PAPER

Treatment of canine idiopathic immune-mediated haemolytic anaemia with mycophenolate mofetil and glucocorticoids: 30 cases (2007 to 2011)

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Objective: To compare short-term outcome and frequency of adverse events for dogs with idiopathic immune-mediated haemolytic anaemia treated with glucocorticoids and mycophenolate mofetil *vs* alternate immunosuppressive protocols.

Methods: A retrospective study of medical case records of dogs with immune-mediated haemolytic anaemia was conducted. Data collected included signalment, clinicopathological data, medications administered, duration of hospitalization, short-term survival and adverse events. Variables were compared between dogs treated with glucocorticoids and mycophenolate mofetil (mycophenolate mofetil group) *vs* dogs treated with other two-drug immunosuppressive protocols (combined group).

Results: Sixty-four cases of idiopathic immune-mediated haemolytic anaemia were identified. Two dogs were euthanased without treatment, three received glucocorticoids alone, and seven received two additional drugs. Fifty-two dogs received glucocorticoids and additional immunosuppressive medications: 30 mycophenolate mofetil, 15 cyclosporine, 6 azathioprine and 1 human immunoglobulin. There was no significant difference between the discharge rate, 30-day or 60-day survival rates between the mycophenolate mofetil and the combined groups (Fisher's exact; P=0·272, 0·518, 1·000, respectively). The sole adverse event observed in the mycophenolate mofetil group was diarrhoea (n=5).

Clinical Significance: Administration of mycophenolate mofetil appears safe in dogs with idiopathic immune-mediated haemolytic anaemia. The combination of glucocorticoids and mycophenolate mofetil has similar efficacy to alternate immunosuppressive protocols used to treat this disease.

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INTRODUCTION

Canine immune-mediated haemolytic anaemia (IMHA) presents considerable therapeutic challenges. IMHA may result from intravascular haemolysis of erythrocytes following activation of complement and/or extravascular haemolysis subsequent to immunoglobulin opsonization and phagocytosis by the reticulo-endothelial system (McCullough 2003, Mitchell & Kruth 2010). IMHA occurs secondarily to infections, toxins, drugs, parasites and neoplasia although vaccines have also been implicated (Bloom *et al.* 1988, Reimer *et al.* 1999, Mellor *et al.* 2005, Mitchell & Kruth 2010, Swann & Skelly 2011, Whitley & Day 2011). Idiopathic or primary IMHA, reported in 60 to 75% of canine IMHA cases, is a diagnosis of exclusion (Reimer *et al.* 1999, Mitchell & Kruth 2010). Mortality rates for idiopathic IMHA range from 22 to 80%, with significantly increased mortality early in the disease course (Bloom *et al.* 1988, Reimer *et al.* 1999, McCullough 2003, Piek *et al.* 2008, Whelan *et al.* 2009, Ishihara *et al.* 2010, Mellett *et al.* 2011, Swann & Skelly 2011).

The mainstay of treatment for idiopathic IMHA is immunosuppression. Glucocorticoids, cyclophosphamide, azathioprine and cyclosporine have been used commonly; danazol, human

intravenous immunoglobulin (hIVIG), leflunomide, clodronate and mycophenolate mofetil (MMF) have also been used (Klag *et al.* 1993, Reimer *et al.* 1999, Weinkle *et al.* 2005, Piek *et al.* 2008, Whelan *et al.* 2009, Swann & Skelly 2011, 2013, Whitley & Day 2011). MMF is the prodrug of mycophenolic acid (MPA), an inhibitor of the enzyme inosine 5′-monophosphate dehydrogenase (IMPDH). IMPDH is necessary in lymphocytes for de novo synthesis of guanosine monophosphate for purines (Braun *et al.* 2002, Dewey *et al.* 2010). MPA inhibits T- and B-lymphocyte production, suppressing both cell-mediated and humoral immune responses (Storb *et al.* 1997, Yu *et al.* 1998, Barten *et al.* 2002, Braun *et al.* 2002, Howard *et al.* 2002, Lupu *et al.* 2006, Bacek & Macintire 2011).

