Use of albumin quotient and IgG index to differentiate blood- vs brain-derived proteins in the cerebrospinal fluid of cats with feline infectious peritonitis

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Key Words
Albumin quotient, antibody specific index, blood–brain barrier, cat, feline infectious peritonitis, IgG index

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Background: Inflammation of the central nervous system (CNS) is a frequent condition in cats but etiology often remains unsolved. Routine cerebrospinal fluid (CSF) analysis can be extended through the calculation of the albumin quotient (Q_{alb}), a marker of the integrity of the blood–brain barrier (BBB), and IgG index, an estimate of intrathecal IgG synthesis.
Objectives: The purpose of this study was to validate nephelometric methods for CSF protein analysis, and to use the Q_{alb} and IgG index to discriminate blood- and brain-derived immunoglobulin fractions in cats with feline infectious peritonitis (FIP).
Methods: Cats presented to our clinic between 2001 and 2005 were included in the study based on clinical and laboratory data and histopathologic findings at necropsy. Cats were grouped as having nonneurologic disease (controls; $n = 37$), brain tumors ($n = 8$), FIP involving the CNS ($n = 12$), and extraneural FIP ($n = 12$). CSF-total protein (TP) was measured and albumin and IgG concentrations were measured in paired CSF/serum samples; Q_{alb} and IgG index were calculated. Intraassay and interassay precision of the nephelometric assays were determined using pooled samples.
Results: Coefficients of variation for the nephelometric assays ranged from 2.7% to 7.2%. In control cats, CSF-TP concentration ranged from 0.06 to 0.36 g/L, Q_{alb} ranged from 0.6 to 5.7×10^{-3}, and IgG index ranged from 0.3 to 0.6. Q_{alb} and IgG index were significantly higher in cats with brain tumors and cats with CNS-FIP compared with other groups. Compared with control cats, pleocytosis was evident in 8 of 12 (67%) cats and CSF-TP was increased in 3 of 12 (25%) cats with CNS-FIP.
Conclusion: Nephelometry is a reliable method for measurement of CSF protein, albumin, and IgG in cats. The Q_{alb} and IgG index did not identify a CSF protein pattern specific for BBB dysfunction or intrathecal IgG synthesis in cats with CNS-FIP.

Introduction

Cerebrospinal fluid (CSF) analysis is the method of choice to diagnose central nervous system (CNS) inflammation and thus provides the basis for appropriate treatment in human and veterinary medicine. In cats, inflammatory CNS disease is frequently diagnosed, but despite intensive investigations, the specific etiology may remain unsolved in up to 37% of cases.[1] The major challenge is to identify the origin of the disease antemortem.

In human medicine, CSF analysis is aimed at discriminating blood-derived immunoglobulin from brain-derived fractions.[2] Routine work-up includes calculation of the CSF/serum albumin quotient (Q_{alb}) as an estimate of the functional integrity of the blood–brain barrier (BBB). Historically, intrathecal

immunoglobulin synthesis was proven by calculation of the IgG index.[3,4] More recently, a hyperbolic function reflecting laws of diffusion combined with individual CSF flow rate was finally confirmed as the best description of CSF protein profiles in humans.[5] By using these "Reiberdiagrams," the CSF/serum IgG quotient (Q_{IgG}) is compared with the Q_{alb} and used as a benchmark of intrathecal IgG synthesis and BBB dysfunction. Furthermore the Q_{IgG} and the CSF/serum-specific antibody quotient (Q_{spec}) have been used to calculate the antibody-specific index ($ASI = Q_{spec}/Q_{IgG}$) in people. This index discriminates between blood-derived antibody and pathologic, brain-derived-specific antibody fractions in CSF and takes changes in BBB function into account.[6] In animals, the application of similar coefficients has been investigated but has not yet gained unequivocal acceptance.[7–10]

