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The Reibergram for immunoglobulin A in dogs: Evaluation of intrathecal IgA synthesis using a quotient graph in dogs with neurological diseases

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Abstract

Background: Increased cerebrospinal fluid (CSF) protein concentration is a common finding in neurological diseases of dogs. Distinguishing between intrathecally-produced proteins and proteins that have passed the blood-CSF barrier because of barrier disruption facilitates diagnosis. Albumin is a microprotein mainly produced extrathecally that can be used as a reference marker for blood-CSF barrier dysfunction.

Objectives: Develop a quotient graph based on the CSF/serum quotient of albumin and immunoglobulin A (IgA; Reibergram) to visualize intrathecal IgA synthesis and blood-CSF barrier dysfunction.

Animals and Methods: Retrospective single-center cohort study. A hyperbolic function was developed using data from 6 healthy Beagles and 38 dogs with neurological diseases in which an isolated blood-CSF barrier dysfunction was expected. The function was validated using data from 10 dogs with expected intrathecal IgA synthesis and was visualized as a quotient graph. Finally, the graph was used to evaluate data of 118 dogs with various neurological diseases.

Results: Within the Reibergram, the function $\mathrm{QLim}\,(\mathrm{IgA}) = 0.13\,\sqrt{(\mathrm{QAlb})^2 + 11.9 \cdot 10^{-6}} - 1.01 \cdot 10^{-3}$ describes the upper values of physiological IgA quotients. It detects diseases with expected intrathecal IgA synthesis with higher sensitivity (85%) and specificity (89%) than the IgA index. The upper value of the physiological albumin quotient is 2.22 and detects diseases with expected blood-CSF barrier dysfunction (sensitivity: 81%; specificity: 88%).

Conclusion and Clinical Importance: The canine Reibergram can detect blood-CSF barrier dysfunction and intrathecal IgA synthesis in the majority of cases. The

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Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; CV, coefficient of variation; FCE, fibrocartilaginous embolism; HNPE, hydrated nucleus pulposus extrusion; Ig, immunoglobulin; IVDH, intervertebral disc herniation; IVS, idiopathic vestibular syndrome; MUO, meningoencephalitis of unknown origin; QAlb, CSF/serum quotient of albumin; QIgA, CSF/serum quotient of IgA; QLim(IgA), upper values of physiological of CSF/serum quotient of IgA; SRMA, steroid responsive meningitis-arteritis.

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graphical visualization simplifies data evaluation and makes it a feasible tool in routine CSF diagnostic testing.

KEYWORDS

albumin, barrier dysfunction, blood-CSF barrier, cerebrospinal fluid, CSF, immunoglobulin

1 | INTRODUCTION

Cerebrospinal fluid (CSF) diagnostic testing helps narrow down the differential diagnoses for central nervous system (CNS) diseases.[1] Increased total protein concentration in CSF is nonspecific and can occur in a wide variety of etiologies.[2,3] Inflammatory diseases often are associated with intrathecal synthesis of immunoglobulin (Ig).[4] Other pathologies cause isolated blood-CSF barrier dysfunction, which results in increased passage of proteins into the CSF.[4,5] Both mechanisms might occur in combination, which makes it difficult to estimate the Ig fraction synthesized within the meninges.[5,6] For a more differentiated examination of proteins in the CSF, albumin (a microprotein mostly produced in the liver) can be used as reference molecule.[7] Blood-CSF barrier dysfunction increases the permeability for albumin and Ig.[5,8] By correlating the CSF/serum quotient of albumin (QAlb) to the CSF/serum quotient of Ig, the amount of intrathecally synthetized Ig can be detected.[9] The linear IgG index (IgG index $= \frac{Ig\,CSF}{Ig\,Serum} \cdot \frac{Alb\,CSF}{Alb\,Serum}$) is used in veterinary medicine.[10] However, diffusion of proteins into the CSF does not follow a linear model, and this equation may result in up to 50% false-positive interpretations.[11] Hydrodynamic radii and concentration gradients vary between larger Ig molecules and the smaller albumin molecules and therefore result in lower concentration of Ig in the CSF.[2,5,12] Relatively more Ig can pass a severely disturbed blood-CSF barrier compared to albumin.[13,14] Therefore, to describe blood-CSF barrier permeability, a hyperbolic function is superior in comparison to the linear index.[11] Reiber investigated and visualized a hyperbolic CSF/serum quotient diagram in human medicine.[1] He postulated that the upper reference values of the CSF/serum quotient of Ig (QLim), that differentiate between intrathecal synthesis and physiological findings, follow the function $QLim(Ig) = \frac{a}{b}\sqrt{(QAlb)^2 + b^2} - c.$[1] The upper reference value of the CSF/serum quotient of albumin is represented by a vertical line and differentiates between a blood-CSF barrier dysfunction and physiological findings.[1] Both lines transferred into a quotient graph divide the diagram into 4 sections: normal, isolated blood-CSF barrier dysfunction, isolated intrathecal synthesis and combined blood-CSF barrier dysfunction with intrathecal synthesis.[8] The graphical visualization of this function is also called a Reibergram, according to the original description.[8] This hyperbolic function allows differentiation of blood-CSF barrier dysfunction from intrathecal synthesis more reliably than linear methods, helps narrow down differential diagnoses, and provides a better understanding of pathological processes in neurological diseases.[1,15]

Our aim was to develop a Reibergram for dogs after measuring IgA and albumin in CSF and serum and compare it to the IgA index. The hypothesis to be proven was that the Reibergram for dogs could

differentiate intrathecal IgA synthesis from blood-CSF barrier dysfunction in clinical cases. For this purpose, the reference range of the CSF/serum quotient of albumin and the IgA index, as well as a hyperbolic function with coefficients specifically developed for dogs were determined. Our objective was to create a graph that enables a differentiated and simple evaluation of CSF protein, thus providing an additional method in veterinary CSF diagnostic testing in a clinical setting.

2 | MATERIAL AND METHODS

2.1 | Data collection

Our retrospective study included data from dogs presented to the Department for Small Animal Internal Medicine and Surgery, University of Veterinary Medicine Hannover Foundation between 2017 and 2020. All dogs that underwent CSF sampling were identified using the patient administration software "easyVET" (VetZ, Isernhagen, Germany). Patients with neurological diseases were included in the study if data from a complete diagnostic evaluation was available, if dogs were not previously treated with glucocorticosteroids, and if results for albumin, total protein, and IgA concentrations were available in paired suboccipital CSF and serum samples collected at intervals of no more than 4 days apart. All examinations were performed with the owners´ written consent as part of a routine diagnostic evaluation. Clinical examinations were performed by at least 1 resident or diplomate of the European College of Veterinary Neurology. Additionally, results of 6 healthy clinic-owned beagles were included, in which paired suboccipital CSF and blood tests had been performed as part of other studies (animal experiment reference number: 33.8-42502-05-18A290). In total, 167 dogs were included in the study.

Cerebrospinal fluid puncture was performed with the dogs under general anesthesia (premedicated with dexmedetomidine 0.05% [up to 375 µg/m² body surface area IV] or diazepam 0.5% [0.2-0.6 mg/kg IV] or midazolam 0.5% [0.1-0.3 mg/kg IV] and levomethadone 0.25% [0.25-1 mg/kg IV], induction with propofol [2 mg/kg body weight IV] and maintenance by inhalation anesthesia with a gas mixture of isoflurane with air and oxygen) as part of the routine diagnostic evaluation. The IgA concentration was measured by ELISA as previously described.[16,17] Protein and albumin in serum and CSF were analyzed using commercially available photometric assay tests (cobas c311 analyzer, Hitachi, Roche, Mannheim, Germany), adapted for dogs. Intra- and inter-assay variability of albumin and protein measurements were evaluated using 30 random pooled and stocked CSF samples.