In small animals, MMF (CellCept Intravenous; Genentech) has been used for organ transplantation and immune-mediated diseases (Creevy *et al.* 2003, Broaddus *et al.* 2006, Lupu *et al.* 2006, Yuki *et al.* 2007, Abelson 2009, Dewey *et al.* 2010, Bacek & Macintire 2011). Canine studies show rapid oral absorption with significant enterohepatic circulation with oral bioavailability reported as 54, 65 and 87% after 10, 15 and 20 mg/kg oral dosing, respectively (Langman *et al.* 1996, Lupu *et al.* 2006). Adverse events reported in dogs and cats include weight loss, diarrhoea, papillomatosis and allergic reactions (Hood & Zarembski 1997, Yu *et al.* 1998, Bacek & Macintire 2011). Purported advantages of MMF over other immunosuppressive drugs include the availability of oral and parenteral forms, rapid onset of action and lack of myelosuppression or hepatotoxicity (Langman *et al.* 1996, Yu *et al.* 1998, Whitley & Day 2011).

The aim of this retrospective study was to describe the use of MMF as an adjunctive immunosuppressive agent in client-owned dogs with idiopathic IMHA. It was hypothesised firstly that there would be no difference in the short-term outcome for idiopathic IMHA between dogs treated with glucocorticoids and MMF *vs* other immunosuppressive protocols, and secondly that adverse events would be less frequent in glucocorticoid- and MMF-treated dogs.

MATERIALS AND METHODS

This retrospective study was conducted at The University of Georgia Veterinary Teaching Hospital (VTH). Medical case records from 2007 to 2011 were reviewed to identify dogs diagnosed with idiopathic IMHA. Inclusion criteria included regenerative or non-regenerative anaemia [packed cell volume (PCV)<35%], with or without evidence of haemolysis (hyperbilirubinaemia, haemoglobinuria), and one or more of the following: (1) positive saline agglutination, (2) moderate to severe spherocytosis (Collicutt *et al.* 2012) and/or (3) positive Coombs' test (>1:16). Dogs were excluded if potential underlying causes of IMHA were identified. These included any neoplasia, whether a tumour was definitively diagnosed or metastases visualised on thoracic radiographs, positive serology (*Ehrlichia canis, Borrelia burgdorferi, Rickettsia rickettsia*; University of Georgia Infectious Diseases Laboratory) or polymerase chain reaction (*Anaplasma phagocytophilum, Anaplasma platys, Babesia canis, Babesia gibsoni, Babesia conradae, Babesia* spp., *Bartonella henselae, Bartonella vinsonii, Ehrlichia canis, Ehrlichia* spp., *Mycoplasma hemocanis/hematoparvum, Neorickettsia risticii, Rickettsia rickettsia,* Antech Diagnostics Canine Tick Borne Disease profile FastPanel™ PCR; Antech Diagnostic Laboratory) for tick-borne diseases, septicaemia or recent administration of cephalosporins, penicillins, sulphonamide antibiotics or phenobarbital.

Data collected included signalment, presenting complaint(s), initial vital parameters, bodyweight and body condition score (9-point scale), presenting PCV and total solids, complete blood count (CBC), biochemical profile, urinalysis, Coombs' testing, bone marrow cytology, diagnostic imaging (including thoracic and abdominal radiographs and ultrasound), short-term survival (30- and 60-day), medications, duration of hospitalization and adverse events. Adverse events potentially attributable to immunosuppressive therapy were extracted from the intensive care unit (ICU) flowsheets, communication logs and/or clinicopathological data. Referring veterinarians and owners were contacted for patient outcome and adverse events. Hepatopathy as an adverse event was defined as an alanine aminotransferase (ALT) increase to a value that was more than four times the upper limit of the laboratory's reference interval after therapy was started.

