





STANDARD ARTICLE

Vascular endothelial growth factor concentrations in the cerebrospinal fluid of dogs with neoplastic or inflammatory central nervous system disorders

Christopher L. Mariani^{1,2,3}  | Zachary E. Niman^{1,2} | Lindsay B. Boozer¹ |
Laura K. Ruterbories^{1,2} | Peter J. Early^{2,3}  | Karen R. Muñana^{2,3}  |
Natasha J. Olby^{2,3} 

¹Comparative Neuroimmunology and Neuro-oncology Laboratory, North Carolina State University, Raleigh, North Carolina

²Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina

³Comparative Medicine Institute, North Carolina State University, Raleigh, North Carolina

Correspondence

Christopher L. Mariani, Department of Clinical Sciences, College of Veterinary Medicine, NC State University, 1060 William Moore Drive, Raleigh, NC 27607, USA.
Email: clmarian@ncsu.edu

Present address

Zachary E. Niman, MedVet Chicago, Chicago, IL,

Lindsay B. Boozer, Friendship Hospital for Animals, Washington, DC,

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Abstract

Background: Vascular endothelial growth factor (VEGF) is a key molecular driver of angiogenesis and vascular permeability and is expressed by a wide variety of neoplasms. Although blood VEGF concentrations have been quantified in intracranial tumors of dogs, cerebrospinal fluid (CSF) VEGF concentration might be a more sensitive biomarker of disease.

Objective: Concentrations of VEGF in CSF are higher in dogs with central nervous system (CNS) neoplasia compared to those with meningoencephalomyelitis and other neurologic disorders.

Animals: One hundred and twenty-six client-owned dogs presented to a veterinary teaching hospital.

Methods: Case-control study. Cerebrospinal fluid was archived from dogs diagnosed with CNS neoplasia and meningoencephalomyelitis. Control dogs had other neurological disorders or diseases outside of the CNS. A commercially available kit was used to determine VEGF concentrations.

Results: Detectable CSF VEGF concentrations were present in 49/63 (77.8%) neoplastic samples, 22/24 (91.7%) inflammatory samples, and 8/39 (20.5%) control samples. The VEGF concentrations were significantly different between groups ($P < .0001$), and multiple comparison testing showed that both neoplastic and inflammatory groups had significantly higher concentrations than did controls ($P < .05$), but did not differ from each other. Gliomas and choroid plexus tumors had significantly higher VEGF concentrations than did the control group ($P < .05$).

Conclusions and Clinical Importance: Cerebrospinal fluid VEGF concentrations may serve as a marker of neoplastic and inflammatory CNS disorders relative to other conditions.

Abbreviations: ANOVA, one-way analysis of variance; CNS, central nervous system; CSF, cerebrospinal fluid; FLAIR, fluid-attenuated inversion recovery; MRI, magnetic resonance imaging; NCC, nucleated cell count; RBC, red blood cell; VEGF, vascular endothelial growth factor.

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KEYWORDS

biomarker, brain tumor, meningoencephalitis, meningomyelitis

1 | INTRODUCTION

Angiogenesis is a tightly controlled process under normal physiological conditions, but becomes dysregulated in neoplastic tissue and is a critical step in carcinogenesis.^{1,2} A key molecular driver of angiogenesis is vascular endothelial growth factor (VEGF), which promotes endothelial survival, stimulates endothelial cell proliferation, and incites vascular permeability.³ Vascular endothelial growth factor is overexpressed in a wide variety of neoplasms in dogs,⁴⁻⁸ including intracranial tumors.⁹⁻¹¹ Additionally, the expression of VEGF in intracranial tumors of dogs has been correlated with histological grade,^{9,11} as well as survival time.¹⁰

Histological confirmation of neoplastic or other disease processes is the current gold standard for diagnosis, but is challenging for disorders affecting the brain. Although progress is being made, barriers to obtaining antemortem histological samples from the brain include reluctance from pet owners, financial burdens associated with these interventions, and the morbidity and potential mortality of the procedure.¹²⁻¹⁴ An easily obtainable, safe, surrogate biomarker of disease would be useful to clinicians not only for diagnostic purposes but also potentially as a mechanism for monitoring disease burden, assessing prognosis, and potentially for identifying actionable therapeutic targets.

A common diagnostic dilemma is the differentiation of neoplastic from inflammatory conditions affecting the central nervous system (CNS) of dogs.¹⁵⁻¹⁷ Several studies in dogs have found increased serum or plasma VEGF concentrations in neoplastic versus non-neoplastic processes outside of the CNS,^{7,18-23} and several reports have found that increased serum or plasma VEGF concentration suggests a higher tumor grade^{18,21} or is a poor prognostic indicator in dogs.^{7,24,25} Although plasma VEGF concentration is increased in some dogs with intracranial tumors,¹¹ cerebrospinal fluid (CSF) is considered to be a more sensitive indicator of CNS disease and is routinely sampled in dogs.