Nephelometry is a preferred method for measuring CSF protein concentrations and establishing CSF/serum quotients in people[11]; to the authors' knowledge this method has not yet been used for protein measurement in cats and only a few studies have been published in dogs and horses.[12–14] Nephelometry is based on an optical detection system that measures the intensity of scattered light emanating from an illuminated volume. The signal of the scattered light is transformed into an electrical signal that is proportional to the concentration of the dispersed particles and therefore also to the antigen concentration. Measurements are done using the "fixed-time-method," whereby a difference is calculated between 2 values measured at different times. Nephelometric analysis is always performed in the presence of antibody excess according to the principles of Heidelberger and Kendall,[15] thus ensuring precise results, creating minimal need for repeated measurements, and covering a wide range of measurement with the antibody excess curve.[12] At present, there is no standardization of measurement of CSF total protein (CSF-TP) in animals, and a variety of methods and standards are utilized in veterinary laboratories. Historically, high-resolution protein electrophoresis has been used for CSF albumin and IgG determination. Nephelometry has been proven to be a precise method for measurement of CSF proteins in dogs and horses but requires expensive equipment.[12,13]

The purpose of this prospective study was first to validate nephelometry for CSF-TP, albumin, and immunoglobulin concentrations in cats. As feline infectious peritonitis (FIP) is characterized by marked vasculitis, which may influence BBB integrity and intrathecal IgG production, the second aim of the study was to evaluate the use of Q_{alb}, IgG index, and ASI as parameters of BBB function, intrathecal IgG production, and specific intrathecal antibody synthesis in cats with FIP and clinical controls.

Material and Methods

Patient selection and sample collection

Client-owned cats referred to the Clinic for Small Animal Medicine, Ludwig Maximilian University of Munich, that were euthanized between August 2001 and June 2004 because of neurologic or nonneurologic diseases were randomly selected for use in the study. Breed, sex, and age were recorded. Clinical and neurologic examination, CBC, serum biochemistry panel, urinalysis, and CSF analysis were performed in each cat. Pre- and postprandial bile acids and/or ammonia concentrations were measured in serum and plasma, respectively, to exclude hepatic dysfunction in all cats with forebrain signs and in cats with evidence of hepatic disease. Thoracic radiographs, abdominal ultrasound, and computed tomographic or magnetic resonance imaging of the brain were performed as indicated. Based on the results of clinical and neurologic examination, laboratory and CSF analyses, necropsy, and histopathologic examination of the CNS, cats were grouped into 4 categories: group 1: control cats with nonneurologic diseases; group 2: cats with brain tumors; group 3: cats with CNS-FIP; group 4: cats with extraneural FIP. Inclusion criteria for group 1 cats were no abnormalities in neurologic examination, liver function tests, CSF analysis (< 3 WBCs/μL; < 30 RBCs/μL),[16–18] and histopathologic examination of the CNS, and no gross or histologic signs of FIP at necropsy. Inclusion criteria for groups 2–4 were a laboratory work-up, CSF analysis, neurologic examination, and postmortem histopathologic confirmation of the diagnosis.

Cats were anesthetized using a combination of diazepam and propofol, intubated, and maintained on isoflurane/O_2. CSF was collected in sterile tubes (Fa. Braun Vet Care GmbH, Tuttlingen, Germany) without anticoagulant from the cerebellomedullary cistern and analyzed within 30 minutes. Routine CSF analysis included TP concentration by nephelometry, and total nucleated cell count (TNCC), RBC count, and differential nucleated cell count using a Fuchs-Rosenthal hemacytometer and cytologic evaluation of cytocentrifuged preparations. A minimum of 500 μL of CSF was placed into a cuvette and centrifuged at 300 *g* for 3 minutes followed by 700 *g* for 1 minute (Hettich Zentrifugen, Tuttlingen, Germany). At the time of CSF collection, venous blood samples were

collected into tubes without anticoagulant. Serum was separated and divided in 300-μL aliquots that were stored at −70 °C until assayed. Additionally, a portion of the collected CSF was stored at −70 °C for later analysis.

Protein analyses

TP, albumin, and IgG concentrations were determined in paired CSF and serum samples from all cats. Serum and corresponding CSF samples were thawed at 4 °C and then at room temperature (~22 °C). After thawing, samples were centrifuged for 5 minutes to eliminate potential cloudiness from cryoglobulins.