TABLE 1 Number of dogs for each of the groups used for calculations in the study

Study population, n = 167	
Cohort with highly probable isolated blood-CSF barrier dysfunction to calculate the hyperbolic curve of the Reibergram	38
Cohort with healthy dogs to calculate the hyperbolic curve of the Reibergram	6
Cohort with highly probable intrathecal IgA synthesis to validate the hyperbolic curve of the Reibergram	10
Cohort with dogs with various neurological diseases that was evaluated with the developed Reibergram	118
Cohorts for the calculation of sensitivity and specificity	
Cohort with diseases with expected intrathecal IgA synthesis (MUO, SRMA)	20
Cohort with diseases without expected intrathecal IgA synthesis (polyradiculoneuritis, IVDH, CNS infarctions, CNS anomalies, intracranial degenerations, IE, idiopathic vestibular syndrome, healthy dogs)	88
Cohort with diseases with expected blood-CSF barrier dysfunction (MUO, SRMA and CNS infarction)	27
Cohort with diseases with expected intact blood-CSF barrier function (IE, healthy dogs)	33

Note: Some dogs are included in several cohorts.
Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; IE, idiopathic epilepsy; IgA, immunoglobulin A; IVDH, intervertebral disc herniation; MUO, meningoencephalitis of unknown origin; n, number of dogs; SRMA, steroid-responsive meningitis-arteritis.

The following variables were recorded for the 167 dogs: breed, age, sex, weight, medical history, time of onset of clinical signs, course of disease, diagnosis, CSF and serum total protein concentrations, CSF and serum albumin concentrations, CSF and serum IgA concentrations, CSF and blood cell count including erythrocytes, leukocytes, and differential cell count. The diagnoses were based on clinical examination, results of blood and CSF tests, results of advanced diagnostic imaging examinations (magnetic resonance tomography [MRI; 3.0 T scanner, Achieva, Philips Medical Systems, Best, The Netherlands], computed tomography [CT; 64-slice multidetector scanner, Brilliance 64, Philips Medical Systems, Cleveland, Ohio, USA]) and histopathological examination or necropsy, if available. Clinical diagnoses were achieved according to the current state of knowledge.[3,6]

The 167 animals were grouped according to their diagnosis into the following categories: meningoencephalitis of unknown origin (MUO), steroid-responsive meningitis-arteritis (SRMA), idiopathic polyradiculoneuritis, acute intervertebral disc herniation (IVDH) and hydrated nucleus pulposus extrusion (HNPE), degenerative CNS disease (including canine cognitive dysfunction, storage diseases), CNS anomaly (including hydrocephalus and Chiari-like malformation), CNS ischemic infarction (including intracranial infarctions and fibrocartilaginous embolic myelopathy [FCE]), metabolic encephalopathy (including intoxication, hypoparathyroidism), intracranial neoplasia (including primary, secondary, and invasive neoplasia), idiopathic epilepsy (IE), idiopathic vestibular syndrome and healthy (Tables 1-3).

Histopathological confirmation of diagnosis was available in 4 dogs (meningioma, bone tumor [chondroma rodens], malignant lymphoma and granulomatous meningoencephalitis).

For all dogs, the IgA CSF/serum quotient ($QIgA = \frac{IgA\ CSF}{IgA\ Serum} \cdot 10^3$), the albumin CSF/serum quotient ($QAlb = \frac{Alb\ CSF}{Alb\ Serum} \cdot 10^3$) and the IgA index ($IgA\ index = \frac{IgA\ CSF}{IgA\ Serum} \cdot \frac{Alb\ Serum}{Alb\ CSF}$), modified as previously described, were calculated.[4,15]

Reference values for the albumin CSF/serum quotient and the IgA index were defined as the central 95% range of observed values from healthy beagle dogs.[18,19]

To discriminate the intrathecally-synthetized IgA fraction from extrathecally-produced IgA that passed the blood-CSF barrier, the hyperbolic function $QLim (IgA) = \frac{a}{b} \sqrt{(QAlb)^2 + b^2} - c$ was used.[1] To determine the coefficients of the function, results of healthy dogs and those with diseases that are accompanied by an isolated blood-CSF barrier dysfunction were used (Table 1; Figure S1).[1] This approach was used because results across the entire spectrum of QAlb (mild to severe cases of isolated blood-CSF barrier dysfunction) are needed to calculate (quantile regression) the curve with a low level of chance for errors.[1] To develop a suitable cohort, the study used the method for which the Reibergram had been originally developed in humans as an orientation.[1] According to the literature, the following diagnoses, partly adapted to dogs, are classified as an isolated blood-CSF barrier dysfunction: tumor of the CNS (excluding malignant lymphoma, leukemia, metastatic carcinoma, meningioma), acute CNS infarctions (excluding systemic autoimmune and infectious disease), acute idiopathic polyradiculoneuritis, degenerative diseases of the CNS, normal pressure hydrocephalus and IVDH.[1,4,20,21] Dogs in which CSF cell count was >3 cells/μL and erythrocytes >500 cells/μL were excluded to avoid erroneous results caused by blood contamination.[15,22,23] Thus, 38 dogs were included with highly probable isolated blood-CSF barrier dysfunction (Table 1; Figure S1 and Table S1).

The canine IgA Reibergram was validated using a cohort of dogs that had a high probability of intrathecal IgA synthesis as described in previous studies.[23] Ten dogs with the inflammatory diseases SRMA or MUO and CSF pleocytosis >3 cells/μL and total CSF protein concentration >25 mg/dL were included in this validation cohort (Table 1; Figure S1 and Table S1).[6,16,17,24]

The resulting quotient graph was used to evaluate data of 118 dogs with various neurological diseases (Tables 2-5).

Sensitivity and specificity of the canine Reibergram to detect diseases with potential intrathecal IgA synthesis and potential blood-CSF barrier dysfunction were calculated and compared to the ability of the IgA index to detect diseases with potential intrathecal IgA synthesis. Meningoencephalitis of unknown origin and SRMA were considered as diseases with expected intrathecal IgA synthesis, whereas polyradiculoneuritis, IVDH, CNS infarctions, intracranial degenerations, IE, idiopathic vestibular syndrome and healthy dogs were not expected to be accompanied by intrathecal IgA synthesis (Table 1).[1,4,9,16,20,25] Diseases with expected blood-CSF barrier dysfunction were MUO, SRMA, and CNS infarction, whereas healthy dogs and those with IE were considered to have an intact blood-CSF barrier (Table 1).[1,9,26]

TABLE 2 Routine cerebrospinal fluid analysis in dogs with various neurological diseases (n = 118) and healthy dogs (n = 6)

Diagnosis	Albumin in CSF (mg/dL) Mean (min-max)	Total protein in CSF (mg/dL) Mean (min-max)	Leukocytes (cells/μL) Mean (min-max)	Erythrocytes (cells/μL) Mean (min-max)	IgA in CSF (mg/L) Mean (min-max)
Healthy	4.83	12.36	0.17	0.78	0.04
n = 6	(2.23-9.1)	(9.74-16.72)	(0-1)	(0-2)	(0.01-0.11)
MUO	66.88	87.96	62.51	514.92	4.3
n = 13	(4.4-463.84)	(12.46-477.19)	(0-533)	(0-5333)	(0.14-29.06)
SRMA	38.37	71.53	210.43	447.43	1.54
n = 7	(10.24-79.49)	(20.77-161.42)	(4-510)	(4-1792)	(0.16-4.66)
Polyradiculoneuritis	12.92	25.71	0.67	140	0.28
n = 5	(3.09-34.49)	(13.31-56.03)	(0-2)	(0-693)	(0.02-0.81)
IVDH	8.94	19.82	3.68	195.12	0.15
n = 27	(2.32-23.38)	(8.94-40.86)	(0-46)	(0-1301)	(0.01-0.42)
Degeneration CNS	7.05	19.48	1.25	757	0.12
n = 4	(3.99-11.51)	(14.38-28.57)	(0-2)	(0-3024)	(0.01-0.28)
Anomaly CNS	11.11	21.06	0.95	53.81	0.38
n = 7	(3.4-18.86)	(14.05-31.34)	(0-2)	(0-272)	(0.04-0.93)
Infarction CNS	15.5	27.54	11.38	5.76	1.46
n = 7	(3.43-29.44)	(12.01-44.43)	(0-53.67)	(0-19)	(0.02-8.34)
Metabolic encephalopathy	12.42	21.6	2	11.61	0.23
n = 6	(2.9-43.64)	(11.12-51.97)	(0-5.67)	(0-40)	(0.01-0.88)
Neoplasia CNS	33.66	49.21	467.57	44.33	1.09
n = 10	(2.46-210.57)	(10.3-277.92)	(0-4667)	(0-421)	(0.03-8.54)
IE	5.54	13.84	0.85	133.81	0.24
n = 27	(2.13-21.36)	(8.7-26.51)	(0-4.33)	(0-1005)	(0.01-3.44)
IVS	8.42	22.39	0.07	2.73	0.09
n = 5	(3.13-20.05)	(12.62-33.42)	(0-0.33)	(1-10)	(0.04-0.18)

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; IE, idiopathic epilepsy; IgA, immunoglobulin A; IVDH, intervertebral disc herniation; IVS, idiopathic vestibular syndrome; MUO, meningoencephalitis of unknown origin; SRMA, steroid-responsive meningitis-arteritis.

