STANDARD ARTICLE

Journal of Veterinary Internal Medicine

ACVIM
American College of
Veterinary Internal Medicine

Retrospective evaluation of cyclosporine in the treatment of presumed idiopathic chronic hepatitis in dogs

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Abstract

Background: The etiology of idiopathic chronic hepatitis (ICH) in dogs is poorly understood, but evidence supports an immune-mediated pathogenesis in some dogs.

Objectives: To describe a case series of dogs with presumed ICH treated with cyclosporine (CsA) with or without concurrent medications and to document the incidence of biochemical remission and factors associated with failure to attain remission.

Animals: Forty-eight client-owned dogs diagnosed with presumed ICH, treatment of which included CsA.

Methods: Two-institution, retrospective case series of dogs between 2010 and 2017. All dogs were treated with CsA with or without concurrent medications for ≥2 weeks. Data were collected from medical records.

Results: Biochemical remission (<1.1 times the upper limit of normal for alanine aminotransferase activity) was attained in 79% of dogs (38/48). Median dose of CsA at remission was 7.9 mg/kg/d (range, 2.5-12.7 mg/kg/d) and median time to remission was 2.5 months (range, 0.75-18 months). Concurrent hepatoprotectant treatment was not associated with likelihood of remission. Clinical score, ascites, hypoalbuminemia, hyperbilirubinemia, prolonged coagulation times, dose, and duration of treatment were not associated with the probability of remission or time to remission. Common adverse effects of CsA were gastrointestinal signs in 38% (18/48) and gingival hyperplasia in 25% (12/48) of treated dogs.

Conclusion and Clinical Importance: A treatment regimen including CsA and frequent hepatoprotectant use resulted in biochemical remission of ICH in most dogs. None of the evaluated factors, including hepatoprotectant use, were significantly associated with likelihood of remission. Future prospective studies are indicated to evaluate CsA monotherapy in ICH dogs.

KEYWORDS
alt, canine, immunosuppressive, liver disease, remission, therapy

1 | INTRODUCTION

Idiopathic chronic hepatitis (ICH) is a common and important hepatic disorder in dogs.[1-6] In 1 study, 64% of chronic hepatitis (CH) cases were diagnosed as idiopathic.[6] Chronic hepatitis is defined by persistent increases in alanine aminotransferase (ALT) activity >2 times normal in the presence of histopathological changes that include a mixed, primarily monocellular, inflammatory cell infiltrate accompanied by evidence of hepatocyte cell death and variable amounts of fibrosis, ductular response, and regenerative nodule formation.[7,8] Cases are diagnosed as idiopathic after ruling out infectious, metabolic and toxic (including copper) causes of CH.[6] Previous reports of ICH include middle-aged to older dogs that can present with subclinical disease or with a range of clinical signs.[2-6] Common breeds include the Labrador Retriever,[9] Doberman Pinscher,[10] American and English Cocker Spaniel,[11,12] English Springer Spaniel,[13] and Standard Poodle.[14] Treatment is essential to prevent progression to cirrhosis, liver failure, and death. Median survival time is variable but averages approximately 1.5 years.[6,9,12,15-20]

A growing body of evidence supports an immune-mediated pathogenesis for ICH in dogs. Such evidence includes abnormal major histocompatibility complex Class II protein expression on hepatocytes,[21-23] presence of autoantibodies in serum,[24-27] lymphocytic infiltrates in the liver,[6,28] association with other autoimmune diseases,[9] female sex and breed predispositions,[2,9-14,24] and a positive response to immunosuppressive drugs.[11,15,16,21] A few studies have suggested that dogs with CH can respond to immunosuppressive doses of corticosteroids,[11,15,16,21] but corticosteroids can be ineffective in many cases. One study observed that only 11/36 dogs with ICH attained clinical remission with corticosteroid treatment.[16] Corticosteroids also cause substantial, often intolerable adverse effects (eg, polyuria, polydipsia, panting, muscle atrophy, thrombosis, gastrointestinal ulceration). They also induce a vacuolar hepatopathy and increases in serum liver enzyme activity.[29] The latter makes laboratory monitoring of response to treatment particularly challenging. Finally, corticosteroids are contraindicated in patients with cardiac disease, diabetes mellitus, and hypertension. These drawbacks justify seeking alternative treatments for dogs with ICH.

Cyclosporine (CsA) is an immunomodulatory drug that inhibits the activation and proliferation of T-lymphocytes.[30] Cyclosporine has proven well tolerated and effective in dogs in the management of immune-mediated disorders including atopic dermatitis,[31-34] immune-mediated thrombocytopenia,[35] inflammatory bowel disease,[36] and immune-mediated polyarthritis.[37] The most commonly reported adverse effects associated with CsA in dogs are gastrointestinal signs[31-34] and, less commonly, opportunistic infections,[38-41] gingival hyperplasia,[42] papillomatosis, and hirsutism.[31-34] Although prednisone and azathioprine are first-line immunosuppressive drugs for immune-mediated hepatitis in humans, CsA has been successfully used as a second-line treatment in patients who are refractory or intolerant of prednisone and azathioprine.[43,44] To date, no study has evaluated the use of CsA in the treatment of ICH in dogs.

Our primary aims were to describe the adverse effects associated with CsA and the frequency of biochemical remission in a group of dogs with presumed ICH treated with CsA with or without concurrent medications. A secondary aim was to identify if any negative prognostic factors such as ascites,[6,19] clinical score,[9] hyperbilirubinemia,[6,45] hypoalbuminemia,[6,15] or prolongations in prothrombin time (PT) and activated partial thromboplastin time (aPTT)[6,9,15] were associated with decreased likelihood of biochemical remission or longer time to biochemical remission.