CSF-TP, CSF and serum albumin concentrations, and CSF and serum IgG concentrations were measured with a Behring Nephelometer 100 (Behringwerke AG, Marburg, Germany). CSF-TP concentration was analyzed following precipitation with trichloroacetic acid (AppliChem GmbH, Biochemica, Darmstadt, Germany). Albumin and IgG concentrations were measured by immunonephelometry using species-specific (anti-cat) anti-albumin and anti-IgG antibodies (Bethyl Laboratories, Montgomery, TX, USA). A human-based CSF-protein standard (Behringwerke AG), cat albumin standard, and cat IgG standard (Sigma-Aldrich Chemie GmbH, Munich, Germany) were used as controls. Serum TP concentration was measured by a biuret method (Hitachi 911, Roche, Grenzach, Germany). Q_{alb} and IgG index were calculated in all cats from CSF and corresponding serum samples according to the following formulas: Q_{alb} = CSF albumin/serum albumin; Q_{IgG} = CSF IgG/serum IgG; IgG index = Q_{IgG}/Q_{alb}. Minimum and maximum values in control cats (group 1) served as upper and lower reference limits for comparison with values from cats in other groups.

Reference curves for CSF-TP, albumin, and IgG concentrations were established from standard reagents using multiple-point calibration at various dilutions. For optimal reaction conditions, N-diluent and N-buffer (OUMT 60/61; Behringwerke AG) were added. Protein concentrations were calculated following precipitation with 20% trichloroacetic acid and automatically came under scrutiny for quality assurance. Only aberrations $< 5\%$ were accepted. The same assumptions were valid for calibration of reference curves for albumin and IgG. Albumin and IgG reference curves were obtained using the cat albumin standard (Sigma-Aldrich Chemie GmbH) precipitated with goat anti-cat albumin (Bethyl Laboratories) and cat IgG standard (Sigma-Aldrich Chemie GmbH) precipitated with goat anti-cat IgG (Bethyl Laboratories).

Assessment of precision

Intraassay precision was assessed by repeated measurements ($n = 10$) of CSF-TP, albumin, and IgG concentrations. In addition, interassay precision was assessed using a serum and a CSF sample. Samples for interassay precision were stored frozen at 20 °C and aliquots were thawed daily for 10 days for analysis. Coefficients of variation (CV) were calculated as SD × 100/mean. Recovery was determined by dilution and measurement of standard reagents of known concentration including a human-based CSF protein (72.8 g/L) (Behringwerke AG), cat albumin standard (2500 mg/dL), and cat IgG standard (1000 mg/L) (Sigma-Aldrich Chemie GmbH). Five dilutions were made for CSF-TP and 8 for albumin and IgG measurements. For routine quality control, standard reagents (human CSF-protein standard, cat albumin standard, and cat IgG standard) and pooled serum and CSF samples were measured before each analytical run. A maximum CV of 10% was considered as acceptable.

Antibody-specific titers and ASI

Anti-feline coronavirus (FCoV)-antibody titers were measured in serum and CSF of 7 cats by an indirect immunofluorescence assay (IFAT) as described in a previous study.[19] Briefly, feline coronavirus ATCC VR-990 FIP virus strain WSU-1 146 was used as antigen.[19] Anti-coronavirus antibodies were detected by fluorescein-labeled anti-cat IgG of goats (ICN FITC goat anti-cat IgG; whole molecule). Starting dilution was 1:32 for CSF and 1:128 for serum. The Q_{spec} was calculated as CSF anti-coronavirus IgG/serum anti-coronavirus IgG. The ASI was calculated as Q_{spec}/Q_{IgG}. An ASI > 4 was taken as evidence of specific intrathecal antibody production.[6]

Postmortem examinations

All cats included in the study underwent complete necropsy and histopathologic examination of the CNS, heart, visceral organs (lung, intestine, pancreas, liver, kidneys, spleen, omental fat), and hemolymphatic tissues (lymph nodes, spleen). Brain dissection focused on the areas determined by neurolocalization and whole-brain sections were made transversally through the medulla oblongata, pons and mid-cerebellum, mesencephalon, diencephalon, and at the level of the rostral commisure. Tissue samples were fixed in 10% neutral buffered formalin, processed in an automatic tissue processor, embedded in paraffin, sectioned at 5 μm, and stained with H&E and Giemsa.