2.2 | Statistical analysis

Descriptive statistics with frequencies of breed, sex, age, weight and diagnoses were calculated using SAS Enterprise Guide 7.1 and SAS software 9.4 (SAS Institute Inc., Cary, North Carolina, USA). This software also was used for the determination of 95% reference intervals after confirming normal distribution using the Kolmogorov-Smirnov test and Shapiro-Wilk test with visualization by Q-Q-plots.

The analysis of the 38 dogs with highly probable isolated blood-CSF barrier dysfunction and 6 healthy dogs to determine the coefficients of the hyperbolic function $QLim(IgA) = \frac{a}{b}\sqrt{(QAlb)^2 + b^2} - c$ was performed using the programming language R, 64-bit version 4.0.3, (R Core Team [2020]. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria) including the R package "readxl" (Hadley Wickham and Jennifer Bryan [2019]. readxl: Read Excel Files. R package version 1.3.1.). The coefficients for the 98% quantile of the hyperbolic function (QLim) were calculated by means of quantile regression using the

values of CSF/serum quotient of albumin and IgA.[1] QLim (IgA) and the upper physiological reference value of the albumin CSF/serum quotient were graphically visualized on a logarithmic scale as a Reibergram (Figure 1). The graphing and quantile regression were rendered using a self-written R code. The application of the scripts and the evaluation of the data were carried out using the integrated development environment and graphical user interface RStudio (R Studio In., Boston, Massachusetts, USA).

The CSF/serum quotients of albumin and IgA of the 167 dogs were plotted in the canine IgA Reibergram by means of the program R. The appearance in the 4 different areas of the Reibergram (reference range, intrathecal IgA synthesis, blood-CSF barrier dysfunction or combined intrathecal IgA synthesis and blood-CSF barrier dysfunction) and CSF results then were analyzed using frequencies in Microsoft Excel 2016 (Microsoft Corporation, Redmond, Washington, USA).

As part of the quality assurance for the albumin and total protein results in the CSF, the coefficient of variation (CV) was calculated using Microsoft Excel: $CV = \text{standard deviation} \cdot \frac{100}{\text{mean}}$.

TABLE 3 Cerebrospinal fluid/serum-quotients in dogs with various neurological diseases (n = 118) and healthy dogs (n = 6)

Diagnosis	QAlb Mean (min-max)	QIgA Mean (min-max)	IgA index Mean (min-max)
Healthy	1.28	0.34	0.31
n = 6	(0.69-2.22)	(0.2-0.59)	(0.12-0.56)
MUO	18.25	24.81	3.06
n = 13	(1.38-125.02)	(1.05-103.5)	(0.37-11.47)
SRMA	12.58	9.9	0.76
n = 7	(2.92-28.19)	(1.55-35.98)	(0.18-2.55)
Polyradiculoneuritis	3.54	1.82	0.37
n = 5	(0.87-9.32)	(0.34-7.3)	(0.13-0.78)
IVDH	2.38	0.93	0.53
n = 27	(0.63-6.66)	(0.1-3.12)	(0.03-2.29)
Degeneration CNS	2.18	0.43	0.22
n = 4	(1.21-3.47)	(0.16-0.71)	(0.1-0.42)
Anomaly CNS	3.17	1.86	0.66
n = 7	(1.12-5.6)	(0.59-5.05)	(0.3-1.9)
Infarction CNS	4.57	6.71	0.91
n = 7	(1.09-8.41)	(0.15-40.6)	(0.11-4.83)
Metabolic encephalopathy	3.56	1.78	0.91
n = 6	(0.98-12.43)	(0.26-5.33)	(0.07-4.29)
Neoplasia CNS	9.29	10.51	0.68
n = 10	(0.66-53.31)	(0.25-89.16)	(0.16-1.96)
IE	1.56	1.61	1.53
n = 27	(0.55-5.85)	(0.06-28.44)	(0.04-29.54)
IVS	2.26	0.33	0.2
n = 5	(0.74-5.55)	(0.15-0.53)	(0.09-0.37)

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; IE, idiopathic epilepsy; IgA, immunoglobulin A; IVDH, intervertebral disc herniation; IVS, idiopathic vestibular syndrome; MUO, meningoencephalitis of unknown origin; QAlb, CSF/serum quotient of albumin; QIgA, CSF/serum quotient of IgA; SRMA, steroid-responsive meningitis-arteritis.

TABLE 4 Cerebrospinal fluid results in relation to their results in the canine IgA Reibergram in dogs with various neurological diseases (n = 118)

	Area of Reibergram			
	1	2	3	4
	Normal	Isolated barrier dysfunction	Combined barrier dysfunction and intrathecal IgA synthesis	Isolated intrathecal IgA synthesis
Routine CSF findings	(n)	(n)	(n)	(n)
Normal CSF protein (<25 mg/dL) with …	54	16	6	6
…normal cell count	52	13	5	6
…increased cell count	2	3	1	0
Increased CSF protein (>25 mg/dL) with …	1	15	20	0
…normal cell count	1	12	3	0
…increased cell count	0	3	17	0

Note: Normal cell count ≤3 leukocytes/μL; increased cell count >3 leukocytes/μL.
Abbreviations: CSF, cerebrospinal fluid; IgA, immunoglobulin A; n, number.

Statistical analysis of difference in means was performed using analysis of variance with Kruskal Wallis test using GraphPad Prism 5 (GraphPad software Inc., San Diego, California, USA). $P < .005$ was considered significant.

TABLE 5 Blood contamination in relation to their results in the canine IgA Reibergram in dogs with various neurological diseases (n = 118)

	Area of Reibergram				
	1	2	3 Combined barrier dysfunction and intrathecal IgA synthesis	4 Isolated intrathecal IgA synthesis	
	Normal	Isolated barrier dysfunction			Total
Erythrocytes in CSF Mean (min-max) [cells/μL]	87 (0-1005)	169 (0-3024)	440 (0-5333)	319 (0-1253)	198 (0-5333)
Number of CSF with … [n]					
…no blood contamination	21 (38%)	10 (32%)	5 (16%)	1 (17%)	37
…mild blood contamination (1-50 erythrocytes/μL)	26 (47%)	15 (48%)	12 (46%)	3 (50%)	56
…medium blood contamination (51-1000 erythrocytes/μL)	7 (13%)	4 (13%)	6 (23%)	1 (17%)	18
…marked blood contamination (>1000 erythrocytes/μL)	1 (2%)	2 (6%)	3 (12%)	1 (17%)	7
All dogs [n]	55 (100%)	31 (100%)	26 (100%)	6 (100%)	118

Abbreviations: CSF, cerebrospinal fluid; IgA, immunoglobulin A; n, number of dogs.

Sensitivity and specificity were assessed by calculating the mean of a 2 × 2 contingency table, with $sensitivity = \frac{true\ positive}{(true\ positive + false\ negative)}$ and $specificity = \frac{true\ negative}{(true\ negative + false\ positive)}$.[27]

3 | RESULTS

After the database search, 167 dogs could be included in the study, of which 6 dogs were in the healthy cohort; 38 dogs with highly probable isolated blood-CSF barrier dysfunction were in the cohort to calculate the hyperbolic curve of the Reibergram; 10 dogs were in the validation cohort with highly probable intrathecal IgA synthesis, which was used to validate the developed hyperbolic function; and, 118 dogs with various neurological diseases were in the cohort that was evaluated using the Reibergram (Tables 1-5).

The cohort of healthy beagle dogs was on average 35 months (range, 23-65 months) old and had a mean body weight of 13 kg (range, 10.1-14.6 kg).