2 | MATERIALS AND METHODS

2.1 | Case selection

Electronic medical records from Colorado State University Veterinary Teaching Hospital and Foster Hospital for Small Animals at Cummings School of Veterinary Medicine at Tufts University between the years 2010 and 2017 were reviewed. Dogs diagnosed with CH by a board-certified veterinary pathologist and treated with CsA with or without concurrent medications for at least 2 weeks were included. Biopsies were performed laparoscopically (40/48), using ultrasound guidance (4/48; 2 with 18-gauge and 2 with 16-gauge needles), and surgically (4/48). Inclusion criteria were: (1) increased serum ALT activity, (2) liver histopathology results consistent with CH based on World Small Animal Veterinary Association (WSAVA) criteria,[8] and (3) exclusion of other causes of CH. Patient presentation, serum biochemistry results, liver histopathology reports, liver and gall bladder culture results, leptospirosis testing, hepatic copper concentration, and treatment response to CsA with or without hepatoprotectants (s-adenosylmethionine [SAMe], silybin, ursodiol, vitamin E, or some combination of these) were reviewed by 1 of 2 board-certified internists (D.C.T., C.L.W.) to ensure adherence to WSAVA histologic criteria[8] and limit inclusion to dogs with presumed ICH. Dogs with increased hepatic copper concentrations (≥1000 μg/g dry weight [dw]) were included only if the hepatic copper staining was not centrilobular or chelation treatment with penicillamine for 5 months had not normalized serum ALT activity before treatment with CsA. Dogs on concurrent medications such as antibiotics, penicillamine, immunosuppressive drugs, and hepatoprotectants were not excluded. Most dogs were on hepatoprotectants before referral or placed on these medications at the time CsA treatment was initiated (42/48, 87.5%). This approach limited our ability to evaluate CsA as monotherapy but was unavoidable because of the popularity of these supplemental medications in veterinary medicine. Comorbidities in the dogs were recorded but not considered as exclusion criteria.

2.2 | Data collection

Data regarding signalment, body weight, clinical signs at presentation, presence of ascites (determined by abdominal ultrasound examination), hepatic encephalopathy (ie, increased serum ammonia concentration or presence of neurologic signs responsive to lactulose, metronidazole, or neomycin, low protein diet, or some combination of these), concurrent medications, diet, dose of CsA, possible adverse effects of CsA, PT, aPTT, serum alkaline phosphatase (ALP) activity, ALT activity, serum aspartate aminotransferase (AST) activity, serum gamma-glutamyl transferase (GGT) activity, and serum albumin, total bilirubin, and globulin

concentrations at the time of initial presentation to the hospital were extracted from electronic medical records for each dog. Individual clinical scores (a numeric scoring system used to infer prognosis by assigning points for the following clinical factors: clinical signs [polyuria, polydipsia, anorexia, lethargy, vomiting], hepatic encephalopathy, ascites, hypoalbuminemia, hypoglobulinemia, hyperbilirubinemia, and prolongation of PTT) were calculated using patient data at the time of liver biopsy.[18] Histopathology results including results of hepatic copper staining, hepatic copper quantification using flame atomic absorption spectroscopy (μg/g dw; Colorado State University Diagnostic Laboratory, Fort Collins, Colorado), and aerobic and anaerobic culture results were recorded. Results of leptospirosis testing (Colorado State University Diagnostic Laboratory or Fort Collins, Colorado, or IDEXX, Westbrook, Maine) were recorded.

The time of initial presentation for liver biopsy was established as baseline (t = 0), before initiating treatment with CsA. Results for serum ALP, ALT, AST, and GGT activity as well as serum albumin and total bilirubin concentrations were collected pretreatment (t = 0) and at sequential time points when the patient was reevaluated. Because dogs were reevaluated at variable time points, the data were categorized into 7 time intervals after baseline 0:1 (2-4 weeks), 2 (1-3 months), 3 (3-6 months), 4 (6-9 months), 5 (9-12 months), 6 (1-2 years), and 7 (> 2 years). If >1 data set was available per time interval, the more complete or recent data set was used. Because of variations in reference ranges among laboratories (Colorado State University Clinical Pathology Laboratory, Cummings Veterinary Medical Center at Tufts University Clinical Pathology Laboratory, IDEXX Laboratories, Antech Laboratories, and in-house machines of referring veterinarians), each biochemical result except for albumin, was converted to a factor times the upper limit of normal (×ULN) to standardize the data. Serum albumin concentrations were converted to factor times the lower limit of normal (×LLN). The dose of CsA (mg/kg/d) and duration of treatment (months post-initiation of CsA) were documented at each time point. Clinical adverse effects suspected to be associated with CsA administration such as inappetence, vomiting, diarrhea, gingival hyperplasia, secondary infections, hirsutism, papillomatosis, neoplasia, nephrotoxicity, hepatotoxicity, and diabetes mellitus were recorded. Hepatotoxicity or nephrotoxicity was defined as a 2-fold increase in ALT activity or increase in serum creatinine concentration combined with urine specific gravity <1.030, respectively, 1 to 3 months after initiation of CsA treatment. If hepatic or renal test results improved or resolved without discontinuation of CsA, the case was not classified as hepatotoxicity or nephrotoxicity, respectively.

Biochemical remission of disease was defined as a decrease in serum ALT activity to <1.1 ×ULN. Dogs that relapsed (ALT activity increased to >1.1 ×ULN) upon tapering of the CsA dose were recorded and, of those, dogs that achieved biochemical remission again after a dose increase also were recorded. Outcomes for each dog were categorized as alive, lost to follow-up, death, or euthanized because of hepatic or nonhepatic disease. If euthanasia was attributed to increased liver enzyme activity, decreased liver function test results, or progression in clinical signs (eg, gastrointestinal signs, ascites, hepatic encephalopathy, coagulopathy, thromboembolic disease), the outcome was categorized as euthanasia because of hepatic disease.