We hypothesized that VEGF concentrations in the CSF would be higher in dogs with CNS neoplasia compared to those with meningoencephalomyelitis and other neurologic disorders, and that dogs with gliomas would have higher concentrations than those with other CNS tumors. We designed a preliminary study to explore these hypotheses, with a primary objective to compare CSF VEGF concentrations in dogs with a diagnosis of CNS neoplasia, CNS inflammatory disease, and other neurologic disorders. A secondary objective was to compare CSF and serum VEGF concentrations in a small subset of dogs with these CNS disorders.

2 | MATERIALS AND METHODS

2.1 | Sample identification and medical record review

Cerebrospinal fluid and serum samples from dogs with a diagnosis of inflammatory or neoplastic CNS disease were identified by review of

archived samples within a biobank. All clinical data in the medical record (including history, clinical signs, clinicopathological data, imaging reports, and magnetic resonance [MR] images) was reviewed by one of the authors (C.L. Mariani) to determine the suitability of these cases for inclusion in the study. This author was not blinded to the VEGF concentrations. Control cases were identified in a similar manner. Signalment, weight, diagnostic imaging findings, results of CSF analysis, infectious disease test results, histopathological diagnoses, and history of glucocorticoid administration were obtained from the medical record. Because glucocorticoid administration before CSF collection varied widely in terms of specific medications, doses, and routes of administration, this information was recorded as a binary yes or no for each case.

2.2 | Diagnostic groups

Samples from dogs with a histological diagnosis of CNS neoplasia, meningoencephalitis, or meningomyelitis were prioritized for analysis in the study, although some patients that had presumptive clinical diagnoses made using presentation, history, physical and neurological examinations, magnetic resonance imaging (MRI) of the CNS, and CSF analysis were included. These were cases with typical clinical presentations and diagnostic imaging findings or CSF abnormalities in which there was a high degree of confidence in the diagnosis. Presumptive, image-diagnosed meningiomas were extra-axial masses with broad dural contact and intense, homogeneous enhancement after IV contrast administration (gadoversetamide, 0.1 mmol/kg [Optimark, Mallinckrodt Inc, St. Louis, Missouri]).^{26,27} Presumptive gliomas were variably contrast-enhancing, intra-axial masses that had imaging characteristics incompatible with hemorrhage on spin echo and gradient echo sequences.²⁷⁻²⁹ Presumptive choroid plexus tumors were strongly contrast-enhancing masses that were located in the lateral, third, or fourth ventricles.^{27,30} Presumptive pituitary tumors were noted to arise from the pituitary gland, and were strongly contrast-enhancing.²⁷ Dogs with CNS lymphoma were diagnosed by histology or by finding neoplastic lymphoblasts on cytologic examination of the CSF. All other tumors ("miscellaneous") were diagnosed by histopathology after necropsy examination.

For samples from dogs with inflammatory CNS disorders, those coming from patients with histological confirmation of disease again were prioritized, but in most of these cases, a presumptive diagnosis was based on MRI and CSF evaluation.^{31,32} Magnetic resonance imaging typically showed multifocal hyperintense lesions on T2-weighted and fluid-attenuated inversion recovery (FLAIR) imaging, with variable degrees of meningeal and parenchymal enhancement after IV contrast administration. Diffuse, ill-defined T2 and FLAIR hyperintense lesions also were noted. Cerebrospinal fluid evaluation had to show pleocytosis (nucleated cell count [NCC] >5 cells/ μ L) for inclusion in

the study. Infectious disease tests performed were variable in this cohort. Dogs without histological confirmation but with mononuclear pleocytosis and consistent MRI findings were classified as having meningoencephalitis of unknown etiology (MUE). Dogs without histological confirmation but with neutrophilic pleocytosis were classified as having neutrophilic meningoencephalitis. Dogs with meningomyelitis but otherwise meeting the above criteria also were included.³³ An exception for CNS imaging was made for dogs with steroid-responsive meningitis-arteritis, which was diagnosed in some cases by characteristic clinical presentation, CSF analysis, and response to treatment.³⁴

A control group also was utilized in the study, consisting of dogs with either non-neoplastic, noninflammatory CNS disorders (idiopathic or unknown epilepsy, vascular disorders, and hydrocephalus), dogs with secondary tumors arising from the vertebrae or skull, peripheral nervous system disorders (including peripheral vestibular dysfunction) or dogs ultimately diagnosed with a disease process not involving the nervous system. All dogs with idiopathic or unknown epilepsy, vascular disorders, hydrocephalus, and peripheral vestibular disease were diagnosed based on a combination of history, physical and neurological examinations, CNS imaging studies, and CSF evaluation in accordance with previously published criteria.³⁵⁻³⁹ For other controls (secondary tumors, other peripheral nervous system disorders, and disorders not involving the nervous system), CNS imaging

and routine CSF analysis, although performed in many cases, were not required for inclusion.

2.3 | Sample collection

Cerebrospinal fluid was collected using standard techniques from either the cerebellomedullary cistern or the lumbar subarachnoid space as part of routine diagnostic testing in animals evaluated for neurological disorders at the NC State Veterinary Hospital. Routine analysis included NCC and red blood cell (RBC) counts, total protein concentration, and cytological examination. In some cases, CSF was obtained immediately after euthanasia without subsequent standard analysis. Serum also was obtained from a limited number of dogs by conventional means. After collection, CSF and serum samples were aliquoted and stored at -80°C until analysis.