The cohort with highly probable isolated blood-CSF barrier dysfunction, which was used to develop the hyperbolic function, comprised the following breeds: n = 10/38 mixed-breed dogs, n = 7/38 Dachshunds, n = 2/38 each Boxer and Hanover Hounds, and n = 1/38 each German Shepherd, Beagle, Labrador Retriever, Chow Chow, Pug, Bolonka, Chihuahua, Fox Terrier, Miniature Schnauzer, French Bulldog, Poodle, German Spaniel, Rottweiler, Australian Shepherd, Yorkshire Terrier, Irish Wolfhound, and Jack Russell Terrier. The following diagnoses were included: n = 20/38 IVDH, n = 5/38 intracranial neoplasia, n = 5/38 CNS anomalies, n = 3/38 CNS degenerations, n = 3/38 CNS infarctions, and n = 2/38 polyradiculoneuritis. Mean age of the dogs was 90 months (range, 7-191 months) with a mean body weight of 18 kg (range, 2.8-74 kg).

The cohort with highly probable intrathecal synthesis of IgA, which was used to validate the developed hyperbolic function, comprised
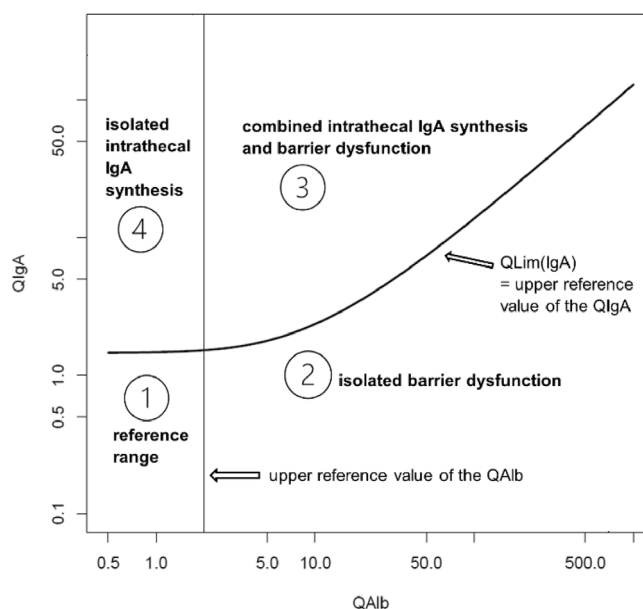
FIGURE 1 The Reibergram. Cerebrospinal fluid (CSF)/serum quotient graph for CSF/serum quotient of albumin (QAlb) and immunoglobulin A (QIgA) on a logarithmic scale, modified according to Reiber et al.[1] Thick line: upper reference values of the CSF/serum quotient of IgA (QIgA) = hyperbolic curve = QLim (IgA) = 0.13 $\sqrt{(QAlb)^2 + 11.9 \cdot 10^{-6}} - 1.01 \cdot 10^{-3}$. Vertical line: upper reference value of the CSF/serum quotient of albumin (QAlb). Area 1 = intact blood-cerebrospinal fluid barrier without intrathecal IgA synthesis; Area 2 = isolated blood-cerebrospinal fluid barrier dysfunction; Area 3: combined blood-cerebrospinal fluid barrier dysfunction with intrathecal IgA synthesis; Area 4: isolated intrathecal IgA synthesis

dogs of the following breeds: n = 5/10 mixed-breed dogs and n = 1/10 each Boxer, Chihuahua, Yorkshire Terrier, Havanese and Labrador Retriever. The following diseases were included: 7/10 with MUO

and 3/10 with SRMA. The mean age was 74 months (range, 10-138 months) and body weight was 17 kg (range, 2.3-34.4 kg).

The cohort used for evaluation of the Reibergram comprised dogs of the following breeds: n = 28/118 mixed-breed dogs, n = 9/118 Dachshunds, n = 5/118 each Labrador Retriever, Jack Russel Terrier, Australian Shepherd, Chihuahua, n = 4/118 each Chihuahua and Boxer, n = 3/118 each Miniature Schnauzer, Yorkshire Terrier and French Bulldog, and n = 44/118 other breeds. They included 11 diagnostic groups (see Tables 2 and 3). A group of healthy dogs also was evaluated. For each of the diagnostic groups and the healthy dogs, the results of the amount of albumin, total protein, leukocytes, erythrocytes and IgA in the CSF and the calculated results of the CSF/serum quotient of albumin, the CSF/serum quotient of IgA and the IgA index can be found in Tables 2 and 3. Inflammatory diseases mostly showed a combined increased cell count and total protein concentration in the CSF (MUO n = 8/13 and SRMA n = 5/7). The 2 remaining animals with SRMA had an isolated increased cell count. A combined increased cell count and total protein concentration in the CSF was found in 2/5 dogs with intracranial infarctions, 2/10 dogs with CNS neoplasia (lymphoma and meningioma) and 3/27 dogs with IVDH (n = 2 HNPE and n = 1 acute intervertebral disc extrusion). An albuminocytologic dissociation was seen in 2/5 dogs with polyradiculoneuritis, 2/5 dogs with idiopathic vestibular syndrome, 2/7 dogs with CNS anomaly, 2/7 dogs with CNS infarctions (n = 1 for intracranial infarction and FCE), 2/10 dogs with CNS neoplasia (n = 1 for bone tumor and meningioma), 1/4 dogs with CNS degeneration, and 3/27 dogs with IVDH. For an overview of the CSF results in the 118 dogs with various neurological diseases in relation to their results in the canine IgA Reibergram, see Tables 4 and 5.

3.1 | Development and validation of the canine IgA Reibergram and calculation of reference values

The intra-assay CV was 1.55% for albumin and 1.05% for total protein. For the inter-assay CV, the results were 0.83% for albumin and 1.92% for total protein. Hence, the reproducibility of the albumin and protein measurements was excellent.

The albumin CSF/serum quotient in healthy dogs had a 95% interval (reference range) of 0.68 to 2.22, with a mean value of 1.28. Therefore, the vertical line in the canine Reibergram was drawn at 2.2 and represents the upper reference value of the albumin CSF/serum quotient.

The IgA index in healthy dogs had a 95% interval (reference range) from 0.12 to 0.56, with a mean value of 0.31.

The quantile regression in R yielded the following canine coefficients for $QLim\,(IgA)$: $a = 0.4398$, $b = 3.4549$, $c = 1.0134$. Thus, the following hyperbolic function was determined: $QLim\,(IgA) = 0.13\ \sqrt{(QAlb)^2 + 11.9 * 10^{-6}} - 1.01 * 10^{-3}$.

3.2 | Validation of the Reibergram

Data of dogs with highly expected intrathecal IgA synthesis and blood-CSF barrier dysfunction (i.e., dogs with MUO and SRMA with
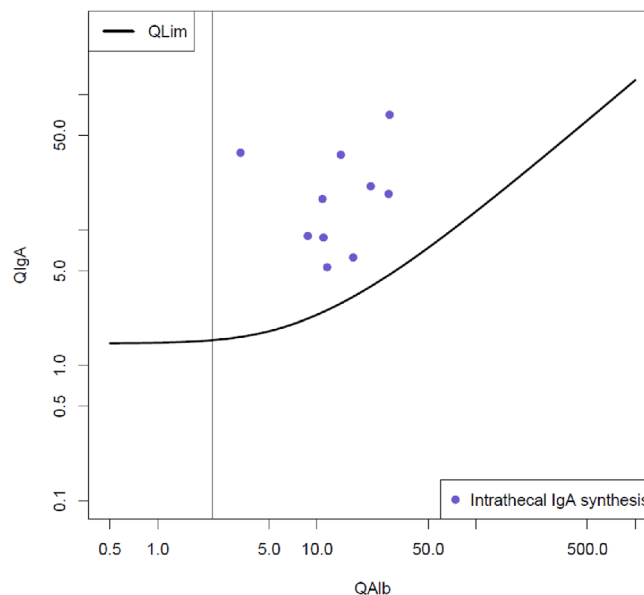

FIGURE 2 Validation of the canine Reibergram. Canine Reibergram for cerebrospinal fluid (CSF)/serum quotient of albumin (QAlb) and immunoglobulin A (QIgA) on a logarithmic scale. Thick line: upper reference values of the CSF/serum quotient of IgA (QIgA) = hyperbolic curve = $QLim\,(IgA) = 0.13\ \sqrt{(QAlb)^2 + 11.9 \cdot 10^{-6}} - 1.01 \cdot 10^{-3}$. Vertical line: upper reference value of the CSF/serum quotient of albumin (QAlb). The dots correspond to dogs of the validation cohort with expected intrathecal IgA synthesis (meningoencephalitis of unknown origin or steroid-responsive meningitis-arteritis and CSF pleocytosis). N = 10/10 are located in the area of combined intrathecal IgA synthesis and blood-cerebrospinal fluid barrier dysfunction

CSF pleocytosis) in the validation cohort all were located in the area of combined barrier dysfunction with intrathecal IgA synthesis in the Reibergram (Figure 2).