2.3 | Statistical analysis

Descriptive statistics were performed for the following variables: age (years), sex, breed, weight (kg), possible adverse effects of CsA, diet, concurrent medications, hepatic or gall bladder culture results or both, leptospirosis results, clinical score, presence of ascites, hypoalbuminemia, hyperbilirubinemia, rhodanine hepatic copper staining score, hepatic copper concentrations (μg/g dw), PT, aPTT, pretreatment and posttreatment serum ALP, ALT, AST, and GGT activity, total bilirubin (×ULN), albumin (×LLN), dose of CsA at biochemical remission (mg/kg/d), duration of treatment until biochemical remission (months), attainment of biochemical remission, and sustained biochemical remission or relapse upon tapering CsA. Frequencies and percentages were calculated for categorical variables. Means and medians with standard deviations or ranges, respectively, were calculated for continuous variables depending on data distribution.

Liver variable data for serum ALP, ALT, AST, and GGT activities, and serum total bilirubin and albumin concentrations were assessed for normality using the Shapiro-Wilk test. If the data were not normally distributed, a log transformation was performed. A univariable linear regression model was used to compare the serum liver test results at each time interval to baseline (t = 0) and identify statistical significance (P-value < .05). A generalized estimating equation was used to take into account repeated measures on each individual dog. A multivariable linear regression model was performed to evaluate the separate effects of dose or time interval on each liver variable. Type III P-values were reported to describe the change in liver variables across all time intervals during CsA treatment with or without concurrent medications.

The association of each clinical factor (ascites, hyperbilirubinemia, hypoalbuminemia, hepatic Cu > 1000 μg/g dw, prolonged PT, prolonged aPTT, pretreatment ×ULN ALT activity, clinical score, CsA dose, hepatoprotectant administration, institution, age, sex, and weight) with the probability of biochemical remission was analyzed using a Cox proportional hazards model of survival analysis. Dogs that did not achieve biochemical remission or were lost to follow-up were censored in the analysis. Hazard ratios were calculated for each clinical factor.

A log-rank test was used to compare time to remission within each categorical variable (ascites, hyperbilirubinemia, hypoalbuminemia, hepatic Cu > 1000 μg/g dw, prolonged PT, prolonged aPTT, and hepatoprotectant administration).

The association of duration of CsA treatment and biochemical remission was analyzed using a Wilcoxon 2-sample test. The change in serum ALT activity (×ULN) during treatment with or without hepatoprotectants was evaluated using a paired t test. All statistical analyses were performed by a statistician using a commercial software package (SAS v9.4, SAS Institute Inc, Cary, North Carolina).

3 | RESULTS

3.1 | Patient population

Forty-eight dogs were included in the study, of which 26/48 (54%) were female spayed, 20/48 (42%) were male castrated, and 2/48 (4%) were male intact. Median age was 8.5 years (range, 0.7-14 years).

Median weight was 19.4 kg (range, 2.2-45 kg). Twenty-two different breeds were represented. The most common were mixed breed (12), Labrador Retriever (10), and Standard Poodle (4). A complete list of breeds can be found in Supplemental Table S1.

3.2 | Medications

All dogs were treated with a microemulsified form of CsA (Nutramax Laboratories, Lancaster, South Carolina) approved for use in dogs. Only 1 dog was switched to an unknown brand of generic CsA after the dog attained biochemical remission. Cyclosporine was administered q12h (35/48, 73%) or q24h (13/48, 27%). In 4 of 13 dogs (31%), CsA subsequently was increased to q12h after ensuring the dog could tolerate q24hr administration. Forty-two of 48 (88%) dogs were receiving hepatoprotectants concurrently; including SAMe (Atopica, Elanco, Greenfield, Indiana) with or without silybin (79%, 38/48), ursodeoxycholic acid (60%, 29/48), vitamin E (29%, 14/48), or silybin (2%, 1/48). Nineteen of 48 dogs (40%) received a course of antibiotic treatment (combinations or monotherapy of amoxicillin, clindamycin, amoxicillin/clavulanic acid, metronidazole, or enrofloxacin, or some combination of these) before (7/19) or shortly after liver biopsy (12/19) was performed. Thirteen dogs (27%) were treated with prednisone or prednisolone before CsA and were tapered off corticosteroids completely before (1/13) or within 6 months of CsA initiation (12/13). The median starting dosage of prednisone or prednisolone was 1 mg/kg/d (range, 0.25-2 mg/kg/d) and median duration of corticosteroid treatment before CsA was 2 months (range, 0.5-5 months). Nine of 13 (69%) dogs were tapered off prednisone or prednisolone and transitioned to CsA because of a lack of improvement in serum liver enzyme activity. Two of the remaining 4 dogs received corticosteroids for a short period of time (2 weeks) before being changed to CsA. Serum liver enzyme activity results while on corticosteroids before starting CsA were not available for the other 2 dogs. One dog each was on azathioprine or mycophenolate concurrently, which were tapered and discontinued 7 months and 4 months after initiation of CsA, respectively.

Three dogs were being treated for ascites (spironolactone, furosemide, or both). One dog had been started on losartan and 1 on colchicine.

3.3 | Copper quantification and treatment

Copper-associated CH was ruled out in all dogs based on quantitative hepatic Cu determination or evaluation of qualitative Cu staining patterns. All dogs had either hepatic Cu quantification (45/48, 94%) or rhodanine Cu staining (34/48, 71%) performed. Median hepatic Cu was 576 μg/g (range, 106-1990 μg/g dw). Eleven dogs had hepatic Cu concentrations >1000 μg/g dw and 2 of these 11 had concentrations ≥1500 μg/g dw. On rhodanine staining, 5/11 had mild and 6/11 had moderate Cu accumulation. None of the dogs had staining predominately localized in the centrilobular areas. Seven of 11 dogs with high hepatic Cu were treated with penicillamine for a median of 6.7 months (range, 2-11 months) without normalization of biochemical results.