Diagnosis of FIP was based on the presence of characteristic histopathologic findings.[20] Specimens with histopathologic changes consistent with FIP were also examined for expression of intracellular FCoV antigen by ABC-enhanced immunohistochemistry (mouse anti-feline coronavirus, clone FIPV3-70, Abd Serotec, Düsseldorf, Germany) and a Histogreen detection kit (Linaris, Wertheim, Germany).

Statistical analysis

Results were reported as minimum, maximum, and median values for each patient group. Gaussian distribution of CSF-TP, Q_{alb}, and IgG index was assessed by the Kolmogorov–Smirnov test. Influence of age and sex on CSF-TP, Q_{alb}, and IgG index was assessed by ANOVA (Kruskall–Wallis) for cats in all groups. Differences in the concentrations of CSF-TP, Q_{alb}, and Q_{IgG} between patient groups were assessed by Fisher's exact test. Regression analysis was used to test for correlations between CSF-TP, Q_{alb}, and IgG index within each patient group. TNCC and RBC were not compared statistically. Level of significance was set at $P < .05$ for all tests (SPSS for Windows, Version 11.5, München, Germany).

Results

Of 637 cats presented to the clinic during the specified time interval, 69 cats were included in the study. Altogether 568 cats were excluded because either postmortem examination or CSF analysis was not available. Thirty-seven cats were classified as controls (group 1), 8 had brain tumors (group 2), 12 had CNS-FIP (group 3), and 12 had extraneural FIP (group 4).

Breeds included 53 domestic shorthair, 6 Persian, 4 Maine Coon, 3 Siamese, 1 Russian Blue, 1 British Shorthair, and 1 Devon Rex cat. Forty-four cats were male (32 neutered males) and 25 were female (19 neutered females). Ages ranged from 3 months to 22 years. Mean age was 10.5 years for group 1 (minimum–maximum, 4 months to 21 years), 14.0 years for group 2 (9–22 years), 3.0 years for group 3 (4 months to 6 years), and 4.5 years for group 4 (4 months to 12 years). Cats with FIP were significantly younger ($P < .01$) than cats with brain tumors or control cats.

Interassay and intraassay precision were $< 10\%$ for all analytes (Table 1). Reproducibility was 98.7% for the CSF-TP standard, 99.1% for the albumin standard, and 97.9% for the IgG standard.

Group 1: Controls

Control cats had no gross or histologic changes in the CNS. Based on necropsy examination, cats were diagnosed with the following extraneural diseases: hypertrophic cardiomyopathy (11), malignant lymphoma (5), chronic renal disease (5), pulmonary adenocarcinoma (4), pneumonia (4), parvovirus enteritis (2), chronic rhinitis (2), pyometra (1), mandibular squamous cell carcinoma (1), *Aelurostrongylus abstrusus* infestation (1), and necrotizing dermatitis (1).

The results of CSF analysis were tabulated (Table 2). CSF-TP concentration and Q_{alb} were positively correlated ($P < .05$; $r = .593$). Influence of age, sex, and group on CSF-TP, Q_{alb}, and IgG index was assessed by ANOVA for all cats.

Group 2: Cats with brain tumors

Of the 8 cats with brain tumors, 5 were diagnosed with intracranial meningiomas, 2 had metastatic squamous cell carcinoma, 1 had a grade III astrocytoma, and 1 had lymphoma. One cat had an extraaxial meningioma in combination with a squamous cell carcinoma affecting the peri- and endoneurium of the facial nerve.