3.3 | Evaluation of the canine IgA-Reibergram

Dogs with inflammatory diseases (MUO n = 10/13 and SRMA n = 6/7), with lymphoma (n = 1/1) and meningioma (n = 2/3) were mostly situated in the area of a combined blood-CSF barrier dysfunction and intrathecal IgA synthesis (Figure 3). The majority of dogs with polyradiculoneuritis, IVDH, infarction in the CNS, metabolic encephalopathy, degeneration in the CNS, anomaly in the CNS and idiopathic vestibular syndrome were found in the reference range (n = 29/61) or in the area of an isolated blood-CSF barrier dysfunction (n = 22/61). Dogs with IE (n = 22/27) and gliomas (n = 3/4) were detected mostly in the region for normal values (Figure 3).

Of the 118 dogs of the cohort with various neurological diseases, 16 had albuminocytologic dissociation (≤3 cells/μL, >25 mg/dL protein) in routine CSF diagnostic testing (Table 4). Twelve of these 16 dogs had isolated blood-CSF barrier dysfunction based on the Reibergram (n = 3 IVDH; n = 2 CNS neoplasia, and n = 1 each polyradiculoneuritis, CNS anomaly, CNS degeneration, idiopathic vestibular
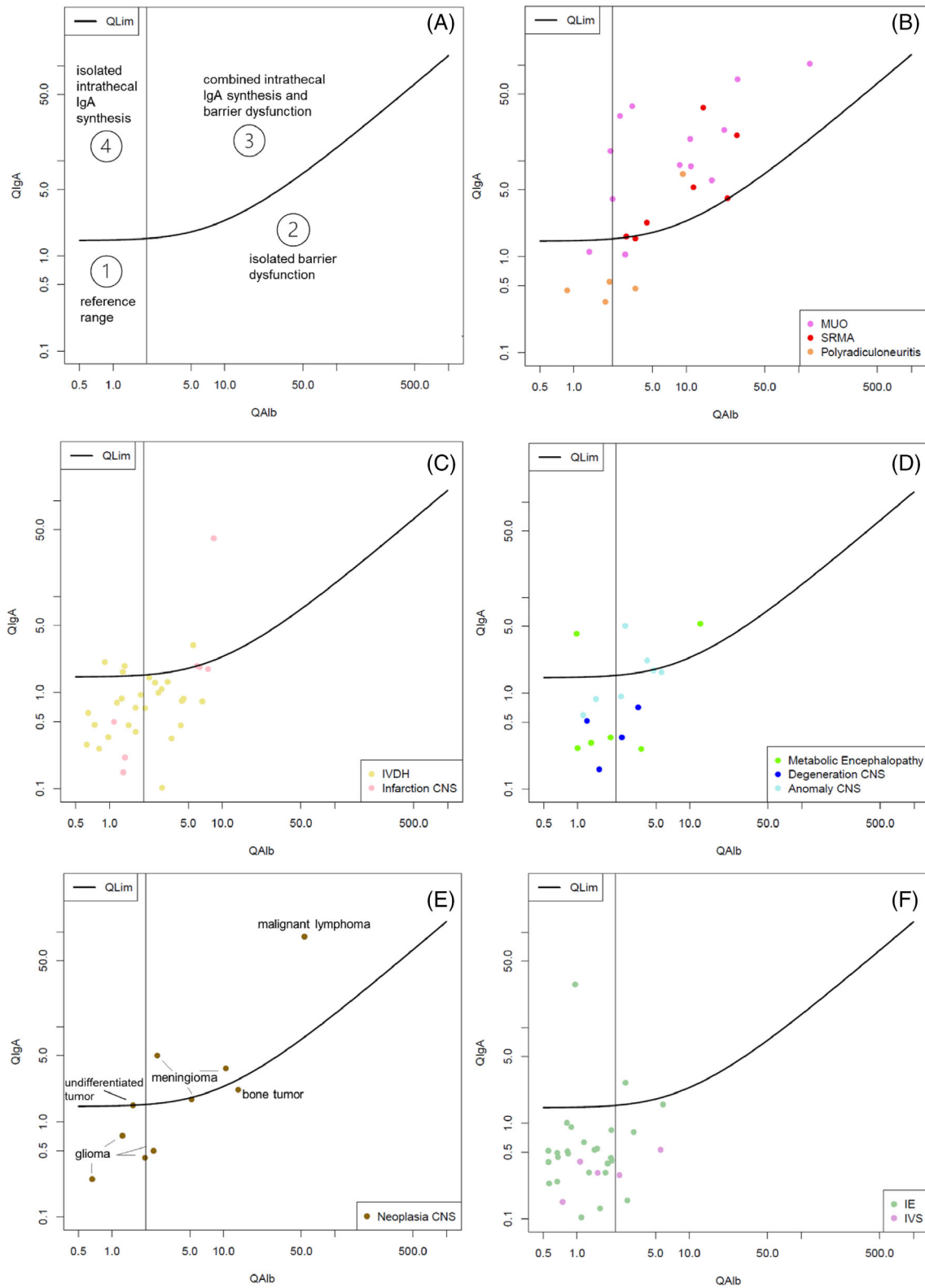
FIGURE 3 Legend on next page.

syndrome, IE, FCE, and intracranial infarction). Three of 16 dogs with albuminocytologic dissociation showed combined barrier dysfunction and intrathecal IgA synthesis (CNS anomaly, polyradiculoneuritis and metabolic encephalopathy) whereas 1 dog was in the reference range (idiopathic vestibular syndrome).

All dogs (n = 6/118) that were within the area of isolated intrathecal IgA synthesis had normal CSF protein concentration and cell count (n = 3 IVDH, n = 1 IE, n = 1 MUO, n = 1 metabolic encephalopathy).

Blood contamination was present in 81/118 CSF samples: 56/118 CSF samples contained 1-50 erythrocytes/μL (mild blood

contamination); 18/118 CSF samples contained 51-500 erythrocytes/μL (moderate blood contamination); 7/118CSF samples contained 501-5333 erythrocytes/μL (marked blood contamination). No significant difference was found in the number of CSF erythrocytes between dogs in different Reibergram areas ($P = .09$; Table 5).

3.4 | Sensitivity and specificity of the canine IgA Reibergram and IgA index

Of all dogs in the study, 20 dogs had diseases with expected intrathecal IgA synthesis (n = 13 MUO, n = 7 SRMA). In the Reibergram, 17/20 dogs were above the hyperbolic curve and therefore in the area of intrathecal IgA synthesis. The canine IgA index was >0.56 in 14/20 dogs indicating intrathecal IgA synthesis.

Of all dogs in the study, 88 dogs had diseases without expected intrathecal IgA synthesis (n = 27 IVDH, n = 7 infarction CNS, n = 7 anomaly, n = 5 polyradiculoneuritis CNS, n = 4 intracranial degeneration, n = 27 IE, n = 5 idiopathic vestibular syndrome, n = 6 healthy). In the Reibergram, 78/88 dogs were below the hyperbolic curve and therefore in the area without intrathecal IgA synthesis. The canine IgA index was <0.56 in 65/88 dogs, indicating no intrathecal IgA synthesis.

Of all dogs in the study, 27 had diseases with expected blood-CSF barrier dysfunction (n = 13 MUO, n = 7 SRMA, n = 7 infarction CNS). In the Reibergram, the albumin CSF/serum quotient of 22/27 dogs was >2.2 indicating blood-CSF barrier dysfunction.

Of all dogs in the study, 33 had diseases with expected intact blood-CSF barrier function (n = 27 IE, n = 6 healthy). In the Reibergram, the albumin CSF/serum quotient of 29/33 dogs was <2.2 indicating intact blood-CSF barrier function.

The canine Reibergram detected diseases with expected intrathecal IgA synthesis with a sensitivity of 85% and a specificity of 89%, whereas the IgA index detected these with a sensitivity of 70% and a specificity of 74%. The canine Reibergram could detect diseases with expected blood-CSF barrier dysfunction with a sensitivity of 81% and a specificity 88%.