Dogs treated with glucocorticoids plus MMF comprised the MMF group (MMF). Dogs treated with glucocorticoids plus a single immunosuppressive drug other than MMF (cyclosporine, azathioprine or hIVIG) were analysed as a single Combined group (COMB). Dogs treated with glucocorticoids plus two other immunosuppressive agents, and dogs treated with glucocorticoids alone were excluded from further analysis.

Comparison of categorical factors between the MMF and COMB groups was performed by chi-squared or Fisher's exact test as appropriate. Comparison of continuous factors between groups was performed by a Student's *t*-test. The folded form *F* statistic evaluated whether variances were equal between groups. If unequal, then Satterwaithe's approximation for degrees of freedom for the Student's *t*-test was used. All hypothesis tests were two-sided and the significance level was P=0·05. Descriptive statistics are reported with mean ±sd. All statistical analyses were performed using commercially available software (SAS version 9.2; SAS Institute Inc.).

RESULTS

The retrospective analysis of the medical case records yielded 93 IMHA cases. Upon review, 22 cases had secondary IMHA [positive titre for *Ehrlichia canis* (n=3), neoplasia (n=6), septicaemia (n=3), acute pancreatitis (n=1), systemic mycosis (n=1), recent administration of cephalosporins (n=4), sulphonamide antibiotics (n=2), phenobarbital (n=2)]. Fifty-two dogs fulfilled the inclusion criteria yielding 30 in the MMF and 22 in the COMB groups, respectively.

There were no significant differences in age, bodyweight or condition score, heart or respiratory rate, or number of dogs vaccinated in the preceding 31 days between the two groups (data not shown). There were significantly (P=0·0041) more neutered

females in the MMF (n=18) compared with the COMB (n=6) group. Of the owner-reported clinical signs, lethargy (n=41), inappetence (n=34) and icterus (n=14) were the most prevalent. The COMB dogs were more likely to have inappetence (n=18; P=0·0329), collapse (n=5; P=0·0060) and dyspnoea (n=4; P=0·0151) than the MMF dogs (n=16, 0, 0, respectively).

There were no significant differences in CBC parameters between the two study groups (data not shown). Intravascular haemolysis was significantly (P=0·0120) more common in the MMF (n=10) than in the COMB (n=1) group. The MMF dogs were also more likely to have increased total bilirubin, cholesterol and blood urea nitrogen (BUN) than the COMB dogs [123 ±188 *vs* 22·2 ±2·55; reference interval 2 to 4 µmol, (P=0·0077), 7·35 ±2·94 *vs* 5·70 ±1·99; reference interval 2·58 to 5·85 mmol/L, (P=0·0306), and 8·78 ±4·36 *vs* 6·21 ±2·82; reference interval 3·6 to 7·1 nmol/L, (P=0·0135), respectively].

Bone marrow cytology was evaluated in seven MMF and nine COMB dogs. Four MMF and one COMB dog exhibited evidence of immune-mediated destruction of erythroid precursors. Thoracic radiographs were obtained in 28 of 30 MMF dogs, and 27 of 28 had no abnormalities detected. One dog had a focal interstitial-to-alveolar lung pattern, assessed to be most consistent with pulmonary thromboembolism (PTE). Other findings supporting PTE included echocardiographic evidence of pulmonary hypertension and increased D-dimer concentration. Thoracic radiographs obtained in 19 of 22 COMB dogs were all normal. Abdominal imaging was performed in 28 of 30 MMF dogs and 12 of 28 were considered unremarkable. Abnormal findings included splenomegaly (n=6), hepatomegaly (n=4), mild peritoneal effusion (n=4), enlarged hypoechoic pancreas (n=2), arterial thrombus (n=2), small adrenal glands (n=1) and nephrolithiasis (n=1). Abdominal imaging was performed in 20 of 22 COMB dogs, and 13 of 20 were considered unremarkable. Abnormal findings included splenomegaly (n=3), hepatosplenomegaly (n=1) and mild peritoneal effusion (n=3).