2.4 | VEGF analysis

Serum and CSF samples were analyzed using a solid-phase ELISA designed for the detection of human VEGF (Quantikine ELISA, R&D Systems, Minneapolis, Minnesota), according to the manufacturer's

TABLE 1 Physical characteristics of dogs in the neoplastic, inflammatory, and control groups

Diagnostic category	Number of dogs	Age (years)	Weight (kg)	Sex			
				M	MC	F	FS
Meningioma	20	10.0 (4.0-13.0)	24.6 (4.1-46.2)	1	11	0	8
Glioma	19	8.0 (5.0-15.0)	19.8 (5.0-42.0)	0	5	2	12
Choroid plexus tumor	8	7.5 (5.0-11.0)	28.0 (11.1-43.3)	0	2	0	6
Round cell tumor	7	5.0 (0.8-12.0)	34.5 (11.3-51.0)	2	1	1	3
Pituitary tumor	6	8.0 (4.0-12.0)	28.7 (11.0-43.0)	0	5	0	1
Other tumors	3	9.0 (4.0-12.0)	20.7 (8.0-22.0)	1	1	0	1
Total neoplastic	63	8.0 (0.8-15.0)	25.2 (4.1-51.0)	4	25	3	31
MUE	10	4.0 (0.8-10.0)	18.7 (4.4-30.0)	1	3	1	5
GME	5	5.0 (2.0-10.0)	22.8 (7.5-30.6)	2	2	0	1
NME	2	2.0 (1.0-3.0)	6.7 (4.5-8.9)	0	1	0	1
Neutrophilic ME	3	1.0 (0.8-11.0)	26.7 (2.6-35.0)	0	1	0	2
SRMA	2	0.8 (0.8-0.8)	20.3 (18.3-22.2)	0	1	1	0
Other inflammatory	2	3.0 (2.0-4.0)	27.1 (24.0-30.2)	0	1	1	0
Total inflammatory	24	3.0 (0.8-11.0)	19.0 (2.6-35.0)	3	9	3	9
Idiopathic/unknown epilepsy	11	3.0 (1.0-8.0)	14.0 (3.8-56.6)	0	5	2	4
Vascular	7	11.0 (4.0-12.0)	23.1 (6.5-45.0)	0	3	0	4
Hydrocephalus	4	0.6 (0.3-2.0)	17.5 (7.2-40.8)	1	2	1	0
Secondary tumor	7	12.0 (3.0-12.0)	31.6 (26.3-41.7)	0	5	0	2
Miscellaneous	11	7.0 (0.7-13.0)	22.5 (5.5-37.6)	3	4	0	4
Total controls	39	7.0 (0.3-12.0)	23.6 (3.8-56.6)	3	19	3	14
All cases	126	7.0 (0.3-15.0)	23.6 (2.6-56.6)	10	53	9	54

Note: Age and weight values are expressed as median (range).

Abbreviations: F, female; FS, female spayed; GME, granulomatous meningoencephalitis; M, male; MC, male castrated; ME, meningoencephalomyelitis; MUE, meningoencephalomyelitis of unknown etiology; NME, necrotizing meningoencephalitis; SRMA, steroid-responsive meningitis-arteritis.

directions. This ELISA measures the human VEGF₁₆₅ isoform (165 amino acids) which is analogous to the canine VEGF₁₆₄ isoform, and has been previously validated for canine serum and CSF.^{22,40} Absorbance was quantified using a microplate reader (Sunrise microplate reader with Magellan software, Tecan, Baldwin Park, California) at a wavelength of 450 nm with wavelength correction at 540 nm. Absorbance values were plotted on a standard curve generated with the use of VEGF standards provided in the kit. Samples were analyzed in duplicate, and results reported in pg/mL.

2.5 | Statistical analysis

Data from all groups were assessed for normality using a D'Agostino and Pearson omnibus normality test. Because most of the data were not normally distributed, nonparametric tests were used for all analyses. The VEGF concentrations between groups were compared using a Kruskal Wallis one-way analysis of variance (ANOVA), followed by Dunn's test for multiple comparisons. Correlations between conventional CSF parameters (NCC, RBC, protein) and CSF

VEGF concentrations and between CSF and serum VEGF concentrations were assessed using a Spearman correlation coefficient (ρ). The Mann-Whitney test was used to compare VEGF concentrations in dogs that did or did not receive glucocorticoids before CSF collection. All tests were 2-tailed and a P value $<.05$ was considered significant for all analyses.