4 | DISCUSSION

A Reibergram is a graph of double logarithmic scale that relates the quotient of albumin in CSF and serum to the quotient of an Ig

in CSF and serum.[1,8] It includes a hyperbolic curve that can detect intrathecal Ig synthesis.[1] This curve is met by a vertical line at the upper physiological reference value of the albumin quotient. Together, both lines form 4 sections which indicate different conditions of the blood-CSF barrier or intrathecal inflammatory reaction.[8]

In our study, a Reibergram for IgA in dogs including the function $\mathrm{QLim}(\mathrm{IgA}) = 0.13 \sqrt{(\mathrm{QAlb})^2 + 11.9 * 10^{-6}} - 1.01 * 10^{-3}$ was developed resulting in a hyperbolic curve and the upper physiological reference value of the albumin quotient of 2.2. This Reibergram could detect diseases with potential intrathecal IgA synthesis more reliably than the IgA index and can be recommended for diagnostic purposes. The Ig index was used in veterinary and human medicine to detect intrathecal Ig synthesis but might render up to 50% false-positive results.[4,11,28] Immunoglobulin A measurement is an important diagnostic marker in veterinary medicine for diseases such as SRMA and consequently is available in standard examinations.[16] Immunoglobulin A, as a dimer, has a higher molecular weight than a monomer (e.g., IgG) and is physiologically present in lower concentrations in the CSF.[2,29] Thus, in the case of impaired barrier function, relatively more proteins may cross over into the CSF or even CNS tissue after the stronger concentration gradient, especially in a severely damaged blood-CSF barrier.[21] Moreover, the Reibergram contains information about potential blood-CSF barrier dysfunction because the upper physiological reference value of the albumin quotient is available and can be evaluated at the same time point.

Although in human medicine lumbar CSF is used for the Reibergram, the developed canine IgA Reibergram is only applicable for sub-occipitally collected CSF samples.[1] Dogs have a higher variability in dorsal length ("flow distance") than humans. Composition of CSF proteins changes because of the rostro-caudal flow, causing a higher variability in the results of CSF analysis in dogs of different size and back length.[6,15,30] However, in some diseases localized at the caudal spinal cord level, pathological CSF changes might only be visible in lumbar samples and might therefore be overlooked in the present Reibergram.[31]

In addition, the present IgA Reibergram is only applicable for adult dogs. The function was not developed for different age groups. Up until now, no age-dependent reference values for QAlb exist in dogs. It is known from human medicine that children have significantly higher albumin quotients in the first months of life which then decrease and finally slowly increase again with age.[10,32]

FIGURE 3 Different neurological diagnostic groups in the canine IgA Reibergram. Canine Reibergram for cerebrospinal fluid (CSF)/serum quotient of albumin (QAlb) and immunoglobulin A (QIgA) on a logarithmic scale. Thick line: upper reference values of the CSF/serum quotient of IgA (QIgA) = hyperbolic curve = $\mathrm{QLim}(\mathrm{IgA}) = 0.13 \sqrt{(\mathrm{QAlb})^2 + 11.9 \cdot 10^{-6}} - 1.01 \cdot 10^{-3}$. Vertical line: upper reference value of the CSF/serum quotient of albumin (QAlb). Diagnostic areas in the Reibergram (A). Area 1 = intact blood-cerebrospinal fluid barrier without intrathecal IgA synthesis; Area 2 = isolated blood-cerebrospinal fluid barrier dysfunction; Area 3: combined blood-cerebrospinal fluid barrier dysfunction with intrathecal IgA synthesis; Area 4: isolated intrathecal IgA synthesis. Different diseases in the canine Reibergram (B-F): The dots correspond to dogs with different neurological diseases. MUO = meningoencephalitis of unknown origin, n = 13; SRMA = steroid-responsive meningitis-arteritis, n = 7; IVDH = intervertebral disc herniation, n = 27; CNS = central nervous system; IE = idiopathic epilepsy, n = 27; IVS = idiopathic vestibular syndrome, n = 5; polyradiculoneuritis, n = 5; infarction CNS, n = 7; metabolic encephalopathy, n = 6; degeneration CNS, n = 4; anomaly CNS, n = 7; neoplasia CNS, n = 10

Some of the dogs in our study had macroscopically visible blood contamination in CSF or had an increased erythrocyte count in CSF. Moderate blood contamination of a CSF sample does not seem to have substantial effects on the leukocyte count.[24] However, blood contamination potentially could lead to increased protein and Ig concentrations, because these molecules could increasingly diffuse from blood to CSF, indicating false positive intrathecal Ig synthesis or barrier dysfunction.[22,33] To avoid this potential effect, dogs with increased erythrocyte counts in CSF were excluded from the cohort used to develop the Reibergram. In the later evaluation of the canine Reibergram, animals with high erythrocyte counts of up to 5333 erythrocytes/μL also were included to reflect the heterogeneity of CSF samples obtained in a clinical setting. No significant difference in erythrocyte count was found between dogs in each area of the Reibergram. It seems that the developed canine Reibergram is suitable to minimize the impact of blood contamination on CSF examination results to some extent.

To calculate the hyperbolic curve of the present Reibergram a cohort of healthy dogs and dogs with an expected isolated blood-CSF barrier dysfunction according to the literature were used.[1,4,20] Other cohorts for the calculation of the hyperbolic curve gave less accurate results.[34] To validate the calculated hyperbolic curve, a cohort of dogs with MUO and SRMA with highly likely intrathecal Ig synthesis was included.[4,16,17] These dogs of the validation cohort were selected by their diagnosis and by routine CSF results (increased leukocyte count or total protein concentration). No direct measurement of blood-CSF barrier-function or intrathecal IgA synthesis was performed in any of the cohorts. This approach might have caused inaccuracy in the calculated Reibergram. Nevertheless, this approach already has been successfully used by other working groups in human medicine to calculate a Reibergram for different Igs.[1] In our study, this approach seems superior to conventional mathematical models (e.g., IgA index) to describe the processes at the blood-CSF barrier. Although the results of our study are promising, the function QLim should be calibrated using a larger sample size of patients.

To test the suitability of using the developed Reibergram in a clinical setting, the CSF/serum quotients of albumin and IgA of 118 dogs were plotted in the hyperbolic curve. Most of them were found in the expected area of the Reibergram based on the known pathogenesis of their disease.

The MUO and SRMA patients were in the area of combined intrathecal IgA synthesis and barrier dysfunction (n = 16/20). In MUO, the involvement of complex mechanisms that can trigger an excessive self-reinforcing autoimmune response (IgA synthesis) involving T cells and microglia with ensuing damage to the blood-CSF barrier could be an explanation.[35] In SRMA, an upregulation of metalloproteases can lead to a barrier dysfunction and enhance the neutrophilic pleocytosis.[36,37] Combined intrathecal IgA synthesis and barrier dysfunction in MUO and SRMA patients has been described previously.[6,17]

Dogs with meningioma and malignant lymphoma also showed a combined intrathecal IgA synthesis and barrier dysfunction in the canine IgA Reibergram. Studies in human medicine have shown that upregulation of metalloproteases occurs in tumors.[38] This upregulation

can lead to barrier dysfunction by release of growth factors and subsequent angiogenesis, remodeling of the extracellular matrix of blood vessels, and splitting of the basal lamina.[38] Secondary infiltration of plasma cells and lymphocytes or strong intrathecal proliferation of lymphocytes could be responsible for the IgG synthesis in meningiomas, which could be extrapolated to IgA.[4,16] In malignant lymphomas, the intrathecal IgA could be produced by the tumor itself or in the course of inflammation by migrating plasma cells or by both mechanisms.[29,39] The barrier dysfunction in this case also can be explained by neovascularization with permeable vessels and infiltrative growth.[40]

Acute polyradiculoneuritis in humans and dogs frequently is observed with strong albuminocytologic dissociation, especially in lumbar CSF.[41-43] Suboccipital CSF results of the dogs in our study were mostly in the normal range or a mild isolated barrier dysfunction was found.

