PAPER

Treatment of presumptive primary immune-mediated thrombocytopenia with mycophenolate mofetil versus cyclosporine in dogs

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Objectives: The objective of this study was to compare hospitalisation duration, survival times, adverse events and cost of therapy in dogs with presumptive primary immune-mediated thrombocytopenia undergoing therapy with mycophenolate mofetil and corticosteroids versus cyclosporine and corticosteroids.

Methods: A retrospective study of medical case records of dogs with presumed primary immune-mediated thrombocytopenia was conducted. Data collected included signalment, presenting complaints, haematologic and biochemical profiles, vector-borne disease testing, thoracic and abdominal radiographs, abdominal ultrasound, medications administered, duration of hospitalisation, 30- and 60-day survival, adverse events and cost of therapy. Variables were compared between dogs treated solely with mycophenolate mofetil and corticosteroids or cyclosporine and corticosteroids.

Results: A total of 55 dogs with primary immune-mediated thrombocytopenia were identified. Eighteen were excluded because multiple immunosuppressive medications were used during treatment. Hospitalisation times, 30-day survival and 60-day survival times were similar between both groups. Dogs in the mycophenolate mofetil/corticosteroid group experienced fewer adverse events than the cyclosporine/corticosteroid group. Therapy with mycophenolate mofetil was less expensive than that with cyclosporine.

Clinical Significance: These results suggest that using the combination of mycophenolate mofetil and corticosteroids appears to be as effective as cyclosporine and corticosteroids in the treatment of presumed primary immune-mediated thrombocytopenia in dogs. Adverse events were less common and cost of therapy was lower in the mycophenolate mofetil group. Additional larger prospective, controlled, double-masked, outcome-based, multi-institutional studies are required to substantiate these preliminary findings.

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INTRODUCTION

Immune-mediated thrombocytopenia (IMTP) results in the premature destruction of platelets secondary to auto-antibodies on their surface (Lewis & Meyers 1996). IMTP is classified as either secondary or primary. Secondary IMTP is the result of an underlying infectious, inflammatory, neoplastic or drug-induced aetiology. In contrast, primary IMTP is idiopathic in origin and diagnosed only after excluding primary causes. A definitive diagnosis of IMTP is made by detecting antiplatelet antibodies on

the surface of platelets; however, the currently available assays are not readily available and do not differentiate between primary and secondary IMTP (Kristensen *et al.* 1994, Scott *et al.* 2002). Patients with IMTP generally have moderate to severe thrombocytopenia, with counts being less than 50,000/μL (Jans & Armstrong 1990). However there have been reports of dogs with primary IMTP with platelet counts of 111,000/μL (Putsche & Kohn 2008). Platelets count of <30 k/μL have been associated with spontaneous bleeding (Williams & Maggio-Price 1984). Clinical signs associated with severe thrombocytopenia include bruising, gingival bleeding, melaena, haematemesis, haematochezia, haematuria, epistaxis, hyphaema, scleral haemorrhage and haemarthrosis (Lewis & Meyers 1996, Walton *et al.* 2014). The cause of the tendency for bleeding in primary IMTP, even in cases of significant thrombocytopenia is largely unknown, and may be related to increased levels of the anti-inflammatory cytokine, interleukin-10 (LeVine *et al.* 2014). Negative prognostic indicators for survival in dogs with IMTP include melaena and increased blood urea nitrogen (BUN) (O'Marra *et al.* 2011). Infectious agents that have been associated with IMTP include rickettsial, protozoal, nematodal and viral diseases (Axthelm & Krakowka 1987, Gould & McInnes 1999, Boozer & Macintire 2003, Bexfield *et al.* 2005). Drug-induced thrombocytopenia has been associated with sulpha-based antibiotics (Trepanier *et al.* 2003). Multiple malignant neoplasms have been associated with thrombocytopenia, with a higher risk of occurrence seen with haemangiosarcoma, lymphoma and melanoma (Grindem *et al.* 1994). The goal of therapy for IMTP is to maintain a normal platelet count after discontinuing immunosuppressive drugs and treatment of any secondary causes of thrombocytopenia (Jans & Armstrong 1990, Lewis & Meyers 1996).