3 | RESULTS

Cerebrospinal fluid samples were available from 126 dogs, with a variety of breeds represented. Descriptive statistics for age, weight, and sex of the diagnostic groups and the entire cohort are shown in Table 1. One hundred and sixteen CSF samples (92.1%) were collected from the cerebellomedullary cistern and 10 (7.9%) from the lumbar cistern. Routine CSF analysis was performed on 93 samples and characteristics of these analyses for each diagnostic group are shown in Table 2. Because of limited sample volume, total protein concentration was not available for one case. Histological confirmation was available for 43/63 (68%) dogs with CNS neoplasia,

TABLE 2 Cerebrospinal fluid parameters of dogs in the neoplastic, inflammatory, and control groups

Diagnostic category	Collection site		Nucleated cell count (cells/ μ L)	Red blood cell count (cells/ μ L)	Protein (mg/dL)
	CCSF	LCSF			
Meningioma	14	0	1 (0-5)	10 (0-550)	31.9 (8.1-83.8)
Glioma	8	2	0 (0-12)	7.5 (0-245)	21.0 (16.9-104.6)
Choroid plexus tumor	3	2	3 (0-10)	169 (0-528)	84.7 (26.2-735.0)
Round cell	4	1	129 (2-5139)	135 (10-775)	88.5 (49.5-128.2)
Pituitary	3	0	4 (2-4)	0 (0-3)	25.5 (23.5-52.6)
Other tumors	1	0	9	130	29.9
Total neoplastic	33	5	2 (0-5139)	12.5 (0-775)	31.9 (8.1-735.0)
MUE	9	1	239.5 (12-1390)	63 (0-2397)	58.2 (10.1-537.5) ^a
GME	3	0	219 (52-388)	242 (5-363)	65.0 (54.0-290.2)
NME	1	0	151	0	61.7
Neutrophilic ME	3	0	237 (204-467)	418 (25-11 500)	75.1 (24.6-82.6)
SRMA	2	0	669.5 (444-895)	20 013 (25-40 000)	97.9 (57.6-138.1)
Other inflammatory	1	1	145 (117-173)	436 (7-865)	232.4 (225.3-239.4)
Total inflammatory	19	2	219 (12-1390)	63 (0-40 000)	70.1 (10.1-537.5)
Idiopathic/unknown epilepsy	11	0	1 (0-2)	3 (0-275)	11.6 (8.4-39.8)
Vascular	7	0	3 (1-156)	25 (0-8150)	28.0 (18.1-44.8)
Hydrocephalus	4	0	0 (0-2)	1.5 (0-5)	10.9 (8.4-35.7)
Secondary tumor	2	2	2 (0-11)	200 (23-3430)	134.3 (23.2-295.4)
Miscellaneous	7	1	1.5 (0-3)	3 (0-185)	23.9 (10.1-62.6)
Total controls	31	3	1 (0-156)	3 (0-8150)	19.0 (8.4-295.4)
All cases	83	10	2 (0-5139)	15 (0-40 000)	30.5 (8.1-735.0)

Note: Nucleated cell count, red blood cell count and protein values are expressed as median (range).

Abbreviations: CCSF, cerebellomedullary cistern cerebrospinal fluid; GME, granulomatous meningoencephalitis; LCSF, lumbar cerebrospinal fluid; ME, meningoencephalomyelitis; MUE, meningoencephalomyelitis of unknown etiology; NME, necrotizing meningoencephalitis; SRMA, steroid-responsive meningitis-arteritis.

^aIn 1 sample, the quantity was not sufficient to analyze protein concentrations.

consisting of 11/20 (55%) meningiomas, 15/19 (79%) gliomas (8 oligodendrogliomas, 7 astrocytomas), 5/8 (63%) choroid plexus tumors, 3/6 (50%) pituitary tumors, 6/7 round cell tumors (86%, 3 CNS lymphoma, 2 histiocytic sarcomas, 1 unclassified round cell tumor; an additional lymphoma case was diagnosed by CSF cytology) and 3/3 (100%) miscellaneous tumors (2 primitive neuroectodermal tumors and 1 metastatic hemangiosarcoma). Histological confirmation was available for 9/24 (38%) dogs diagnosed with inflammatory CNS disease, consisting of 5 dogs with granulomatous meningoencephalitis, 2 with necrotizing meningoencephalitis, 1 dog with eosinophilic meningoencephalitis (diagnosed at necropsy with distemper and heartworm disease), and 1 dog with amoebic meningoencephalitis. The control group consisted of 39 samples from dogs with various disorders including idiopathic or unknown epilepsy (11), cerebrovascular accidents (7), vertebral, skull and nasal tumors (7), hydrocephalus (4), and a variety of other conditions (Table S1).

Cerebrospinal fluid VEGF concentrations by diagnostic group are shown in Table 3. Detectable VEGF concentrations were present in 49/63 (77.8%) neoplastic samples, 22/24 (91.7%) inflammatory samples, and 8/39 (20.5%) of controls. The VEGF concentrations were significantly different between groups ($P < .0001$), and multiple comparison testing showed that both neoplastic and inflammatory groups had significantly higher concentrations than controls ($P < .05$), but did not differ

from each other. When comparing CSF VEGF concentrations in specific tumor subtypes to each other and to controls, these groups were significantly different ($P < .0001$), and multiple comparison testing showed that gliomas, choroid plexus tumors, and miscellaneous CNS tumors had significantly higher VEGF concentrations than the control group ($P < .05$). No difference in CSF VEGF concentrations was found between tumor groups and no clear distinction was identified when comparing oligodendrogliomas and astrocytomas (Table S2). No differences in CSF VEGF concentrations were found between dogs that did or did not receive glucocorticoids before CSF collection for the CNS neoplasia ($P = .49$) or inflammatory CNS disease ($P = .39$) cohorts (Figure S1). Correlations between CSF parameters and CSF VEGF concentrations for dogs in the neoplastic and inflammatory groups are shown in Table 4.