The MMF dogs received a mean dose of 2·6 mg/kg/day (range 1·3 to 4·0 mg/kg/day) prednisone, orally, or a mean dose of 0·3 mg/kg/day (range 0·1 to 0·4 mg/kg/day) dexamethasone sodium phosphate (dexSP), intravenously (iv). The COMB dogs received a mean dose of 2·9 mg/kg/day (range 1·5 to 4·4 mg/kg/day) prednisone, orally, and/or a mean dose 0·4 mg/kg/day (range 0·3 to 0·6 mg/kg/day) dexSP, iv. All dogs given both glucocorticoids received dexSP first, followed by prednisone (6 of 30 MMF and 2 of 22 COMB dogs) once they could tolerate oral medications. There was no significant difference between the prednisone (P=0·371) or dexSP dose (P=0·298) between the two groups. The MMF dogs received a mean dose of 20·5 mg/kg/day (range 13 to 50 mg/kg/day) MMF orally and/or parenterally . Fifteen COMB dogs received a mean dose of 10·6 mg/kg/day (range 5·0 – 20·0 mg/kg/day) cyclosporine, orally, six dogs received a mean dose of 1·9 mg/kg/day (range 0·7 to 2·7 mg/kg/day) azathioprine, and one dog received 0·5 g/kg hIVIG, iv.

Twenty-six MMF dogs (87%) received anticoagulants, including a mean dose of 0·6 mg/kg (range 0·5 to 1·3 mg/kg) aspirin, orally, every 24 hours; a mean dose of 300 U/kg (range 100 to 600 U/kg) heparin, subcutaneously (sc), every 8 hours; or a mean dose of 1·9 mg/kg (range 1 to 3·3 mg/kg) clopidogrel (Plavix®; Bristol-Myers Squibb/Sanofi Pharmaceuticals Partnership). Two dogs received aspirin and heparin, the other dogs received aspirin and clopidogrel (n=2), heparin and clopidogrel (n=1), aspirin alone (n=11), heparin alone (n=1) or clopidogrel alone (n=9). Twenty-eight MMF dogs (93%) received a gastroprotectant. Sixteen MMF dogs (53%) received an antibiotic and 14 dogs (47%) received an anti-emetic.

Twenty-one COMB dogs (95%) received anticoagulants, including a mean dose of 0·7 mg/kg (range 0·5 to 3·8 mg/kg) aspirin, orally, every 24 hours; a mean dose of 162·5 U/kg (range 100 – 250 U/kg) heparin, sc, every 8 hours; or a mean dose of 3 mg/kg (range 1 to 5 mg/kg) clopidogrel. One dog received all three anticoagulants and the other dogs received aspirin and heparin (n=2), aspirin and clopidogrel (n=2), aspirin alone (n=15) or heparin alone (n=1). Twenty-one COMB dogs (95%) received a gastroprotectant. Ten COMB dogs (45%) received an antibiotic and seven dogs (32%) received an anti-emetic. There were no significant differences between the frequency of administration of gastroprotectants (P=0·809), anticoagulants (P=0·538), anti-emetics (P=0·636) or antibiotics (P=0·700) between the two groups. There was no significant difference between the number of transfusions administered to MMF (1·9 ±1·6; range 0 to 7) and COMB dogs (1·5 ±0·86; range 0 to 3) (P = 0·208).