The remaining 4 dogs did not receive penicillamine, but 3 of 4 (75%) attained remission during CsA treatment.

Of the 14 dogs that did not have Cu staining performed, 13 had hepatic Cu quantification. These dogs had Cu concentrations <800 μg/g dw (median, 447 μg/g dw; range, 274-1350 μg/g dw). The 1 dog with hepatic Cu excess (1350 μg/g dw) and no rhodanine staining had no pigment seen on hematoxylin and eosin-stained sections, was not a breed predisposed to Cu accumulation, and achieved complete remission with CsA treatment.

Rhodanine staining was minimal to mild in 26/34 (75%) dogs and moderate in 8/34 (24%) dogs. In dogs with moderate staining, Cu was present in portal areas or in areas of inflammation and fibrosis without a clear centrilobular pattern in any dogs. In the 3 dogs without Cu quantification, Cu staining helped rule out Cu-associated hepatitis because these dogs showed mild accumulation (2/3) or negative Cu staining (1/3).

3.4 | Concurrent diet

Nineteen of 46 dogs (43%) with available diet information were on commercially available therapeutic hepatic diets (18/19; (Hill's Prescription Diet Canine l/d, Topeka, KS, or Royal Canin Veterinary Diet Canine Hepatic, Saint Charles, Missouri) or home-cooked low Cu diets (1/19). In 6 of 19, the diet was started a median of 3 months (range, 1-15 months) before CsA treatment. In 13 of 19 dogs, the diet was started before or within 2 weeks of starting CsA treatment.

3.5 | Comorbidities

Twenty-nine of 48 dogs had comorbidities, the most common being renal disease (3/29), idiopathic epilepsy (3/29), and urinary bladder uroliths (3/29). Additional information on comorbidities is included in Supplemental Materials.

3.6 | Liver culture results

Four of 40 (10%) aerobic liver cultures were positive. Organisms grown were *Acinetobacter* (1/4), *beta hemolytic streptococcus* (1/4), and *Staphylococcus* (2/4). One of the 37 (2.7%) anaerobic liver cultures was positive (*Clostridium septicum*). One dog had a bile culture performed, which was negative for aerobic and anaerobic growth.

3.7 | Leptospirosis results

Seventeen of 48 dogs (35%) had leptospirosis microscopic agglutination serology performed, of which 4 had positive reactions. All 4 dogs were vaccinated for leptospirosis, 1 month (2/4), 7 months (1/4), or 2.5 years (1/4) before serologic evaluation. Three dogs failed to show improvement in serum liver enzyme activity after treatment (ampicillin IV for 3 days or doxycycline PO for at least 2 weeks). The fourth dog had lower titers 4 months after initial testing without any antimicrobial treatment.

TABLE 1 Fold change in biochemical indices of liver injury at various time points post-CsA treatment

Time points	0	1	2	3	4	5	6	7	Type III P-value	Controlling for dose type III P-value
Time intervals	0	2-4 weeks	1-3 months	3-6 months	6-9 months	9-12 months	1-2 years	>2 years		
ALT (IU/L)	6.46 (2.1-27.0)	2.53 (0.36-58.0)*	1.15 (0.15-18.3)*	0.98 (0.23-8.1)*	0.91 (0.18-3.1)*	0.92 (0.17-4.3)*	0.79 (0.21-13.6)*	0.67 (0.2-4.4)*	<.0001[a]	<.0001[b]
	n = 48	n = 32	n = 36	n = 35	n = 31	n = 25	n = 27	n = 19		
AST (IU/L)	2.29 (0-10.7)	1.02 (0.35-27.6)*	0.66 (0.38-5.5)*	0.73 (0.20-2.8)*	0.56 (0.39-3.3)*	0.71 (0.44-2.3)*	0.76 (0.28-2.3)*	0.6 (0-2.3)*	<.0001[a]	.003[b]
	n = 42	n = 22	n = 26	n = 27	n = 24	n = 17	n = 23	n = 14		
ALP (IU/L)	2.68 (0.44-41.2)	1.56 (0.21-42.2)*	0.81 (0.09-13.8)*	0.89 (0.17-16.8)*	0.61 (0.11-3.1)*	0.51 (0.14-4.3)*	0.64 (0.15-45.8)*	0.61 (0.21-3.0)*	<.0001[a]	.004[b]
	n = 48	n = 30	n = 35	n = 33	n = 29	n = 23	n = 27	n = 19		
GGT (IU/L)	1.46 (0-35.1)	1.33 (0-33.6)	0.56 (0-9.2)*	0.42 (0-5.6)*	0.33 (0-4.8)*	0.43 (0-1.2)*	0.20 (0-22.9)*	0.26 (0-1.6)*	.002[a]	.004[b]
	n = 42	n = 23	n = 24	n = 25	n = 23	n = 19	n = 22	n = 16		
Total bilirubin (mg/dL)	1.25 (0.33-54)	1.50 (0-8.7)	1.00 (0.11-3.5)*	0.67 (0.33-3.5)*	0.67 (0.11-3)*	0.58 (0.11-2)*	0.50 (0-6)*	0.50 (0.11-5)*	.004[a]	.003[b]
	n = 46	n = 26	n = 29	n = 31	n = 25	n = 20	n = 27	n = 18		
Albumin (g/dL)	1.17 (0-1.6)	1.15 (0.6-1.4)	1.18 (0.9-1.8)	1.2 (0.85-1.5)	1.11 (0.85-1.7)	1.16 (0.82-1.8)	1.20 (0.93-1.6)	1.10 (0.69-1.6)	.21	.09
	n = 44	n = 27	n = 32	n = 31	n = 26	n = 21	n = 27	n = 18		

