive neuroectodermal tumor

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