The Q_{alb} and IgG index of cats with brain tumors were significantly higher than those of control cats and cats with extraneural FIP (Table 2). Based on the upper limit of values for control cats, 4 (50%) cats with brain tumors had a mild increase in CSF TNCC (4–20 leukocytes/μL), 2 had an elevated RBC count (148 RBC/μL, 1387 RBC/μL), and 4 (50%) had increased CSF-TP concentrations. The Q_{alb} was increased in 3 cats with brain tumors, 2 of which also had an increased IgG index. Two cats had an increased IgG index without an increase in Q_{alb}. The ASI was > 4 in the cat with meningioma (Table 3). There was no correlation between CSF-TP, Q_{alb}, and IgG index in this group. The ASI of 2 cats (nos. 3 and 13) were calculated with Q_{lim} rather than Q_{IgG} because the IgG index was increased.

Group 3: Cats with CNS-FIP

Histologic findings in cats with CNS-FIP consisted of disseminated infiltrates consisting of plasma cells, macrophages, neutrophils, and lymphocytes within the CNS. Locations of the infiltrates were typical for FIP and included marked vasculitis with perivascular cuffing within the superficial brain parenchyma, leptomeninges, meninges, ependyma, choroid plexus, and spinal cord.[19,21,22] One cat had involvement of the neuroparenchyma confined unilaterally to the thalamus. Eleven of 12 cats with FIP-CNS also had extraneural

pyogranulomatous infiltrates involving the kidneys, liver, spleen, and less commonly the pancreas and lung. Peritoneal effusion was noted in 4 cats.

Immunohistochemical staining was positive for FCoV antigen within the CNS in 8 cats, including the cat with lesions confined to one side of the thalamus (Table 4). Immunohistochemical results were also positive in extraneural tissues in 8 of 12 cats. Three cats tested negative and 1 was not evaluated because of lack of sufficient brain tissue (Table 4).

The CSF of cats with CNS-FIP had higher TNCCs compared with control cats, cats with brain tumors, and cats with extraneural FIP. Compared with the upper control value, pleocytosis (11–295 TNCC/μL) was found in 8 of 12 (67%) cats, RBC counts were elevated in 3 (25%) cats (84–403 RBC/μL), and CSF-TP was increased in 3 (25%) cats. The Q_{alb} exceeded the upper control value in 4 cats, of which 2 cats also had an increased IgG index. One additional cat had an increased IgG index with a normal Q_{alb}.

The Q_{alb} of cats with CNS-FIP was significantly higher than that of control cats and cats with extraneural FIP (Table 2). The Q_{alb} in 3 cats were considered as outliers: 31×10^{-3}, 18×10^{-3}, and 16×10^{-3}. IgG index in cats with CNS-FIP was not significantly different from that of control cats and cats with extraneural FIP. The IgG indexes in 3 cats were considered as outliers (1.03, 0.77, 5.57). There was a strong positive correlation between Q_{alb} and IgG index within this group ($P < .01$; $r = .833$). CSF-TP was not correlated with either Q_{alb} or IgG index. The ASI was > 4 in 1 of 3 cats with CNS-FIP (Table 3).

Group 4: Cats with extraneural FIP

Ten of 12 cats with extraneural FIP had pyogranulomatous serositis with protein-rich effusions in body cavities, and all cats had granulomatous inflammatory lesions affecting predominantly liver and kidneys, but also lung, pancreas, and omentum. Histologic findings in the CNS were unremarkable. All cats with extraneural FIP were positive with immunohistochemical staining for FCoV antigen in macrophages.

CSF results in cats with extraneural FIP did not differ from those in control cats but differed from cats with CNS-FIP or brain tumors (Table 2). CSF results for TNCC, CSF-TP, Q_{alb}, and IgG index did not exceed the upper control values. The CSF in 1 cat had > 30 RBC/μL. CSF-TP and Q_{alb} were positively correlated ($P < .01$; $r = .695$) in cats in this group. The ASI was > 4 in 1 of 3 cats with extraneural FIP (Table 3).

Discussion

Results of the present study suggest that nephelometry is a reliable and accurate method for measurement of CSF-TP, albumin, and IgG concentrations in cats, as has previously been demonstrated in people and in dogs.[11,14,23] Using nephelometric methods, calculation of Q_{alb} and IgG index did not identify a protein pattern specific for CNS-FIP. The results also indicated that disturbance of blood–CSF barrier function and intrathecal IgG synthesis was not a common feature of FIP in cats. The ASI did not support intrathecal immunoglobulin synthesis in cats with FIP.