Notes: Results of the univariable regression model showing the medians and ranges for the fold increases over the upper limit of normal (ULN) for ALT, AST, GGT, ALP, bilirubin or fold decreases from the lower limit of normal (LLN) for albumin at each of the 7 time points post initiation of CsA. The asterisk indicates statistical significance (*P*-value of <.05) when comparing fold increase over the ULN or fold decrease below the LLN at each posttreatment time interval to baseline (t = 0). A type III *P*-value <.05, labeled with superscript "a," indicates an overall statistically significant decrease in liver values during CsA treatment. After controlling for CsA dose using a multivariable regression model, an adjusted type III *P*-value <0.05, labeled with superscript "b," denotes an overall significant decrease in liver values if CsA dose was held constant. "n" is the number of dogs with available liver parameter data at each time interval. Dogs were rechecked at irregular intervals and parameters like AST and GGT were not always evaluated. Also, some dogs were lost to follow-up, died, or were euthanized. Therefore, the "n" varies from time interval to time interval.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CsA, cyclosporine; GGT, gamma-glutamyl transferase.

3.8 | Liver variable changes and remission during CsA treatment with or without concurrent medications

Thirty-eight of 48 dogs (79%) attained biochemical remission of serum ALT activity during treatment with CsA with or without concurrent medications. The median starting dosage of CsA was 7.8 mg/kg/d (range, 1.5-12.7 mg/kg/d), and the median dosage at the time of biochemical remission was 7.9 mg/kg/d (range, 2.5-12.7 mg/kg/d). The median time to biochemical remission was 2.5 months (range, 0.75-12 months). After biochemical remission was reached, CsA was tapered in 33/38 dogs. Twenty-two of 33 (67%) dogs experienced a relapse (ALT ≥1.1 ×ULN)

when the CsA dose was tapered. The tapering protocol was not standardized. Nine of these 22 dogs achieved biochemical remission again when their CsA dose was increased. One dog was tapered off CsA completely and remained in biochemical remission for 12 months.

Twenty-four of 48 dogs (50%) were started on some combination of hepatoprotectant medications a median of 2 months (range, 0.5-24 months) before CsA. Treatment included some combination of SAMe and silybin (22/24, 91%), ursodiol (8/24, 33%), and vitamin E (5/24, 21%), or SAMe alone (2/24, 8.3%). Median ×ULN serum ALT activity was 6.0 (range, 1.8-31.5) pretreatment and 7.2 (range, 1.29-27) posttreatment, before starting CsA (P = .23).
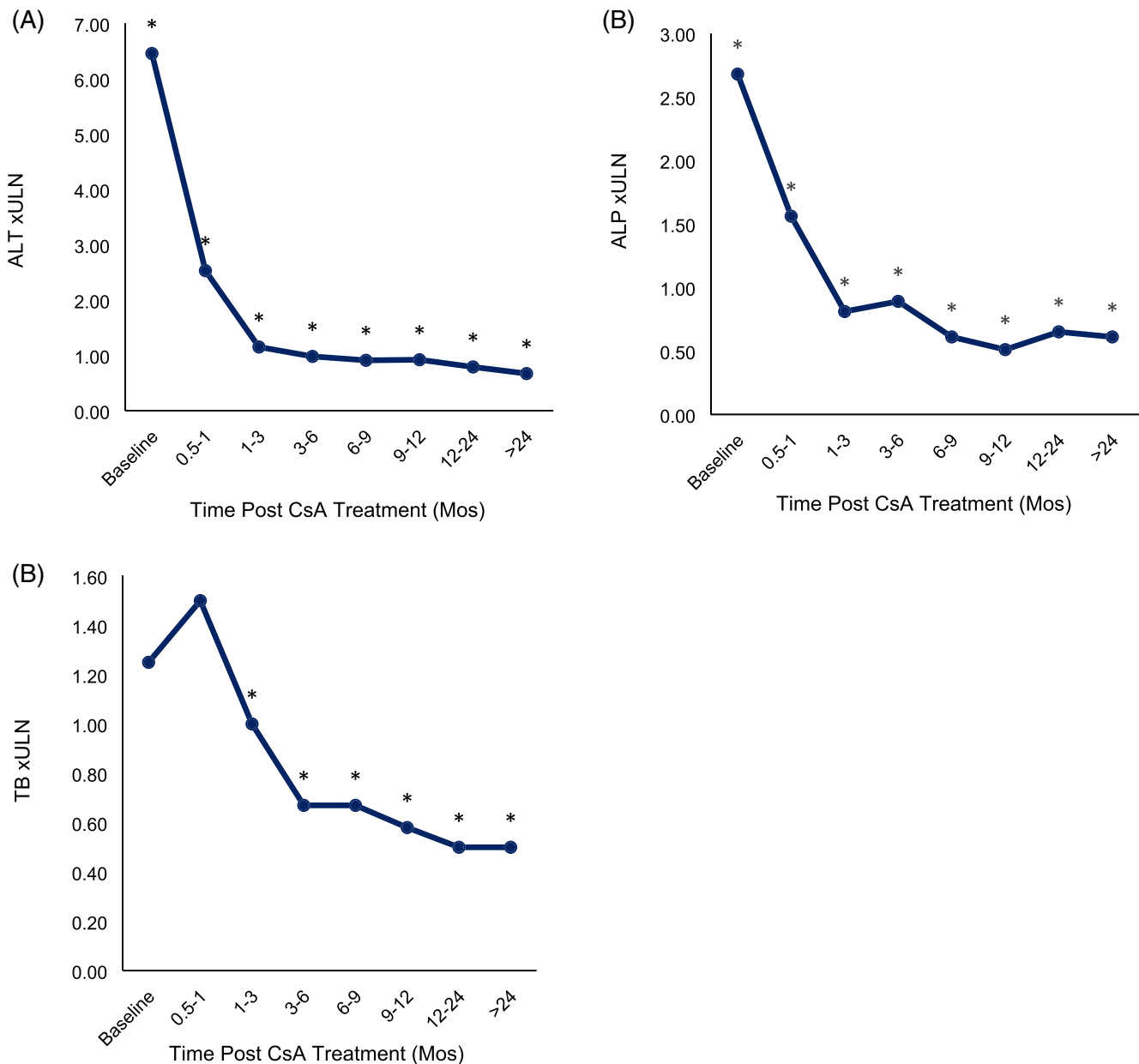
FIGURE 1 A, Median increase over the upper limit of the normal reference range (×ULN) for serum alanine aminotransferase (ALT) activity, B, serum alkaline phosphatase activity (ALP), and C, total bilirubin (TB) at each time interval after starting CsA (cyclosporine). The asterisk indicates the median value of ×ULN of each variable at that time interval was significantly reduced compared to the baseline value. The number of dogs with available data at each time interval is listed in Table 1. Mos, months