Intracranial degenerations (in our study canine cognitive dysfunction syndrome or suspected congenital storage disease) showed no intrathecal IgA synthesis but an isolated barrier dysfunction or normal results as expected. Dogs with IVDH, CNS infarctions, and intracranial anomalies showed similar results with rare examples of intrathecal IgA synthesis. This finding is consistent with earlier studies of IgG.[4,44] Traumatic injuries of the spinal cord may induce a local immune response with infiltration of inflammatory cells, which could explain an increased QIgA.[45] The MRI of 1 dog with an intracranial infarction that had a markedly increased QIgA and moderate barrier dysfunction showed high-grade multiple intracranial lesions that indicated severe ischemic infarctions. The result is ongoing cell necrosis, which in turn stimulates inflammatory cells to produce reactive oxygen species and cytokines, resulting in leukocyte infiltration.[46] Additionally, chronicity of clinical signs might be taken into account as plasma cells might start producing antibodies with a delay of 2 weeks.[9]

The animals with metabolic encephalopathies (mostly suspected intoxication and 1 dog with hypoparathyroidism) showed reactive seizures and are heterogeneously spread over the areas of the canine IgA Reibergram. This obervation is consistent with previous studies in human medicine because the blood-CSF barrier may or may not be damaged depending on the toxin and dosage, and intrathecal Ig synthesis can occur.[47-51]

Idiopathic vestibular syndrome showed mild barrier dysfunction in addition to normal findings. The reason for increased permeability of the blood-CSF barrier is not known.[52] Because of the presumed inflammatory origin and occasional albuminocytologic dissociation, a comparison with inflammatory demyelinating polyneuropathies is discussed, which in turn often is accompanied by barrier dysfunction.[53,54] Recent studies found a suppression in the fluid-attenuated inversion recovery sequence on the affected side in patients with idiopathic vestibular syndrome which could be caused by an alteration in the composition of the endolymph after dysfunction of the blood-labyrinthine barrier.[55] This possibility could be consistent with our findings.

Dogs with gliomas and IE were mostly located in the reference range of the canine IgA Reibergram, which is consistent with previous studies.[9,26,56] Contrary to humans, there seems to be no correlation

between cluster seizures and the rarely occurring mild and transient aberrant CSF findings.[56-60] The pathophysiology of deviant CSF findings is still unclear, but IE may have an inflammatory component in individual cases.[56,61]

In conclusion, most of the results of the Reibergram for each of the diagnostic groups were as expected. However, in some cases the findings deviated. For example, in 2 dogs with MUO no intrathecal IgA synthesis was detected in our study and total protein concentrations and leukocyte counts were in the normal range. Both dogs were diagnosed in the acute phase of the disease. Delayed antibody production by plasma cells could be a reason for the QIgA in the reference range.[9] Although most results of dogs with a CNS anomaly concurred with isolated barrier dysfunction, similar to people with hydrocephalus,[20] 1 dog in our study showed clear intrathecal IgA synthesis and barrier dysfunction. However, this dog also showed severe signs of increased intracranial pressure on the MRI. Increased intracranial pressure can decrease perfusion via compression of blood vessels in the brain parenchyma, leading to cell damage, ischemia, and secondary intrathecal IgA synthesis.[51]

The Reibergram indicated abnormal isolated intrathecal IgA synthesis in 6 dogs, a combined intrathecal IgA synthesis and barrier dysfunction in 5 dogs and an isolated barrier dysfunction in 13 dogs where routine CSF examination of total protein concentration and leukocyte count was unremarkable. Also, in dogs with albuminocytologic dissociation, the Reibergram distinguished between an isolated blood-CSF barrier dysfunction, a combined dysfunction with an intrathecal IgA synthesis and physiological findings, which is very valuable in establishing a clinical diagnosis.

The developed canine IgA Reibergram showed increased sensitivity in comparison to the IgA index and increased specificity. Hence, the hyperbolic approach seems to be superior to the linear approach, because of the nonlinear passage of proteins across the blood-CSF barrier as discussed above.

Overall, the combined evaluation of the developed canine IgA Reibergram allows informed conclusions about processes in the CSF and the blood-CSF barrier, making it useful for the differential diagnosis of neurological diseases. In some cases, the canine Reibergram could detect a blood-CSF barrier dysfunction or intrathecal IgA synthesis or both when the other CSF results were normal. The graphical presentation simplifies the evaluation and interpretation of the data and makes it a feasible tool in routine CSF analysis in dogs.

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CONFLICT OF INTEREST DECLARATION

Andrea Tipold serves as Associate Editor for the Journal of Veterinary Internal Medicine. She was not involved in review of this manuscript. No other authors have conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

The authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The authors declare no IACUC approval was needed. The animal experiment reference number for the CSF and blood tests of the six healthy clinic-owned beagles performed in other studies is 33.8-42502-05-18A290. Archived samples and medical information about the animals were used with written owners' consent and in accordance with the university's ethical guidelines.

HUMAN ETHICS APPROVAL DECLARATION

The authors declare human ethics approval was not needed for this study.

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REFERENCES

1. Reiber H, Felgenhauer K. Protein transfer at the blood cerebrospinal fluid barrier and the quantitation of the humoral immune response within the central nervous system. *Clin Chim Acta*. 1987;163:319-328.
2. Di Terlizzi R, Platt SR. The function, composition and analysis of cerebrospinal fluid in companion animals: part II–analysis. *Vet J*. 2009;180(1):15-32.
3. De Lahunta A, Glass EN, Kent M. *Veterinary Neuroanatomy and Clinical Neurology*. 4th ed. Amsterdam: Elsevier; 2014:600.
4. Tipold A, Pfister H, Vandevelde M. Determination of the IgG index for the detection of intrathecal immunoglobulin synthesis in dogs using an ELISA. *Res Vet Sci*. 1993;54:40-44.
5. Reiber H. The discrimination between different blood-CSF barrier dysfunctions and inflammatory reactions of the CNS by a recent evaluation graph for the protein profile of cerebrospinal fluid. *J Neurol*. 1980;224:89-99.
6. Platt SR, Olby NJ. *BSAVA Manual of Canine and Feline Neurology*. 4th ed. Gloucester: British Small Animal Veterinary Association; 2014:542.
7. Sorjonen DC. Total protein, albumin quota, and electrophoretic patterns in cerebrospinal fluid of dogs with central nervous system disorders. *Am J Vet Res*. 1987;48:301-305.
8. Reiber H. Dynamics of brain-derived proteins in cerebrospinal fluid. *Clin Chim Acta*. 2001;310:173-186.
9. Schindler T. Untersuchungen zur differenzialdiagnostischen Bedeutung des IgG-Index und Albuminquotienten bei neurologisch erkrankten Hunden. [Doctoral dissertation], Lehrstuhl für Innere Krankheiten, Dermatologie und Neurologie der kleinen Haustiere sowie für klinische Labordiagnostik der Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München. München; 2003.
10. Tibbling G, Link H, Öhman S. Principles of albumin and IgG analyses in neurological disorders. I. Establishment of reference values. *Scand J Clin Lab Invest*. 1977;37:385-390.
11. Reiber H, Peter JB. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. *J Neurol Sci*. 2001;184:101-122.
12. Felgenhauer K, Schliep G, Rapic N. Evaluation of the blood-CSF barrier by protein gradients and the humoral immune response within the central nervous system. *J Neurol Sci*. 1976;30:113-128.

13. Felgenhauer K. Protein filtration and secretion at human body fluid barriers. *Pflugers Arch*. 1980;384:9-17.

14. Reiber H, Uhr M. Liquordiagnostik. *Klinische Neurologie*. 3rd ed. Heidelberg: Springer; 2011:143-178.

15. Eckhoff A, Reiber H, Kirbach H, et al. Cerebrospinal fluid analysis in Equine Borna Disease - a new diagnostic approach to measure intrathecal immune response. *Wien Tierarztl Monatsschr*. 2006;93: 235-243.

16. Maiolini A, Carlson R, Schwartz M, Gandini G, Tipold A. Determination of immunoglobulin a concentrations in the serum and cerebrospinal fluid of dogs: an estimation of its diagnostic value in canine steroid-responsive meningitis-arteritis. *Vet J*. 2012;191: 219-224.

17. Tipold A, Pfister H, Zurbriggen A, Vandevelde M. Intrathecal synthesis of major immunoglobulin classes in inflammatory diseases of the canine CNS. *Vet Immunol Immunopathol*. 1994;42:149-159.

18. Horn PS, Pesce AJ. Reference intervals: an update. *Clin Chim Acta*. 2003;334:5-23.

19. Lucke S. Methoden zur Bestimmung von medizinischen Referenzbereichen für labordiagnostische Parameter. [Doctoral dissertation], Universitätsmedizin der Ernst-Moritz-Arndt-Universität Greifswald. Greifswald; 2014.