Adverse events attributed to immunosuppressive therapy were more commonly reported in COMB (15 of 21) than MMF dogs (5 of 30) (P=0·0002). In COMB dogs, these included vomiting (n=3), diarrhoea (n=3), hepatopathy (n=1), systemic mycosis (n=1) and hirsutism (n=1) among the 15 dogs given cyclosporine, and hepatopathy (n=4), vomiting (n=1) and diarrhoea (n=1) among the 6 dogs given azathioprine. Diarrhoea was reported in five MMF dogs. One dog had had diarrhoea at the time of presentation, which resolved by day 5 of hospitalization. Of the other four dogs, one received metronidazole and the diarrhoea resolved within 4 days; one dog was receiving amoxicillin/clavulanic acid (Clavamox®; Pfizer Animal Health) for an oesophagostomy tube site infection, and the diarrhoea resolved within 1 day of discontinuation of the antibiotic; one dog developed a gastric ulcer secondary to parenteral dexamethasone and diarrhoea resolved within 13 days following discontinuation of dexamethasone; and one dog still had diarrhoea when it was euthanized after discharge due to pre-existing epilepsy.

There was no significant difference in duration of hospitalization between the two groups (P=0·801; Table 1). Twenty-three

Table 1. Outcome measures for 52 dogs treated for idiopathic IMHA

Outcome	n	MMF group	n	Combined group	P value
Days hospitalized	30	5·8 ±4·6	22	5·5 ±2·6	0·801
Discharged alive	30	23	22	20	0·272
Survival at 30 days	30	21	21	18	0·518
Survival at 60 days	30	20	21	14	1·000
Adverse drug events	30	5	21	15	0·0002*

Continuous variables are presented as mean ±sd and categorical variables as counts. One dog from the "Combined group" was lost to follow-up after discharge. Statistically significant differences are indicated by *.

MMF (77%) and 20 COMB dogs (91%) survived to discharge. Twenty-one MMF dogs (70%) were alive at day 30, and 20 (67%) were alive at day 60. Eighteen COMB dogs (86%) were alive at day 30, and 14 (67%) were alive at day 60; 1 dog was lost to follow-up. There were no significant differences in discharge rates, 30-day survival or 60-day survival between the treatment groups (P=0·272, 0·518, 1·000, respectively).

Causes of death in the hospitalized MMF group included euthanasia because of poor response to therapy (n=6), thromboembolic disease confirmed by ultrasound examination (n=1) and concurrent disease (pre-existing epilepsy; n=1). Causes of death in the MMF group after discharge included euthanasia because of poor response to therapy (n=1) and thromboembolic disease confirmed by ultrasound examination (n=1); two dogs were lost to follow-up. Causes of death in the hospitalized COMB group included cardiac arrest (n=1) and euthanasia because of poor response to therapy (n=1). After discharge, causes of death in these dogs included cardiac arrest (n=1), and euthanasia because of relapse of IMHA (n=1), development of systemic mycosis (n=1) and septic arthritis (n=1). The cause of death was unknown in three dogs; one dog was lost to follow-up.

DISCUSSION

The results of this retrospective study fulfilled the hypothesis that there was no difference in short-term outcome for dogs with idiopathic IMHA between those treated with glucocorticoids and MMF or glucocorticoids and another adjunctive immunosuppressive agent. This was despite MMF dogs presenting with clinicopathological data shown to be associated with a poorer prognosis, including intravascular haemolysis, increased BUN and increased total bilirubin concentrations (Klag *et al.* 1993, Weinkle *et al.* 2005, Piek *et al.* 2008, Ishihara *et al.* 2010, Swann & Skelly 2011). Adverse events were also less frequent among MMF dogs, supporting the secondary hypothesis. MMF administration was only associated with transient diarrhoea in a small number of dogs.

The discharge rate from the hospital was 77% for MMF and 91% for COMB dogs. Survival at 60 days was equal between the groups (67%). These results are comparable with previously published reports of survival in canine idiopathic IMHA (Weinkle *et al.* 2005, Swann & Skelly 2011).