Corticosteroids, azathioprine, cyclosporine, mycophenolate mofetil and leflumonide have all been used for their immunosuppressive properties. Corticosteroids continue to be a first-line drug in immune-mediated diseases due to their rapid onset of action in controlling disease, parental and oral administration and low cost. Side-effects can be severe, most notably with chronic administration, so additional immunosuppressive medication is often used to avoid using higher doses of corticosteroids (Jans & Armstrong 1990, Lewis & Meyers 1996, Stroup *et al.* 2006, Nakamura *et al.* 2012). Evidence-based support for use of the secondary immunosuppressive drugs is limited in veterinary literature.

Cyclosporine has been used in combination with prednisone to treat dogs with IMTP (O'Marra *et al.* 2011). Cyclosporine, a calcineurin inhibitor, inhibits transcription within activated T lymphocytes thus decreasing interleukin-2 and inflammatory cytokine formation (Whitley & Day 2011). Adverse effects can include vomiting, diarrhoea, inappetance, gingival ulceration, gingival hyperplasia, alopecia, hypertrichosis and an increased prevalence of secondary infections (Seibel *et al.* 1989, Radowicz & Power 2005, Allenspach *et al.* 2006). However, cost may limit the use of cyclosporine in veterinary patients. Mycophenolate mofetil is a newer immunosuppressive drug that is gaining clinical interest because of flexibility in its administration and low cost. Mycophenolate mofetil exerts its immunosuppressive effects by inhibiting inosine monophosphate dehydroxygenase (IMPDH) production by T and B lymphocytes after being hydrolysed in the intestines to the parent compound, mycophenalic acid. By blocking IMPDH, guanosine triphosphate production is decreased ultimately leading to reduction in DNA production (Allison & Eugui 2000). Previous studies have evaluated its use in immune-mediated haemolytic anaemia, IMTP, myasthenia gravis, inflammatory bowel disease, glomerulonephritis and meningoencephalomyelitis with variable success rates (Whitley & Day 2011, Wang *et al.* 2013, Barnoon *et al.* 2016). Gastrointestinal side-effects have commonly been reported with its use (Whitley & Day 2011, West & Hart 2014). In the authors' experience, anecdotal responses have been promising in regards to clinical remission with minimal gastrointestinal signs when dosed at 11 to 17 mg/kg every 24 hours.

The purpose of this retrospective study was to compare duration of hospitalisation, survival times, adverse effects and cost in dogs with presumptive IMTP treated exclusively with mycophenolate mofetil and corticosteroids (MC) versus cyclosporine and corticosteroids (CC). To the authors' knowledge, there have been no published reports comparing these two treatment groups in the management of IMTP in dogs.

MATERIAL AND METHODS

This retrospective study identified client-owned dogs treated at our hospital from January 2011 to October 2015 and diagnosed with presumptive primary IMTP. All dogs were either referred for a second opinion by another veterinarian or the client presented to our hospital as their first option. A search of the internal database was conducted to identify dogs with primary immune-mediated thrombocytopenia. Inclusion criteria included dogs with platelet counts <148,000/μL (reference interval 148,000 to 480,000/μL) without an identifiable aetiology, and that had immunosuppressive therapy initiated at our facility. No dogs had antiplatelet antibody testing performed because this test is not readily available, although it is required to confirm an immune-mediated component. Patients were presumed to have primary IMTP based on their response to therapy with immunosuppressive drugs. Signalment, presenting complaints and physical examination findings and complete blood count were evaluated in all dogs. The packed cell volume, biochemical profile, thoracic and abdominal radiographs, abdominal ultrasound, vector-disease serology, vector-borne disease polymerase chain reaction (PCR), prothrombin time (PT), activated partial thrombin time (APTT), intensive care flow sheets and communication logs (i.e. veterinary and client) were all used for data collection, when available. Physical exam findings, differences in haematologic and biochemical changes, adverse events that occurred after starting therapy, duration of hospitalisation, survival to 30 and 60 days, and cost of therapy were also compared between the two groups. Dogs were excluded if immunosuppressive medications other than mycophenolate mofetil, cyclosporine or glucocorticoids were administered at any point during treatment. Follow-up for all dogs were continued for a maximum of 24 months.