Reference values for CSF-TP were similar to those reported in recent studies in cats,[17,24] but slightly different from those reported by others,[18,25] maybe depending on different group size and composition of variable analytical methods. The Q_{alb} values obtained in control cats in this study resembled those reported previously in dogs and in people.[18,26] To the authors' knowledge only 1 study has described reference values for feline Q_{alb} (0.12 ± 0.04).[27] However, these values differ by a factor of 20 from the results of the current investigation ($2.4 \times 10^{-3} \pm 0.0006$), possibly because only 5 cats were measured in that study and different methods of protein analysis (electrophoresis) and calculation of Q_{alb} were used. In humans and in other species, age-related differences in Q_{alb} likely depend on maturation of the BBB and CNS.[26,28,29] In the present study there was no apparent influence of age on CSF-TP, Q_{alb}, or IgG index although only 3 cats were younger than 4 months of age.

Results of IgG index resembled those in a previous study in cats[17] as well as those reported for people[26]

Table 1. Interassay and intraassay precision of a nephelometric assay for total protein, albumin, and IgG concentrations in cerebrospinal fluid from cats.

	Interassay			Intraassay		
Analyte	*n*	Mean ± SD	CV (%)	*n*	Mean ± SD	CV (%)
Total protein (g/L)	10	0.15 ± 0.039	3.0	10	0.15 ± 0.0044	2.7
Albumin (mg/dL)	10	0.10 ± 0.014	3.2	10	16.1 ± 0.014	3.4
IgG (mg/L)	10	0.19 ± 0.0050	7.0	10	42.5 ± 0.0030	3.2

CV, coefficient of variation.

Table 2. Median, minimum, and maximum values for parameters of cerebrospinal fluid analysis in cats in different disease groups.

Group	TNCC (/μL)	RBCs (/μL)	Total protein (g/L)	Q_{alb} ($\times 10^{-3}$)	IgG index
Control ($n = 37$)	1 (0–4)	2 (0–30)	0.13 (0.06–0.36)	2.0 (0.6–5.7)	0.42 (0.3–0.6)
Brain tumors ($n = 8$)	9 (0–20)	11 (0–1387)	0.47* (0.08–1.37)	4.6* (1.3–10.0)	0.89* (0.30–1.9)
CNS-FIP ($n = 12$)	27 (1–295)	3 (0–403)	0.18 (0.07–1.11)	3.0* (1.0–31.3)	0.48 (0.41–5.57)
FIP-extraneural ($n = 12$)	2 (0–4)	3 (0–84)	0.08 (0.06–0.22)	1.8 (1.0–4.4)	0.44 (0.3–0.5)

*Significantly higher than control cats and cats with extraneural FIP ($P < .01$).
TNCC, total nucleated cell count; TP, total protein; Q_{alb}, albumin quotient; CNS, central nervous system, FIP, feline infectious peritonitis.

but were lower than those calculated in 6 specific pathogen-free cats (0.61–1.65) with all measurements done by ELISA.[30] In the present study, IgG index was characterized as a linear function although empirical data in human patients suggest that increased transfer of blood proteins into CSF is better characterized by a hyperbolic function.[2,29] The application of these diagrams could have provided more precise information regarding discrimination between authentic intrathecal IgG synthesis and simple diffusion of blood-derived IgG into CSF caused by BBB dysfunction, but no data are available for domestic animals.

Brain tumors in animals are usually characterized by altered function of the BBB.[31,32] In the present study, the cat with multiple meningiomas had evidence of BBB dysfunction based on the Q_{alb}, while 2 cats had evidence of both BBB dysfunction and intrathecal IgG synthesis. Loss of endothelial tight junctions, secretion of growth factor, activation of serine proteases, and increase in specific matrix components can cause a breakdown of the BBB.[31,33–35] Thus, passive transfer of blood-derived proteins into CSF can lead to an increase in Q_{alb} as was seen in 3 of 8 tumor-bearing cats in this study.