Glucocorticoids are the cornerstone of therapy for idiopathic IMHA (Whitley & Day 2011). Immunosuppressive doses of prednisone and other glucocorticoids induce numerous adverse effects such as polyuria, polydipsia, panting, weight gain, delayed wound healing, alopecia and muscle wasting (Piek *et al.* 2008, Cohn 2010, Whitley & Day 2011). While there is no definitive evidence that adjunctive immunosuppressive medications improve survival for IMHA patient (Piek *et al.* 2011, Swann & Skelly 2013), they are routinely added to broaden immunosuppressive mechanisms and to enable dose reduction or discontinuation of long-term glucocorticoids (Reimer *et al.* 1999, Burgess *et al.* 2000, Piek *et al.* 2008, Whelan *et al.* 2009, Swann & Skelly 2011, Whitley & Day 2011).

MMF use in the laboratory and veterinary clinical setting has been described previously (Yu *et al.* 1998, Yuki *et al.* 2007, Abelson 2009, Dewey *et al.* 2010, Ginel *et al.* 2010, Bacek & Macintire 2011). Its use with prednisolone in canine idiopathic IMHA induced resolution of anaemia in seven of eight dogs, with mild enteritis seen in 1 dog (Nielsen 2005). Another report described successful treatment of canine aplastic anaemia using 10 mg/kg MMF, every 12 hours (Yuki *et al.* 2007).

MMF is an attractive option for adjunctive treatment of canine idiopathic IMHA because of its oral and parenteral formulations, rapid onset of action, limited side effects profile and relative affordability. The option of a parenteral adjunctive immunosuppressive agent is particularly appealing in IMHA, as these patients often vomit and may experience poor gastrointestinal absorption of medications (Swann & Skelly 2011). If MMF were equivalently effective, these attributes would make it very appealing as an adjunctive treatment of idiopathic IMHA.

Azathioprine is only available in an oral formulation. Documented adverse effects include myelosuppression, hepatotoxicity, pancreatitis and gastrointestinal distress. Its immunosuppressive action may be delayed for several weeks after beginning therapy (Beale 1988, Houston & Taylor 1991, Rinkardt & Kruth 1996, Whitley & Day 2011). The idiosyncratic occurrence of myelosuppression and hepatotoxicity can present a monitoring challenge in IMHA dogs whose alkaline phosphatase is likely increased because of glucocorticoid therapy. In this study, five of six dogs treated with azathioprine experienced adverse events including significantly increased ALT, vomiting and diarrhoea.

The bioavailability of cyclosporine varies depending on formulation; modern microemulsified formulations (Atopica®; Novartis Animal Health US Inc.) have better and more consistent bioavailability (Steffan *et al.* 2004). An intravenous preparation exists, though its administration is labour-intensive because of the need to extensively dilute the carrier vehicle (McEvoy 2012). Adverse effects of cyclosporine include vomiting and diarrhoea, hirsutism, excessive shedding, gingival hyperplasia, papillomatosis, secondary infections and neoplasia (Blackwood *et al.* 2004, Callan *et al.* 2005, Robson 2003a, b, Swann & Skelly 2011). In this study, 10 of 15 dogs given cyclosporine experienced adverse events, including vomiting, diarrhoea, systemic mycosis and hirsutism that was bothersome to the owner.

Adverse effects of hIVIG include anaphylaxis, risk of type III hypersensitivity reactions, hypercoagulability and hypertension (Spurlock & Prittie 2011). A recent prospective study of dogs with IMHA showed no difference in survival to discharge between dogs given hIVIG and dogs not given hIVIG as part of their initial management (Whelan *et al.* 2009).

In this retrospective study, no significant differences in survival to discharge, short-term survival rates, duration of hospitalization or number of transfusions were observed between dogs given glucocorticoids with MMF and dogs given glucocorticoids with azathioprine, cyclosporine or hIVIG. However, fewer incidences of adverse side effects were reported in the MMF group. Previously reported adverse effects of MMF include weight loss, diarrhoea and allergic reactions (Yu *et al.* 1998, Bacek & Macin-

tire 2011). Diarrhoea in IMHA patients may be due to gastrointestinal injury from the primary disease, erosion or ulceration secondary to corticosteroids, and adverse effects of antibiotics or immunosuppressive drugs. In this study, the diarrhoea observed in MMF dogs was mild in all cases and did not warrant discontinuation or dose reduction of the drug.