RESULTS

A total of 55 dogs were identified with presumptive primary IMTP. Of the 55 dogs, 18 dogs did not meet the inclusion criteria and were therefore excluded. Of these 18 dogs, nine were treated with cyclosporine and mycophenolate mofetil, seven were treated with azathioprine and cyclosporine, one was treated with cyclosporine and vincristine and one was treated with azathioprine, cyclosporine, mycophenolate mofetil and leflunomide due to refractory thrombocytopenia. A total of 37 dogs matched the inclusion criteria. Dogs were placed into one of the two groups based on the immunosuppressive regimen started at the time of diagnosis. The first group was treated exclusively with mycophenolate MC. The second group was treated exclusively with CC.

There were 20 dogs in the MC group versus 17 dogs in the CC group. Mean platelet counts for the MC group and CG group was 6000/μL and 7000/μL, respectively. Of 20 (90%) dogs, 18 in the MC group had a platelet count of <30,000/μL. Of 17 (76%) dogs, 13 in the CC group had platelets <30,000/μL. Mean ages for the MC and CC groups were eight and six years, respectively. The mean weight in the MC group was 32·8 kg versus 11·3 kg in the CC group. Signalment and initial platelet counts for both groups are shown in Table 1. A list of presenting complaints for dogs in both groups is shown in Table 2. Initial complete blood count (CBC) and biochemical findings are shown in Table 3. Prothrombin time (PT) and activated partial thrombin time (APTT) tests were performed in five (29%) of the dogs in the CC group and five (25%) dogs in the MC group, and all results were within the reference interval. Thoracic radiographs were taken in 11 (65%) of dogs in the CC group. All radiographs were reviewed by a board-certified radiologist when the dogs were evaluated by the attending clinician. Radiographic findings in this set of dogs included no significant findings (n=8), pleural effusion (n=1), cardiomegaly (n=1) and a diffuse bronchial pattern (n=1). Thoracic radiographs were performed in 14 (70%) of the dogs in the MC group and revealed no significant findings (n=11), pleural effusion (n=2) and a diffuse bronchial pattern (n=1). Abdominal radiographs were performed in five (29%) dogs in the CC group and revealed no significant findings (n=3), hepatomegaly (n=1) and loss of serosal detail in the right cranial quadrant (n=2). Four (20%) of the dogs in the MC group had abdominal radiographs taken. Three were classified as having no significant findings while the remaining dog had splenomegaly and cystic calculi. Abdominal ultrasound was performed in 14 (83%) of the dogs in the CC group. Abnormalities included hepatomegaly (n=3), pancreatitis (n=3), peritoneal effusion (n=1) and a blood clot in the urinary bladder (n=1). Abdominal ultrasound was performed in 17 (85%) of the dogs in the MC group. Abnormalities included pancreatitis (n=5), splenomegaly (n=2), splenic mottling (n=3), adrenomegaly (n=1), cystic calculi (n=1) and ileus (n=1). Vector-borne disease PCR and vector serology was performed in 11 (65%) and 2 (12%) in the MC and CC groups, respectively. Vector-borne disease PCR was performed in 12 (64%) of dogs in the MC group. Results of all infectious disease tests were negative.

Table 1. Signalment and initial platelet counts of dogs

Breed	Sex	Age (years)	Weight (kg)	Initial platelets (000/μL)
MC group				
Golden retriever	FS	8	38·1	10
Greyhound	MN	5	38·5	15
Shepherd mix	FS	9	34	9
Shepherd mix	MN	10	14·8	0
Bernese mountain dog	MN	6	56·8	2
Mixed	MN	8	36·4	0
Labrador retriever	FS	10	44·2	15
Rat terrier	MN	10	8	26
Labrador retriever	FS	6	32·3	0
Doberman pinscher	MN	8	37·2	0
Jack Russell terrier	FS	5	11·4	2
Chihuahua	MN	11	7·7	16
Shih-tzu	FS	9	7·2	31
Beagle mix	FS	6	19·6	3
Hound mix	MN	4	46·4	11
English setter	FS	13	15·2	0
Labrador retriever	FS	8	32·3	81
Labrador retriever	FS	10	28·1	0
Miniature schnauzer	FS	11	8·3	0
German shepherd dog	FS	2	39·5	29
CC group				
Dachshund	MN	12	10·3	5
Miniature schnauzer	FS	11	5·3	51
Rottweiler	MN	3	50	2
Chow chow	FS	5	22·8	0
Schnauzer	FS	6	11·3	7
Maltese	FS	9	4·4	32
Terrier mix	MI	7	6·9	0
Labrador retriever	FS	7	41·2	38
Pekingese mix	FS	3	3·5	0
Retriever mix	FS	15	40·3	11
Golden retriever	FS	5	31·4	56
Shih-tzu	FS	8	5·1	17
English bulldog	FS	5	25	0
Boxer	FS	6	28·1	7
Miniature schnauzer	FS	3	3·9	15
Goldendoodle	FS	3	25·5	1
Cairn terrier	FS	7	9·1	11