20. Brettschneider J, Claus A, Kassubek J, Tumani H. Isolated blood–cerebrospinal fluid-barrier dysfunction: prevalence and associated diseases. *J Neurol*. 2005;252:1067-1073.

21. Felgenhauer K. Differentiation of the humoral immune response in inflammatory diseases of the central nervous system. *J Neurol*. 1982; 228:223-237.

22. You JS, Gelfanova V, Knierman MD, Witzmann FA, Wang M, Hale JE. The impact of blood contamination on the proteome of cerebrospinal fluid. *Proteomics*. 2005;5:290-296.

23. Auer M, Hegen H, Zeileis A, Deisenhammer F. Quantitation of intrathecal immunoglobulin synthesis–a new empirical formula. *Eur J Neurol*. 2016;23:713-721.

24. Tipold A. Cerebrospinal fluid. In: Braund KG, ed. *Clinical Neurology in Small Animals-Localization, Diagnosis and Treatment*. Ithaca: International Veterinary Information Service; 2003.

25. Posselt E. Das idiopathische Vestibularsyndrom beim alten Hund. [Doctoral dissertation], Zentrum für Klinische Tiermedizin der Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München, Lehrstuhl für Innere Medizin der kleinen Haustiere. München; 2017.

26. Bichsel P, Vandevelde M, Vandevelde E, Affolter U, Pfister H. Immunoelectrophoretic determination of albumin and IgG in serum and cerebrospinal fluid in dogs with neurological diseases. *Res Vet Sci*. 1984;37:101-107.

27. Cockcroft P, Holmes M. *Handbook of Evidence-Based Veterinary Medicine*. 1st ed. Hoboken: John Wiley & Sons; 2008:224.

28. Wick DMM. *Ausgewählte Methoden der Liquordiagnostik und Klinischen Neurochemie*. 4th ed. München: Deutsche Gesellschaft für Liquordiagnostik und Klinische Neurochemie e.V; 2020:68.

29. Berg JM, Stryer L, Tymoczko JL. *Stryer Biochemie*. 8th ed. Berlin: Springer-Verlag; 2017:1401.

30. Fishman RA, Chan PH. Metabolic basis of brain edema. *Adv Neurol*. 1980;28:207-215.

31. Lampe R, Foss KD, Vitale S, Hague DW, Barger AM. Comparison of cerebellomedullary and lumbar cerebrospinal fluid analysis in dogs with neurological disease. *J Vet Intern Med*. 2020;34: 838-843.

32. Reiber H. External quality assessment in clinical neurochemistry: survey of analysis for cerebrospinal fluid (CSF) proteins based on CSF-/serum quotients. *Clin Chem*. 1995;41:256-263.

33. Schwenkenbecher P, Janssen T, Wurster U, et al. The influence of blood contamination on cerebrospinal fluid diagnostics. *Front Neurol*. 2019;10:584.

34. Püschel M. *Untersuchung zur Etablierung des Reibergramms in der Diagnostik von neurologischen Erkrankungen bei Hunden* [Doctoral dissertation], Department of Small Animal Medicine and Surgery, University of Veterinary Medicine, Foundation. Hannover; 2021.

35. Coates JR, Jeffery ND. Perspectives on meningoencephalomyelitis of unknown origin. *Vet Clin N Am-Small*. 2014;44:1157-1185.

36. Schwartz M, Puff C, Stein V, et al. Marked MMP-2 transcriptional upregulation in mononuclear leukocytes invading the subarachnoidal space in aseptic suppurative steroid-responsive meningitis–arteritis in dogs. *Vet Immunol Immunopathol*. 2010;133:198-206.

37. Tipold A, Schatzberg S. An update on steroid responsive meningitis-arteritis. *J Small Anim Pract*. 2010;51:150-154.

38. Wolburg H, Noell S, Fallier-Becker P, Mack AF, Wolburg-Buchholz K. The disturbed blood–brain barrier in human glioblastoma. *Mol Aspects Med*. 2012;33:579-589.

39. Burkhardt D, Schipper HI, Kaboth U, Felgenhauer K. IgA producing primary intracerebral lymphoma. *J Neurol Neurosurg Psychiatry*. 1992; 55:623-625.

40. Lai R, Rosenblum MK, DeAngelis LM. Primary CNS lymphoma: a whole-brain disease? *Neurology*. 2002;59:1557-1562.

41. Murray M, Cuddon P. Cerebrospinal fluid analysis in acute canine polyradiculoneuritis: albumin quotient and immunoglobulin G index determination using polyacrylamide gel electrophoresis. *J Vet Intern Med*. 2002;16(3): 318-389.

42. Reiber H. Die diagnostische Bedeutung neuroimmunologischer Reaktionsmuster im Liquor cerebrospinalis. *Lab Med*. 1995;19:444-462.

43. Martinez-Anton L, Marenda M, Firestone S, et al. Investigation of the role of campylobacter infection in suspected acute polyradiculoneuritis in dogs. *J Vet Intern Med*. 2018;32:352-360.

44. Rao ML, Böker D-K. Cerebrospinal fluid and serum levels of albumin, IgG, IgA and IgM in patients with intracranial tumors and lumbar disc herniation. *Eur Neurol*. 1987;26:241-245.

45. Hausmann O. Post-traumatic inflammation following spinal cord injury. *Spinal Cord*. 2003;41:369-378.

46. Kawabori M, Yenari MA. Inflammatory responses in brain ischemia. *Curr Med Chem*. 2015;22:1258-1277.

47. Sorby-Adams AJ, Marcoionni AM, Dempsey ER, Woenig J, Turner R. The role of neurogenic inflammation in blood-brain barrier disruption and development of cerebral oedema following acute central nervous system (CNS) injury. *Int J Mol Sci*. 2017;18:1788.

48. Harms L, Prüß H. Toxische und physikalisch bedingte Enzephalopathien. *Bewusstseinsstörungen und Enzephalopathien*. Berlin, Germany: Springer; 2013:403-417.

49. Kindt GW, Brock M, Altenau LL, et al. Blood/brain barrier and brain oedema in ammonia intoxication. *Lancet*. 1977;1:201.

50. Bouldin TW, Mushak P, O'Tuama LA, et al. Blood-brain barrier dysfunction in acute lead encephalopathy: a reappraisal. *Environ Health Perspect*. 1975;12:81-88.

51. Sundström R, Müntzing K, Kalimo H, et al. Changes in the integrity of the blood-brain barrier in suckling rats with low dose lead encephalopathy. *Acta Neuropathol*. 1985;68:1-9.

52. Kent M, Platt SR, Schatzberg SJ. The neurology of balance: function and dysfunction of the vestibular system in dogs and cats. *Vet J*. 2010;185:247-258.

53. Bartual-Pastor J. Vestibular neuritis: etiopathogenesis. *Rev Int Rhinol Otol Laryngol*. 2005;126:279-281.

54. Jeandel A, Thibaud J, Blot S. Facial and vestibular neuropathy of unknown origin in 16 dogs. *J Small Anim Pract*. 2016;57:74-78.

55. Foth S, Meller S, De Decker S, Volk HA. Unilateral decrease in inner ear signal in fluid-attenuated inversion recovery sequences in previously suspected canine idiopathic vestibular syndrome. *Vet J*. 2021;277:105748.

56. De Risio L, Bhatti S, Muñana K, et al. International veterinary epilepsy task force consensus proposal: diagnostic approach to epilepsy in dogs. *BMC Vet Res*. 2015;11:1-11.

57. Schmidley JW, Simon RP. Postictal pleocytosis. *Ann Neurol*. 1981;9:81-84.

58. Edwards R, Schmidley JW, Simon RP. How often does a CSF pleocytosis follow generalized convulsions? *Ann Neurol*. 1983;13:460-462.

59. Dingledine R, Varvel NH, Dudek FE. When and how do seizures kill neurons, and is cell death relevant to epileptogenesis? *Adv Exp Med Biol*. 2014;813:109-122.

60. Gonçalves R, Anderson T, Innocent G, et al. Effect of seizures on cerebrospinal fluid analysis in dogs with idiopathic epilepsy. *Vet Rec*. 2010;166:497-498.

61. Knebel A, Kämpe A, Carlson R, Rohn K, Tipold A. Th17 cell-mediated immune response in a subpopulation of dogs with idiopathic epilepsy. *PLoS One*. 2022;17(1):e0262285.

SUPPORTING INFORMATION

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