Serum VEGF concentrations were analyzed in 16 dogs, consisting of 9 dogs with CNS neoplasia, 5 dogs with inflammatory CNS disease, 1 dog with multiple myeloma (involving multiple vertebrae but not directly affecting the CNS), and 1 dog with congenital hydrocephalus. Median and mean results for each group are shown in Table 5. Twelve of these dogs had paired CSF and serum samples analyzed, which showed lower VEGF concentrations in serum in all cases except for the dog with congenital hydrocephalus, which had undetectable concentrations in the CSF and a serum concentration of 2.8 pg/mL.

TABLE 3 Cerebrospinal fluid VEGF concentrations in dogs by diagnostic category

Diagnostic category	Number	Median VEGF (range) (pg/mL)	Mean VEGF \pm SD (pg/mL)
Meningioma	20	7.3 (0-98.4)	18.0 \pm 26.1
Glioma	19	14.6 (0-2000.0 ^a)a	229.0 \pm 553.0
Choroid plexus tumor	8	496.0 (0-2000.0 ^a)a	744.0 \pm 835.0
Round cell tumor	7	3.2 (0-109.0)	21.4 \pm 40.3
Pituitary tumor	6	7.2 (0-105.0)	21.4 \pm 40.9
Other tumors	3	80.9 (45.0-223.0)	116.0 \pm 94.0
Total neoplastic	63	13.1 (0-2000.0^a)a	179.0 \pm 473.0
MUE	10	31.5 (0-490.0)	93.4 \pm 149.0
GME	5	16.1 (7.3-275.0)	67.1 \pm 116.0
NME	2	7.2 (0-14.4)	7.2 \pm 10.2
Neutrophilic ME	3	28.2 (15.4-33.5)	25.7 \pm 9.3
SRMA	2	120.0 (72.8-168.0)	120.0 \pm 67.4
Other inflammatory	2	70.0 (50.2-89.8)	70.0 \pm 28.0
Total inflammatory	24	26.9 (0-490.0)a	72.6 \pm 111.0
Idiopathic/unknown epilepsy	11	0 (0-18.6)	3.0 \pm 6.8
Vascular	7	0 (0-49.8)	8.9 \pm 18.6
Hydrocephalus	4	0 (0-0)	0 \pm 0
Secondary tumor	7	0 (0-28.0)	4.8 \pm 10.5
Miscellaneous	10	0 (0-11.2)	2.0 \pm 4.2
Total controls	39	0 (0-49.8)b	3.8 \pm 9.8
Total all cases	126	9.1 (0-2000^a)	105.0 \pm 346.0

Note: Within a column, median values with different letters differ significantly ($P < .05$).

Abbreviations: GME, granulomatous meningoencephalitis; ME, meningoencephalomyelitis; MUE, meningoencephalomyelitis of unknown etiology; NME, necrotizing meningoencephalitis; SD, standard deviation; SRMA, steroid-responsive meningitis-arteritis; VEGF, vascular endothelial growth factor.

^a2000 pg/mL was the maximum concentration of the standard range used in the assay and further dilution was not performed; therefore, maximum concentrations in these categories are greater than 2000 pg/mL.

TABLE 4 Correlations between CSF parameters and CSF VEGF concentrations in dogs with neoplastic or inflammatory disorders

CSF analyte	VEGF subgroup	Number of dogs	Correlation coefficient (ρ) ^a	P value
Nucleated cell count	Neoplasia	38	.405	.01
	Inflammatory	21	.692	.0005
	Total	66	.466	<.0001
Red blood cell count	Neoplasia	38	.350	.03
	Inflammatory	21	.549	.01
	Total	66	.407	.0007
Total protein	Neoplasia	38	.319	.05
	Inflammatory	20	.83	<.0001
	Total	65	.494	<.0001

Abbreviations: CSF, cerebrospinal fluid; VEGF, vascular endothelial growth factor.

^a ρ is the Spearman correlation coefficient.

Diagnostic category	Number	Median VEGF (range) (pg/mL)	Mean VEGF \pm SD (pg/mL)
Neoplastic	9	11.1 (0-44.0)	13.0 \pm 14.6
Inflammatory	5	3.0 (0-29.4)	9.6 \pm 12.8
Other (controls)	2	6.9 (2.8-11.1)	6.9 \pm 5.9
Total	16	7.0 (0-44.0)	11.2 \pm 12.8

TABLE 5 Serum VEGF concentrations in dogs by diagnostic category

Abbreviations: SD, standard deviation; VEGF, vascular endothelial growth factor.