F female, M male, S spayed, N neutered, I intact

Table 2. List of presenting complaints in all dogs

Presenting complaint other than thrombocytopenia	Number of affected dogs
Lethargy	15
Bruising	12
Vomiting	8
Decreased appetite	8
Melaena	7
Hyphaema	5
Epistaxis	4
Febrile	4
Haematuria	3
Haematemesis	3
Diarrhoea	3
Coughing	2
Pancreatitis	2
Seizures	2
Haematochezia	2
Uveitis	1
Neutropoenia	1

Table 3. Haematologic and biochemical findings in both groups

Drug group	Cyclosporine group					Mycophenolate mofetil group				
Variable	n	Mean	sd	Minimum	Maximum	n	Mean	sd	Minimum	Maximum
ALB (g/dL)	13	3·18	0·42	2·2	3·9	18	2·89	0·61	1·8	4
ALB:GLOB	13	1·18	0·57	0·8	2·8	19	0·93	0·32	0·5	1·5
ALKP (U/L)	13	211·31	257·17	17	932	19	237·37	361·20	25	1573
ALT (U/L)	13	88·62	138·44	12	506	19	101·37	175·42	27	810
AMYL (U/L)	12	718·58	258·68	282	1091	14	1802·43	3287·18	383	13,091
BUN (mg/dL)	13	18·54	9·65	7	37	19	17·16	9·44	8	50
BUN:CREA	11	21·86	14·98	9	46	19	17·94	10·07	7	48
CHOL (mg/dL)	10	218·30	115·75	92	472	18	179·00	58·63	84	280
CREA (mg/dL)	13	0·92	0·18	0·7	1·3	19	1·09	0·55	0·4	2·4
Ca (mg/dL)	13	9·98	0·92	8·1	11·7	18	9·48	1·20	7·1	12·8
Cl (mmol/L)	11	115·36	2·66	112	119	19	109·21	18·29	35	119
GGT (U/L)	6	1·83	2·32	0	6	14	2·07	2·89	0	9
GLOB (g/dL)	13	3·20	0·72	1·6	4	19	3·37	0·90	2·3	5·9
GLU (mg/dL)	13	117·77	22·52	80	158	19	114·26	15·97	91	147
HCT (%)	17	35·25	15·62	11·3	65	20	37·10	14·29	7·7	68·2
HGB (g/dL)	17	11·88	5·07	3·2	21	19	13·01	4·26	1·9	19·4
K (mmol/L)	13	3·89	0·43	3·1	4·7	19	4·05	0·41	3·3	4·8
LIPA (U/L)	6	671·67	250·90	391	934	13	849·85	1462·2	34	5626
Na (mmol/L)	13	151·00	8·48	133	163	19	151·42	3·76	145	158
OSM_CALC (mmol/kg)	5	305·40	3·29	300	309	13	269·15	89·50	31	324
PHOS (mg/dL)	13	3·98	0·97	2·5	5·9	18	4·08	1·47	1·5	8·3
PLATELETS (K/uL)	17	14·88	18·24	0	56	20	12·5	19·11	0	81
PT (sec)	5	12·60	0·89	12	14	5	12·58	1·30	12	14·9
PTT (sec)	5	74·20	15·01	52	89	5	89·30	8·81	77	99·5
RBC (M/uL)	17	4·79	1·94	1·52	8·24	20	5·47	2·06	1·19	9·42
TBIL (mg/dL)	12	0·66	1·12	0·2	4·2	16	0·33	0·14	0	0·6
TP (g/dL)	13	6·55	1·15	4·5	8·3	20	6·31	1·04	4·6	8·9
WBC (K/uL)	13	16·6	9·55	6·3	40·04	20	15·54	8·48	1·2	25·5