There are several limitations to this study. The heterogeneity of immunosuppressive therapy within the COMB group prevents determination of a single best two-drug immunosuppressive protocol for canine idiopathic IMHA. There is currently a dearth of published patient-derived information for clinicians to evaluate adjunctive immunosuppressive therapies for IMHA (Piek 2011, Swann & Skelly 2013). This may be in part because large prospective, double-blinded, placebo-controlled studies of this complex condition are unusually difficult to perform. And yet, the high morbidity and mortality of the disease, and the diverse side effects of chronic, high-dose prednisone have led to the frequent use of adjunctive agents (Swann & Skelly 2011, Whitley & Day 2011). In this study, we hoped to determine whether MMF was likely to be equivalent in performance to the protocols commonly used. Not knowing which non-MMF two-drug immunosuppressive protocol was superior, they were analysed as a single group. The variety of two-drug immunosuppressive protocols contained within the COMB group enabled MMF to be compared against the realistic diversity of IMHA treatments commonly employed. However, the lack of a homogenous comparison group for MMF is a limitation of the study, in that the comparative performance of MMF with any individual adjunctive agent cannot be determined.

As another limitation of this retrospective analysis, although there were no differences in the number of dogs administered anticoagulants, antibiotics, anti-emetics or gastroprotectants between groups, the type, duration and dosage of these treatments varied among attending clinicians. While antithromboembolic drugs are routinely used during treatment of IMHA, no one protocol has been proven as superior (Mellett *et al.* 2011, Kidd & Mackman 2013). Additionally, dogs were not randomly assigned to treatment groups. A preponderance of dogs with intravascular haemolysis and increased BUN and total bilirubin concentrations received MMF, as compared to other adjunctive immunosuppressive drugs. Given that these factors have previously been associated with a worse prognosis (Klag *et al.* 1993, Reimer *et al.* 1999, Weinkle *et al.* 2005, Piek *et al.* 2008, Swann & Skelly 2011), this uneven distribution would suggest that dogs with the poorer prognoses were given MMF. That the MMF group did not underperform the COMB group in survival outcomes is even more interesting given this bias.

Significant toxic effects of MMF were not seen in this study. Most but not all study dogs were rechecked at the authors' institution (21 of 23 MMF and 17 of 20 COMB patients); one dog in the COMB group did not have a biochemistry performed after discharge and another dog was lost to follow-up. It remains possible that some adverse events were not recorded and more consistent follow-up laboratory data are recommended in future studies. Because of adverse events associated with long-term immunosuppression such as infections and the development of lymphoma, a gradual taper of MMF to the lowest effective dose appears judicious.

A prospective, randomized, double-blinded study comparing the effects of MMF with another adjunctive immunosuppressive would be ideal for comparison of hospitalization duration, treatment costs and survival (Piek 2011, Swann & Skelly 2013). While random assignment of dogs to different treatment groups does not guarantee better matching in terms of risk factors, larger group sizes may facilitate this. Such a study would require 86 dogs per group to demonstrate a 60-day survival rate difference of 20% with 80% power (nQuery 4.0; Statistical Solutions) based on previously reported 60-day survival rates for idiopathic IMHA (Weinkle *et al.* 2005, Piek *et al.* 2008).

In summary, this case series describes the clinical use of MMF in dogs with idiopathic IMHA. The purpose of the study was to retrospectively compare short-term outcome of canine idiopathic IMHA patients given MMF as an adjunctive immunosuppressive to that of patients given adjunctive azathioprine, cyclosporine or hIVIG, and to document adverse effects seen with MMF use. Based on our findings, immunosuppression with 10 mg/kg MMF, iv or orally, every 12 hours appears safe in dogs with idiopathic IMHA. The combination of glucocorticoids and MMF provides similar short-term outcomes and potentially fewer adverse side effects compared with other immunosuppressive protocols used to treat this disease.

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Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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