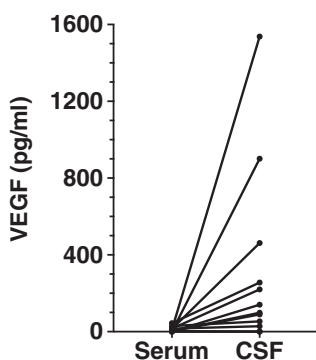


FIGURE 1 Paired serum and cerebrospinal fluid (CSF) samples from 12 dogs (comprising 4 dogs with MUE, 3 with gliomas, 2 with choroid plexus tumors, and 1 each with hydrocephalus, meningioma, and amebic meningoencephalitis) showing differences in vascular endothelial growth factor (VEGF) concentrations

In some cases, serum VEGF was not detectable even with substantial increases in CSF VEGF concentration (Figure 1). No correlation was found between serum and CSF VEGF concentrations ($\rho = .097, P = .76$).

4 | DISCUSSION

In our study, CSF VEGF concentrations were significantly higher in dogs with neoplasia and meningoencephalitis when compared to controls. Subgroup analysis indicated that dogs with gliomas, choroid

plexus tumors, and miscellaneous tumors (primitive neuroectodermal tumors and metastatic hemangiosarcoma) had particularly high concentrations. No significant difference was found between neoplastic and inflammatory samples and we therefore rejected our original hypothesis. Correlations between conventional CSF parameters and VEGF concentrations were weak to moderate when considering all dogs or the neoplastic subgroup but moderate to very strong when considering only the inflammatory subgroup. Analysis of a small cohort suggests that VEGF concentrations are higher in the CSF than in the serum in dogs with neoplastic and inflammatory CNS disorders.

Vascular endothelial growth factor is a potent endothelial cell mitogen, endothelial chemotactic and survival factor, and inducer of vascular permeability.^{3,41-43} As a critical regulator of angiogenesis, its role in tumor progression has been long established, and VEGF is expressed by a wide variety of cancers in humans.⁴⁴⁻⁵² Expression of VEGF also has been documented in a number of cancers in dogs,⁴⁻⁸ including meningiomas,⁹⁻¹¹ gliomas,^{9,11} and choroid plexus tumors.⁵³ In humans, VEGF expression has been associated with both intracranial tumor histology and tumor grade.^{49,54-58} Similarly, in dogs, higher VEGF expression has been documented in gliomas relative to meningiomas or to normal brain and in higher grade gliomas relative to lower grade tumors,^{9,11} although this pattern relative to tumor grade was not noted in another study of choroid plexus tumors.⁵³

Based on its expression in this wide variety of tumors, there has been much interest in the detection of VEGF in the peripheral blood and in other body fluids as a biomarker of neoplastic disease. Concentrations of VEGF in the serum or plasma of dogs with various tumors were higher than in control samples^{7,18-23} One study documented

higher plasma concentrations in dogs with lymphoma versus healthy control dogs and higher concentrations in dogs with T-cell lymphomas and stage V disease compared with lower disease stages.¹⁸ Studies of serum or plasma VEGF concentrations in humans with intracranial meningiomas and gliomas have produced conflicting results, with some finding increased concentrations⁵⁹ and others showing no difference to controls.⁶⁰⁻⁶² One study identified plasma VEGF concentrations in 9/27 dogs with intracranial neoplasms, with a higher proportion in those with astrocytomas, particularly those of a higher grade.¹¹ The literature in dogs describing VEGF in body fluids beyond peripheral blood is limited, although 1 study identified high concentrations in body cavity effusions relative to plasma.⁶³

Our study identified increased concentrations of VEGF in CSF of dogs with neoplastic or inflammatory disease relative to samples from dogs with non-neoplastic, noninflammatory disorders and particularly high concentrations in a subset of dogs with gliomas, choroid plexus tumors, and primitive neuroectodermal tumors. Increased VEGF concentrations in the CSF have been documented in humans with gliomas^{61,64,65} and leptomeningeal metastasis of primary tumors originating outside of the CNS.⁶⁶⁻⁶⁸ Cerebrospinal fluid VEGF concentrations were higher in high-grade gliomas than in low-grade gliomas^{61,65} or other CNS neoplasms.⁶⁴

The reason for the variability in CSF VEGF concentrations in dogs with neoplastic CNS disease is unknown. Concentrations of VEGF in CSF from dogs with gliomas were higher than in dogs with meningiomas, which is consistent with differences in tissue expression of these tumors in dogs.^{9,11} Tissue concentrations also have been shown to vary by tumor subtype and grade in meningiomas^{56,57} and gliomas^{54,55,58} of humans and this has been reflected in differences in CSF concentrations in some studies.^{61,64,65} It is likely that tissue VEGF concentrations varied based on the grade of these tumors and may have been reflected in the CSF concentrations. Consistent and reliable tumor grading was not available in the pathology reports for all dogs included in our study and therefore was not addressed. Limited information is available regarding other CNS tumors, but VEGF expression has been documented in embryonic tumors and choroid plexus tumors in both humans and dogs⁵¹⁻⁵³ and in the CSF of human patients with leptomeningeal metastases.^{67,68}