Surprisingly, the majority of cats with brain tumors had evidence of intrathecal IgG synthesis. This is an uncommon finding in humans with brain tumors, with the exception of CNS lymphoma and meningioma.[36–38] Isolated IgM production was reported in a human patient with non-Hodgkin's lymphoma.[26] In this study, intrathecal IgG was evaluated by using the IgG index, which assumes a linear relationship between Q_{alb} and Q_{IgG}. It remains unclear, however, whether a correction of these values using a hyperbolic function would have unmasked some of these cats as false positives. On the other hand, 1 cat with B-cell lymphoma and 1 with fibrous meningioma had an increased IgG index despite a normal CSF-TP and Q_{alb}. Therefore, passive passage of Igs appears unlikely. Increased IgG index in dogs with CNS lymphoma and meningioma was explained as peritumoral inflammation, as demonstrated histologically.[39,40]

Blood contamination was evident in 2 cats but was not associated with an increased CSF-TP or Q_{alb} in the cat with the largest amount of blood (1387 RBC/μL). On the contrary, the other cat with blood contamination had evidence of intrathecal IgG synthesis and BBB dysfunction. Studies on the effects of iatrogenic blood contamination demonstrated no significant alterations in CSF-TP, Q_{alb}, and IgG index in dogs and rhesus macaques.[41–43] A variable increase in IgG index was seen in horses depending on serum IgG concentrations.[44]

CNS manifestation of FIP is characterized by marked vasculitis within the CNS caused by immune complex deposition, upregulation of cytokines, and viral-induced activation of monocytes and endothelial cells.[21,45,46] Depending on the amount of perivascular inflammation, various inflammatory cells

Table 3. Histologic diagnosis and CSF results in 7 cats in which antibody specific index was measured.

	Anti-coronavirus IgG				
Diagnosis	CSF	Serum	Q_{alb} ($\times 10^{-3}$)	IgG index	Antibody-specific index
Brain tumor (meningioma)	1:256	1:4096	10.0	0.98	9.22
CNS-FIP	1:32	1:16384	6.5	0.55	0.55
CNS-FIP	1:1024	1:8192	31.0	5.57	5.34*
CNS-FIP	1:64	1:16384	2.5	0.41	3.81
Extraneural FIP	1:32	1:16384	2.9	0.29	2.32
Extraneural FIP	1:32	1:4096	4.4	0.54	3.28
Extraneural FIP	1:64	1:4096	3.0	0.42	12.40

*Corrected as noted in the text, because $Q_{IgG} > Q_{lim}$.
CNS indicates central nervous system; FIP, feline infectious peritonitis.

Table 4. Histologic and immunohistochemical results in 12 cats with feline infectious peritonitis (FIP) involving the central nervous system (CNS).

	FIP involving CNS	
Cat no.	Histology	Immunohistochemistry
1	+ (t)	+
2	+ (t)	NE
3	+ (t)	+
4	+ (t)*	–
5	+ (a)*	–
6	+ (t)	+
7	+ (t)	+
8	+ (t)	–
9	+ (t)	+
10	+ (a)	+
11	+ (t)	+
12	+ (t)	+

*Antigen-positive in macrophages.
(t), typical type B-lesions; (a), atypical non A/B-lesions; NE, not examined.

(lymphocytes, neutrophils, macrophages) may be found within the CSF. It has been postulated that CSF-TP > 2 g/L is characteristic of CNS-FIP.[17] Only 3 cats in the present investigation had abnormal CSF-TP. This was similar to observations in another study in which increased CSF-TP was not considered mandatory for a diagnosis of CNS-FIP.[47] Histologically, CNS inflammation varied from mild to marked and was not correlated with CSF-TP. It was therefore concluded that normal CSF-TP does not exclude FIP in the CNS.

Two cats with CNS-FIP had evidence of BBB disturbance, based on high Q_{alb}. Both cats had marked CSF pleocytosis indicative of severe CNS inflammation. Most cats with normal to mildly elevated CSF leukocytes presented with an unchanged CSF protein pattern.