The mean dexamethasone sodium phosphate dose in the CC group was 0·14 mg/kg/day (range 0·14 to 0·29 mg/kg/day). The mean prednisone dose in the CC group was 1·92 mg/kg/day (range 1·44 to 3·53 mg/kg/day). The mean cyclosporine dose was 7·8 mg/kg/day (range 3·6 to 16·4 mg/kg/day). The mean mycophenolate mofetil dose was 14·2 mg/kg/day (range 10·7 to 17·8 mg/kg/day). The mean dexamethasone SP dose in the MC group was 0·15 mg/kg/day (range 0·12 to 0·18 mg/kg/day). The mean prednisone dose in the MC group was 1·24 mg/kg/day (range 0·51 to 2·03 mg/kg/day). Doxycycline was administered to 11 (55%) of dogs in the MC group and 7 (41%) of dogs in the CC group. The mean doxycycline dose in the CC group was 13 mg/kg/day (range 9·1 to 18·6 mg/kg/day). The mean doxycycline dose in the MC group was 11·6 mg/kg/day (range 8·8 to 13·8 mg/kg/day). Doxycycline was discontinued in all dogs once the infectious disease test results were available.

A CBC was performed daily in all dogs that were hospitalised until the time of discharge. Twelve (60%) dogs in the MC group were hospitalised, whereas 13 (76%) dogs in the CC group were hospitalised. In contrast, the initial follow-up for dogs that were never hospitalised ranged between 2 and 5 days. Duration of hospitalisation for the MC group and CC group ranged from 0 to 7 days (median=3 days) and 0 to 6 days (median=3 days), respectively. Sixteen (94%) of the dogs in the CC group were discharged from the hospital and eighteen (90%) of the MC group were discharged. The three dogs that were not discharged were euthanased after starting therapy at the request of the owner. The median periods for platelets to reach >50,000/µL in the MC group and CC group were 2 and 3 days, respectively. Normalisation of platelet counts (>148,000/µL) ranged from 5 to 14 days (median=10 days) in the MC group, and 6 to 14 days (median=9 days) in the CC group. Adverse events were noted in 11 (65%) of the dogs in the CC group after starting therapy and included diarrhoea (n=3), anaemia (n=3), pancreatitis (n=1), panting (n=1), haematuria (n=1), septicaemia (n=1) and lick granuloma (n=1). Nine (45%) of the dogs in the MC group developed adverse events after starting therapy and included pancreatitis (n=3), recurrence of thrombocytopenia (n=3), haematochezia (n=2) and anaemia (n=1). Fifteen (88%) of the dogs in the CC group survived to 30 days and 14 (82%) survived to 60 days. Seventeen (85%) of the dogs in the MC group survived to 30 days and 16 (80%) survived to 60 days. The dog with septicaemia died after initial discharge. Two dogs were lost to follow-up. Comparison of hospitalisation and survival times are shown in Table 4.

In our clinic, approximate monthly cost for a 32-kg dog receiving a dose of 15 mg/kg mycophenolate mofetil orally daily was $45 versus $300 if treated with a dose of 6 mg/kg cyclosporine orally daily).

DISCUSSION

The diagnosis of IMTP continues to be challenging and is generally made by exclusion of other known causes of thrombocytopenia. Unfortunately there is no gold standard available to diagnose

primary immune-mediated thrombocytopenia. The diagnosis is best established with a combination of bone marrow examination and exclusion of other causes of thrombocytopenia. Antiplatelet antibody testing has been used with some success to confirm the diagnosis but the assay is not commercially available and no test has the ability to differentiate between secondary and primary immune-mediated thrombocytopenia (Kristensen *et al*. 1994, Scott *et al*. 2002, Bachman *et al*. 2015). Furthermore, there is no standard cut-off of platelet count by which to diagnose cases of IMTP because secondary causes of thrombocytopenia can also result in moderate to severely decreased platelet counts. The lack of consensus as to the definition of IMTP makes performing a study challenging and case selection a limiting factor in most retrospective studies. Other IMTP studies have used a cutoff of platelets counts ranging from <50,000 to a high of <150,000 for inclusion of cases with a possible IMTP diagnosis (Jans & Armstrong 1990, Putsche & Kohn 2008). Given this large range we elected to use a cut-off of <148,000 platelets to presumptively diagnose IMTP.