Medians and ranges for ×ULN serum ALP, ALT, AST, and GGT activities, bilirubin, and ×LLN albumin in the 7 defined time ranges during CsA treatment with or without concurrent medications are shown in Table 1. All liver variables except albumin showed significant decreases after starting CsA treatment. The fold increases over the ULN for serum ALT and AST activities were significantly decreased at posttreatment intervals 1-7 (P < .003) in comparison to baseline (Figure 1A and Table 1). The fold increases over the ULN for ALP and GGT activities were significantly decreased at posttreatment intervals 2-7 (P < .0001) and interval 3-7 (P < .0001), respectively (Figure 1B and Table 1). The fold increases in bilirubin were significantly decreased for time intervals 2-7 (P < .004). P values were not significant for ×ULN albumin at any time interval (Figure 1C and Table 1).

Type III P-values were used to assess the overall change for each liver variable during CsA treatment with or without concurrent medications. The fold increases over the ULN for serum ALP, ALT, and AST activities (type III P < .0001) and for serum GGT activity (type III P = .002) were significantly decreased posttreatment compared to baseline, but were not significantly different for ×LLN albumin (type III P = .21). When CsA dosage was held constant to remove the effect of this variable, the fold increases over ULN for serum ALP (P = .004), ALT (P < .0001), AST (P = .003), and GGT (P = .004) activities remained significantly decreased posttreatment. Type III P-values are presented in Table 1.

3.9 | Clinical factors and their effect on attaining biochemical remission

Ascites was present in 8/47 (17%), hypoalbuminemia in 6/47 (13%), hyperbilirubinemia in 25/47 (53%), prolonged PT or aPTT in 6/41 (15%), and increased hepatic Cu (>1000 µg/g dw) in 11/45 (24%) dogs. Cox proportional hazards model of survival analysis indicated that the probability of attaining biochemical remission was not significantly associated with the presence of ascites (P = .27), hyperbilirubinemia (P = .54), hypoalbuminemia (P = .11), hepatic Cu > 1000 µg/g (P = .86), or prolongation of PT or aPTT (P = .71). Median clinical score was 1.5 (range, 0-7). Clinical score ≥ 4 (P = .93), institution (P = .96), age (P = .95), sex (P = .93), weight (P = .10), pretreatment ×ULN ALT activity (P = .80), starting CsA dosage (P = .20), and duration of CsA treatment (P = .75) were not significantly associated with the likelihood of biochemical remission. Forty-two of 48 dogs were on hepatoprotective treatment (SAMe, silybin, vitamin E, ursodiol, or a combination of these) concurrently. Concurrent hepatoprotectant administration was not associated with likelihood of remission (P = .40).

3.10 | Clinical factors and their effect on time to biochemical remission

Log-rank analysis showed no significant differences in time to biochemical remission for the following clinical factors: ascites (P = .22), hyperbilirubinemia (P = .51), hypoalbuminemia (P = .08), hepatic Cu > 1000 µg/g dw (P = .85), prolonged PT or aPTT (P = .68), and hepatoprotectant administration (P = .38).

3.11 | Adverse effects

The most common adverse effects associated with CsA treatment were gastrointestinal signs in 18/48 (38%) dogs, including hyporexia, vomiting, diarrhea, or some combination of these (Table 2). Sixteen of 18 dogs experienced gastrointestinal signs that were transient and improved with a decrease in dosage or frequency of administration (7/17), freezing the capsule (2/17), adaptation to CsA treatment over time (ie, within 2 months; 5/17), administration with food (1/17), or a combination of an antiemetic and decreased CsA dosage (1/17). The second most common adverse effect was gingival hyperplasia, which occurred in 12/48 (25%) of dogs. Gastrointestinal signs were observed at a median of 3 weeks after initiation of CsA (range, 2 days to 5 months). Gingival hyperplasia was observed at a median of 13.5 months (range, 5-50 months) after initiation of CsA. Less common potential adverse effects included opportunistic infections (4/48), lymphoma (3/48), acute kidney injury (3/48), hirsutism (2/48), hepatotoxicity (1/48), papillomatosis (1/48), diabetes mellitus (1/48), and head tremors (1/48). The opportunistic infections observed were urinary tract infections (2; *Escherichia coli* and *Proteus*), pyoderma (1; with a *Bacillus* sp. grown on enrichment broth only), and dermatomycosis (1; specific species unknown).

Three dogs were diagnosed with lymphoma 6, 8, and 19 months after starting CsA. Cyclosporine dosages at the time of lymphoma diagnosis were 4.5 , 2.9, and 3.7 mg/kg/d, respectively, for each dog. All dogs had evidence of lymphoplasmacytic infiltrates on their initial liver biopsy samples, but no histologic evidence of lymphoma.