Factors other than the tumor subtype and grade are also likely to play a role in influencing VEGF concentrations in the CSF. The proximity of the neoplasms to the ventricular system and subarachnoid space is one potential influencing factor. Choroid plexus tumors are intimately associated with the CSF within the ventricular system and may contribute to CSF production but are also typically highly vascular tumors. Normal choroid plexus epithelium has been shown to express VEGF in several species, including dogs,^{53,69-71} and VEGF expression has been documented in choroid plexus tumors of dogs.⁵³ In our study, these tumors had the highest median and mean concentrations of all neoplasms, but several tumors had concentrations that were quite low or even undetectable. Although arising from an intraparenchymal location, gliomas may be found adjacent to CSF within the ventricular system or subarachnoid space and oligodendrogliomas have been documented to invade and metastasize via the CSF in dogs.⁷² However, no clear

distinction was seen in CSF VEGF concentrations between oligodendrogliomas and astrocytomas (Table S2). Meningiomas had relatively low CSF concentrations of VEGF in our study. Whether this finding is a function of their tissue expression or the ability to release expressed protein into the CSF is unknown. Expression of VEGF previously has been documented in canine meningioma tissue, typically at concentrations lower than gliomas.⁹⁻¹¹ However, although meningiomas are in close proximity to the subarachnoid space, tissue VEGF may fail to enter CSF because of the presence of basement membranes or other physical barriers. It seems likely that CSF VEGF expression in dogs is influenced by tumor tissue expression, proximity to the ventricular system or subarachnoid space, and potentially other factors.

Similarly, reasons for the variability in VEGF concentrations in dogs with inflammatory CNS disorders are unclear. Almost 92% of dogs with these disorders had detectable CSF VEGF. Human patients with bacterial meningitis (including tuberculous meningitis) frequently have detectable VEGF in the CSF, although it was detected less commonly or in lower concentrations in patients with viral meningitis.^{67,68,71,73,74} Increased concentrations also have been documented in humans with fungal meningitis,⁷⁴ and in several studies of non-infectious inflammatory CNS disorders, including Behcet's disease with neurological involvement,⁷⁵ Guillain-Barré syndrome,⁷⁶ chronic inflammatory demyelinating polyneuropathy,⁷⁶ and multiple sclerosis,⁷⁵ although CSF VEGF could not be detected in another study that included multiple sclerosis patients.⁶⁷ A major source of VEGF in these inflammatory CNS conditions appears to be invading immune cells, including monocytes, lymphocytes, and neutrophils.^{71,73,74} A VEGF index, calculated similarly to an IgG index, has suggested intrathecal VEGF production in some studies of bacterial meningitis⁷¹ but not in others.⁶⁷ Expression of VEGF by astrocytes within inflammatory plaques also has been documented in multiple sclerosis patients.^{77,78}

In our study, CSF VEGF concentrations were weakly to moderately correlated with CSF NCC and protein concentrations in dogs with neoplasia or when considering all dogs together but these correlations were strong for NCC and very strong for protein concentrations in the inflammatory cohort,⁷⁹ supporting a role for inflammatory cells in producing VEGF in dogs with meningoencephalitis and meningomyelitis. A similar pattern has emerged in limited studies of the relationship between CSF VEGF concentrations and NCC in human patients. One study found positive correlations between these parameters in patients with tuberculous meningitis,⁷⁴ and another study showed that patients with bacterial meningitis and detectable CSF VEGF concentrations had higher CSF NCC and protein concentrations (although these did not reach statistical significance).⁷¹ However, yet another study found no correlations between CSF VEGF concentrations and conventional CSF parameters in patients with carcinomatous meningitis.⁶⁷ The source of VEGF in dogs with inflammatory CNS disease likely involves immune cells, at least in part, but may be multifactorial, and this question requires additional study.

We analyzed paired CSF and serum samples in a small subset of dogs, which showed higher VEGF concentrations in the CSF in all dogs with neoplastic or inflammatory CNS disease. The discrepancies in concentrations were most evident in the neoplastic samples, and

were often dramatic. Studies evaluating the correlation of CSF and serum VEGF concentrations in human patients have shown inconsistent results,^{61,67,75} but a number of reports have shown CSF concentrations to be more sensitive than serum concentrations for the presence of both neoplastic and inflammatory CNS disorders.^{61,67,71,73} One of these reports found CSF but not serum VEGF concentrations to be indicative of tumor grade and vascularity, and predictive of overall survival in glioma patients.⁶¹ Other studies have further documented the insensitivity of serum VEGF concentrations for the detection of brain tumors.^{60,80} Studies of VEGF concentrations in the peripheral blood of multiple sclerosis patients are also contradictory, with increased concentrations identified in some studies but not in others.^{67,81-83} Although our sample size was small, and confirmatory studies including larger numbers of dogs are required, our preliminary data suggest that CSF VEGF concentrations may be more representative of neoplastic and inflammatory disorders than serum concentrations in dogs.