The IgG index indicated intrathecal IgG synthesis in 3 cats with CNS-FIP. Intrathecal IgG synthesis can result from a specific immune response within the CNS or a polyspecific immune response directed against multiple antigens.[48,49] In the case of FIP, antigen-loaden macrophages appear in the CNS.[21,47,50] Anti-FCoV IgG and IgM-producing B cells have been demonstrated in the boundary layers of pyogranulomas.[51] It is surprising that only 25% of cats with CNS-FIP had laboratory evidence of intrathecal IgG production, considering that serum hyperglobulinemia is frequently observed in FIP and specific intrathecal antibody production has been documented.[47] In the present study, serum TP concentration was unremarkable in all but 1 cat with FIP; CSF protein pattern in this cat also was unremarkable. Thus, results of the present study suggest that local IgG production is not a characteristic feature of CNS-FIP.

The question arises as to why a normal CSF protein profile was found in 7 of 12 cats with CNS-FIP. Only a few of the cats had CSF alterations typical of FIP, such as yellow-tinged fluid, high TNCCs, and high protein content. However, FIP was confirmed by histopathologic and immunohistochemical examination in the brain and viscera. Four cats with mild lymphocytic meningitis and neuropathologic alterations were interpreted as having possible FIP or viral meningitis of unknown viral origin. One cat had an unusual pattern of distribution of cerebral lesions, with unilateral involvement of the thalamus. However, all 4 cats had extraneural granulomatous lesions typical of FIP. The possibility that CSF examination and histopathology were done too early in the course of the disease with inadequate activation of immune defense systems was excluded. This was based on the knowledge that although clinical signs of FIP may appear acutely in some cats, the pathogenesis of FIP lesions is usually more chronic. Furthermore, IgG might have precipitated in the lesions in the form of immune complexes. This phenomenon also accounts for the fact that about 10% of cats with clinical signs of FIP are antibody-negative in the blood.[52]

Besides increased permeability of the BBB, reduced CSF flow rate is thought to modulate the concentration of protein in the CSF.[29] In CNS-FIP, hydrocephalus can result from impaired resorption of CSF due to deposition of immune complexes around the meninges and the choroid plexus.[53] Interestingly, the second highest values for Q_{alb} and IgG index were found in a cat with hydrocephalus and CNS-FIP.

Measurement of CSF antibody titers has been advocated in cats for the diagnosis of FIP.[7,30] However, when serum antibody titers are increased, it may be difficult to determine whether the CSF antibodies are produced locally or blood-derived. In FIP, both intrathecal synthesis of specific antibodies and passive diffusion of antibodies across the BBB have been demonstrated.[19,47] Thus, meaningful interpretation of CSF antibody titers requires evaluation of the integrity of the BBB as well as calculation of the amount of intrathecally produced antibody. The ASI is a sensitive indicator of local synthesis of specific antibodies in people, based on the fact that Igs of the same class have the same molecular size and ability to cross the BBB.[6,54] Local synthesis of a specific antibody is indicated by an ASI > 1.4 (ELISA) or 4 (IFAT, as in this study).[6,48] In the present investigation, the ASI was > 4 in 3 of 7 cats evaluated, and thus indicated intrathecal synthesis of anti-coronavirus IgG, even in cats without CNS-FIP or FIP at all. Conversely, an ASI of < 4 in 2 cats with CNS-FIP, suggested these antibodies

were derived from blood alone. In humans, the ASI may be falsely low in cases of excessive polyspecific intrathecal IgG synthesis resulting in an increased Q_{IgG} ($Q_{IgG} > Q_{lim}$). In this situation calculation of ASI using only the blood-derived fraction of IgG (Q_{lim}) is recommended.[6] In the present study, recalculation of ASI with use of Q_{lim} instead of Q_{IgG} in 2 cats led to a correction from 0.7 to 5.3. One could argue that it would be better to use a species-specific formula for Q_{lim} but these data are yet not available. Furthermore, calculation of ASI requires a highly sensitive method to quantify CSF antibodies.[6] Therefore, a limitation of the present study is that CSF and serum titers were measured by IFAT and not by ELISA. Thus, imprecision in the CSF/serum quotient has to be considered.

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