Two dogs experienced acute kidney injury 2 and 4 years after being treated with CsA, respectively. In both dogs, the cause was not identified, but in the first dog, necropsy showed severe chronic interstitial nephritis and fibrosis with glomerulosclerosis. One dog experienced clinical deterioration and an abrupt >2 times increase in ALT activity compared with baseline and subsequently was euthanized.

Cyclosporine was discontinued in 3 dogs for vomiting (1), suspected bronchopneumonia (1), and foaming at the mouth after receiving the liquid CsA (1) after 3 months, 4 years, and 2-3 weeks of CsA administration, respectively.

TABLE 2 Potential adverse effects associated with cyclosporine (CsA) administration

Adverse effects	Number of dogs (%)
Gastrointestinal signs	18 (38)
Gingival hyperplasia	12 (25)
Opportunistic infections	4 (8)
Acute kidney injury	3 (6)
Lymphoma	3 (6)
Hirsutism	2 (4)
Hepatotoxicity	1 (2)
Papillomatosis	1 (2)
Diabetes mellitus	1 (2)
Head tremors	1 (2)

3.12 | Outcome

Survival outcomes at the last available time interval (range, 24-65 months) were as follows: alive and doing well (21/48), lost to follow-up (10/48), euthanized because of nonhepatic disease (13/48), euthanized because of hepatic disease (2/48), or died or euthanized for unknown cause (2/48). Causes for euthanasia associated with nonhepatic disease were lymphoma (3), hyperadrenocorticism (1), acute kidney injury and sepsis (1), renal disease (2), diabetes mellitus (1), hypoxemic respiratory disease (1), other neoplasia (3), and decreased quality of life (1).

4 | DISCUSSION

In our retrospective case series, biochemical remission, defined as a decrease in serum ALT activity to <1.1 ×ULN, was attained in 79% of dogs treated with CsA with or without concurrent medications for presumed ICH. The median duration of CsA treatment necessary to obtain biochemical remission was 2.5 months (range, 0.75-12 months) and the median dosage in the dogs that attained biochemical remission was 7.9 mg/kg/d (range, 2.5-12.7 mg/kg/d). Significant improvements in serum ALP, ALT, AST, and GGT activities and serum total bilirubin concentration occurred during multimodal treatment that included CsA. None of the clinical features and biochemical variables previously associated with a poor prognosis in CH, such as ascites,[6,19] hypoalbuminemia,[6,15] hyperbilirubinemia,[6,45] prolonged PT or aPTT,[6,9,15] or higher clinical score[9] were significantly associated with likelihood of biochemical remission or time to biochemical remission. The most common adverse effects with CsA treatment were gastrointestinal upset and gingival hyperplasia. Severe adverse effects were rare and most likely unrelated to CsA administration. Our results provide preliminary evidence that CsA may be an effective treatment for dogs with suspected immune-mediated CH. However, because of its administration with concurrent medications in most cases and lack of a control group, substantiation of the effect of CsA as monotherapy for ICH awaits future prospective, randomized controlled clinical trials.

None of the previously reported negative prognostic factors[6,9,15,45] were significantly associated with the probability of biochemical remission or time to biochemical remission, indicating that treatment of presumed ICH with a multimodal approach, including CsA, may be successful even in dogs with later stage disease. The low incidence of some negative prognostic indicators (6/47 with hypoalbuminemia, 6/41 with prolongations in PT or aPTT, and 8/47 with ascites) however may have limited the statistical power of our study to find significant associations.

The failure of biochemical remission in 21% of the dogs in our study could be explained by individual variability in the pharmacokinetic and pharmacodynamic response to CsA.[24] Intrinsic differences of CsA disposition among dogs are associated with several factors including variations in cytochrome p450 activity, multidrug resistant 1 (MDR1) status, extent of protein binding, and the drug's volume of distribution. Other factors that could have impacted the pharmacokinetics of CsA in our study include administration of CsA with food,[46,47] concurrent medications,[48-50] and freezing the medication.[51] A previous study indicated that administration of CsA with food decreases bioavailability,[46] whereas a second study found no impact on drug efficacy in dogs.[47] Freezing the CsA capsule for 28 days has not been shown to affect integrity of the capsule, absorption of the medication, or plasma concentrations,[51] but the dogs in the previous study did not have hepatic disease.[52] Performing pharmacokinetic or pharmacodynamic testing, such as serum CsA concentrations or T-cell inhibition assays[53,54] would help elucidate the impact of such factors on the bioavailability and immunosuppressive effects of CsA in dogs with hepatic disease. Other potential explanations for failure to attain remission with CsA treatment are owner noncompliance, lack of therapeutic efficacy, comorbidities, or concurrent medications that impacted serum liver enzyme activity. Alternatively, dogs that failed to respond to CsA may not have had immune-mediated CH.

In human medicine, unlike veterinary medicine, immune-mediated hepatitis is a well-described condition with established diagnostic criteria and recommendations for treatment.[44] Prednisone and azathioprine are first line treatments, whereas CsA and mycophenolate are used as rescue agents or in cases refractory to prednisone and azathioprine.[43,44] No standardized diagnostic criteria or treatment approach for suspected immune-mediated hepatitis is available in dogs. Two retrospective studies evaluating the use of corticosteroids to treat CH in dogs have suggested that some dogs have a positive response to immunosuppressive treatment and thus meet 1 of the diagnostic criteria for immune-mediated disease.[15,16] The potential response in this study to CsA, a T-cell inhibitor with more targeted immunosuppressive activity, further supports this hypothesis.