A future role might be envisioned for using CSF VEGF concentrations in decision making regarding tailored treatments (eg, antiangiogenic interventions)⁸⁴ or for the monitoring of tumor progression or recurrence after definitive treatments. Such a role has been suggested in studies of serum or plasma VEGF concentrations in dogs with various cancers.^{18,19,23,85} It is also possible that CSF VEGF might provide useful prognostic information. Serum or plasma VEGF concentrations have been associated with disease-free interval or survival times in dogs with a variety of cancers^{7,20,21,24,25} and VEGF expression in meningiomas in dogs has been associated with prognosis.¹⁰ Cerebrospinal fluid VEGF concentrations have been shown to decrease after treatment for tuberculous meningitis in humans,^{73,74} and human patients with bacterial meningitis and detectable CSF VEGF were more likely to have neurological signs and more likely to die from their disease.⁷¹ Therefore, additional investigation of CSF VEGF concentrations in therapeutic monitoring and prognosis in dogs with neoplastic and inflammatory CNS disorders appears warranted.

We could find no effect of prior glucocorticoid administration on VEGF concentrations in CSF in our study. In fact, some of the highest CSF VEGF concentrations found in our study were in dogs that had received glucocorticoids (Figure S1). Corticosteroids have been shown to decrease VEGF expression in cultured human and rat malignant glioma cells, primarily at a transcriptional level.^{86,87} However, a study of VEGF expression in canine tumor tissue failed to find an effect of prior glucocorticoid administration,⁹ and intracranial tumors have been found to express VEGF regardless of glucocorticoid treatment in both humans^{88,89} and dogs.¹⁰ Our analysis was suboptimal, segregating dogs only as having received or not received glucocorticoids, because of the variety of administered drugs (given both before and after admission to our hospital) at various doses and by different routes of administration. Future prospective investigations into the effects of these drugs on VEGF concentrations in canine CSF likely will be necessary to more definitively investigate this issue.

Our study had a number of limitations. Although we prioritized samples obtained from dogs with histological confirmation of disease, histopathology was not available from all cases and it is possible that some dogs were misdiagnosed. However, we used established clinical,

imaging, and CSF parameters to define these cases, and it is unlikely that incorrect diagnoses in a few dogs would have changed the ultimate findings of our study. It is possible that this preference for histological confirmation resulted in a bias for the selection of more severely affected patients, which may have influenced VEGF concentrations. The inclusion of dogs with hydrocephalus and cerebrovascular disorders in the control group may have been ill-advised, because these conditions have been associated with increased CSF VEGF concentrations in humans.^{90,91} However, all of the CSF samples from hydrocephalic patients and 5/7 of the samples from patients with cerebrovascular CNS disorders had undetectable VEGF concentrations (the exceptions were both hemorrhagic infarcts, Table S1), and this decision likely did not substantially influence the results of our study. The CSF samples used were stored for variable amounts of time and were not centrifuged before freezing. Therefore, it is unclear if the VEGF detected in our study originated from soluble protein or from cell-associated VEGF. The ELISA used detects the canine VEGF₁₆₄ isoform, which is considered the most biologically important soluble isoform³ and is the 1 evaluated in most studies of canine and human patients.^{9,11,40,56,62,64-67,71,73,74,83} However, other VEGF isoforms likely play important roles in pathological angiogenesis and the progression of disease.⁴⁷ These were not evaluated in our study, and further investigation of other VEGF isoform concentrations in CNS disorders in dogs is warranted. Samples with VEGF concentrations above the upper limit of the range established by the standard curve (2000 pg/mL) were not further diluted because of limited sample volumes and some samples may have had markedly higher VEGF concentrations than reported here. Finally, although samples were banked in a prospective manner, clinical data were retrieved retrospectively and reviewed by an unblinded investigator, which may have introduced some bias and led to the omission of some data. Thus, some glucocorticoid administration before presentation at our hospital may have occurred and not been captured in the medical records. However, the results of questioning concerning prior medication administration were explicitly stated in most records, and such omission errors are unlikely to have had a large effect on our study conclusions.

In conclusion, CSF VEGF concentrations were higher in dogs with neoplastic and inflammatory CNS disorders relative to other conditions, and were particularly high in a subset of dogs with gliomas and choroid plexus tumors. Preliminary data suggest that CSF VEGF concentrations may be more sensitive than serum concentrations in the detection of these disorders in dogs. Future investigations of the utility of CSF VEGF concentrations as a disease biomarker, and their role in therapeutic and prognostic monitoring in dogs with CNS neoplasia and meningoencephalomyelitis seem warranted.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflicts 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 conducted on samples previously obtained and archived during the course of a routine diagnostic evaluation; IACUC approval is not required for such studies at North Carolina State University.

ORCID

Christopher L. Mariani  <https://orcid.org/0000-0003-3228-6536>

Peter J. Early  <https://orcid.org/0000-0003-3399-8500>

Karen R. Muñana  <https://orcid.org/0000-0001-9592-6276>

Natasha J. Olby  <https://orcid.org/0000-0003-1349-3484>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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