Although previous studies suggested that corticosteroids were beneficial in some dogs with CH,[11,15,16] a number of associated adverse effects may occur,[29] including induction of increased serum liver enzyme activity (usually ALP > GGT > ALT) and development of vacuolar hepatopathy.[16,55] Corticosteroids also have the potential to worsen hepatic encephalopathy,[56] promote sodium retention and exacerbate ascites,[57] and promote hypercoagulability predisposing to thrombosis.[58] Corticosteroids even may be harmful in dogs with late-stage disease[16,59] and are contraindicated with comorbidities such as hypertension, cardiac disease, and diabetes mellitus. These consequences can be avoided by the administration of CsA. Additionally, the use of CsA makes it easier to monitor serum liver enzyme activity to assess response to treatment.

The most common adverse effects associated with CsA in our study were gastrointestinal signs, which typically were transient. Several studies have documented that gastrointestinal signs are the most common adverse effects in dogs treated with CsA for atopic dermatitis.[31-34] Gingival hyperplasia was the second most common adverse effect observed in our study, but typically it can be managed using azithromycin or gingivectomy if needed.[60,61] Severe adverse events, such as AKI, hepatotoxicity, or lymphoma have an uncertain association with CsA use. Only a single case of nephrotoxicity has been reported with CsA overdose[62] and 1 case of multicentric lymphoma has been associated with CsA administration.[63] Experimentally, CsA is reported to cause liver injury in rats.[64] However, the acute increase in ALT activity in 1 case in our study may have been caused by progression of hepatic disease.

Four cases had positive liver cultures, but biochemical remission was not achieved until the addition of CsA, which suggests that the primary etiology was immune-mediated and not infectious. Furthermore, the inflammatory infiltrate on hepatic histopathology was not consistent with primary bacterial cholangiohepatitis. In future studies, bile cultures and fluorescent in situ hybridization staining could be a better tool to identify bacterial organisms as compared to liver culture.

Our study had several limitations. Because it was a retrospective study, treatment and monitoring protocols were not standardized. The majority of the dogs in the study received concurrent medications, specifically hepatoprotectants. Hepatoprotective treatment before initiation of CsA, however, had no effect on serum ALT activity, and the use of these medications had no association with the likelihood of remission after the addition of CsA. Other medications (antibiotics, penicillamine, other immunodulatory drugs), diets formulated for hepatic disease, the presence of comorbidities, or some combination of these also could have affected liver enzyme activity after starting CsA. The effects of CsA may have been additive or synergistic with these other therapeutic interventions to improve clinicopathologic findings.

Reevaluation appointments were scheduled at different time points and follow-up was variable. Eleven of 48 dogs had unknown outcome data. Because of this lack of follow-up, survival analysis could not be performed. Additionally, there may have been selection bias toward dogs with more positive outcomes. The exclusion of dogs on CsA for <2 weeks and the selection of dogs at tertiary academic institution referral hospitals may have contributed to this bias.

Our study lacked an untreated control group of dogs not receiving CsA. A control group would have been optimal to evaluate CsA efficacy, but not providing immunosuppressive drugs to dogs with presumed ICH, a disease known to progress to cirrhosis and liver failure, might be construed as unethical. Future studies comparing CsA monotherapy with different immunosuppressive drugs such as corticosteroids are warranted.

Although established WSAVA criteria[8] were used for histopathologic diagnosis of CH and all biopsy reports were reviewed by a board-certified internist with expertise in hepatology, all biopsy samples were not interpreted by the same board-certified pathologist, which may have introduced variability in the final diagnosis, particularly when different biopsy methods[65,66] and scoring criteria were used. Interobserver agreement is reported to be fair to poor in the analysis of fibrosis and necroinflammatory activity.[67] Because of these limitations, we did not assess stage (severity of fibrosis) or grade (inflammation and degenerative change) on histopathology.

In humans, the diagnostic criteria for immune-mediated hepatitis are scored based on increased serum ALT activity, presence of positive serum autoantibody titers, increased serum immunoglobulin G concentration, characteristic histopathology findings (ie, interface hepatitis), and exclusion of other etiologies such as viral and alcoholic hepatitis.[43,44] These criteria have not been established for dogs.[68] Although medical records were comprehensively reviewed, given the retrospective nature of the study, it is possible that some cases were not actually ICH. Although we excluded Cu-associated hepatitis by a combination of quantitative Cu determination and qualitative Cu staining as well as

failure to respond to penicillamine chelation, it can be difficult to exclude Cu toxicosis as a cause of CH. It is well established that most commercial dog diets have excess Cu, which can contribute to a mild to moderate increase in hepatic Cu in dogs both with and without CH.[5] In addition, the lobe-to-lobe heterogeneity in hepatic Cu deposition and loss of ability to detect lobular Cu distribution in late stage disease complicates the diagnosis.[5]

Remission criteria are standardized in humans with immune-mediated hepatitis and include normalization of serum ALT activity as well as serum bilirubin and IgG concentrations, alleviation of clinical symptoms, and return to normal hepatic histopathology.[44] We chose serum ALT activity as a marker of biochemical remission because it is the most objective and sensitive indicator of hepatic necroinflammatory disease in the dog.[12,13,16] Serial clinical signs and scores were not extracted from medical records because of the subjective nature of such data and lack of follow-up. A future prospective study evaluating histologic as well as biochemical endpoints will be necessary to evaluate the efficacy of monotherapy with CsA in presumed ICH in dogs.

5 | CONCLUSION

In conclusion, we determined that CsA with or without concurrent medications may help significantly decrease serum ALT activity and attain biochemical remission in dogs with presumed ICH. Previously reported poor prognostic factors in CH were not associated with attaining biochemical remission. These findings provide further support that, in some dogs, ICH is immune-mediated. Because of limitations in this retrospective study in which CsA was not given as monotherapy, we cannot ascribe all changes to CsA. Further prospective studies will be necessary to determine whether CsA monotherapy results in clinical, biochemical, and histopathologic remission of disease. Further studies also are needed to compare CsA with other immunomodulatory medications, such as corticosteroids, and to determine which treatment is safer and more effective in attaining remission and extending survival in dogs with ICH.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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