This retrospective study found that survival times and hospital duration were similar in dogs undergoing therapy for presumptive primary IMTP with CC versus mycophenolate MC. Both 30- and 60-day survival times within the MC group and CC group were 85 and 80, and 88 and 82%, respectively. Survival times were similar to those in a previous report of dogs with IMTP that underwent therapy with mycophenolate mofetil as a sole therapy (Yau & Bianco 2014). Hospitalisation times were similar between groups in this study, ranging from 0 to 7 days (MC) and 0 to 6 days (CC). These times are similar to dogs in previous studies undergoing therapy for presumptive IMTP (Yau & Bianco 2014). Recent reports of survival from hospitalisation to discharge ranged from 84 to 100% (Putsche & Kohn 2008, O'Marra *et al*. 2011, Yau *et al*. 2014). In the current study, 94% (n=16) of the dogs in the CC group survived to discharge, while 90% (n=18) of dogs in the MC group survived to discharge. It is possible that survival rates at discharge were higher because multiple immunosuppressive agents were used and the sample population was smaller. Previously noted negative prognostic indicators included both elevated BUN and melaena (O'Marra *et al*. 2011). Of the three dogs that did not survive to discharge, one had an elevated BUN and one had melena, both of which were noted prior to starting immunosuppressive therapy. Other clinical signs in these dogs included lethargy, hematemesis, bleeding from gums and hyporexia. Additional haematologic and biochemical changes in dogs not surviving to discharge in the current study included anaemia, leucocytosis, and hypoproteinaemia. Pleural effusion was noted on thoracic radiographs in two of these dogs. Pleural and peritoneal effusions are not common findings in dogs with primary IMTP. Based on the bleeding tendencies noted in IMTP, it could be presumed that the fluid was haemorrhagic but samples were not obtained for confirmation, nor were post-mortem examinations performed. In a previous case report of primary IMTP, the dog was diagnosed with haemorrhagic effusion (Middleton 2005). There is also a single case of a dog with idiopathic IMTP with haemarthrosis (Walton *et al*. 2014).

The hepatic and splenic changes that were observed may have been the result of platelet phagocytosis by the reticulendothelial system, extramedullary haematopoiesis, benign hyperplasia or malignancy. No dogs had cytologic or histologic evaluation performed. Although the overall prognosis for IMTP is favourable, it is important to recognise certain clinical signs, haematologic, biochemical and radiographic changes that could potentially influence the owner's decision to continue with treatment if historically associated with a more guarded outcome.

Sixty-five percent of dogs in the CC group experienced adverse events, while 45% exhibited adverse events in the MC group. The most common adverse events reported in the CC group were diarrhoea and anaemia. The anaemia was most likely a result of the thrombocytopenia rather than an adverse drug reaction to cyclosporine. The most common reported findings in the MC group were pancreatitis and recurrence of thrombocytopenia. Mycophenolate mofetil has been associated with gastrointestinal toxicity; with diarrhoea being most commonly reported (Dewey *et al*. 2010, Yau & Bianco 2014). Gastrointestinal side effects associated with a dose of 20 mg/kg mycophenolate mofetil every 12 hours may also be dose-dependent (Barnoon *et al*. 2016). Interestingly, pancreatitis was seen in six (16%) of the dogs in the current study. Two dogs initially presented for evaluation of pancreatitis and thrombocytopenia, while four dogs developed pancreatitis after a diagnosis of IMTP was made. Pancreatitis has previously not been reported to be associated with IMTP. A diagnosis of pancreatitis was confirmed via abdominal ultrasound, canine SNAP pancreatic lipase, or the canine pancreatic lipase immunoreactivity (Texas A&M University Veterinary Medical & Biomedical Sciences). Although not fully understood, severe thrombocytopenia and bleeding into the pancreas may result in active pancreatitis. Another possibility is that there may be primary active pancreatitis, which can result in a systemic inflammatory response and ultimately opsinisation and destruction of platelets. Lastly, pancreatitis may be a sequela of the severe inflammatory response associated with immune-mediated disease. More studies are needed to further evaluate a possible cause and effect.

Diarrhoea was observed in three dogs in the CC group and none of the dogs in the MC group. This is in contrast to previous reports of diarrhoea being a limiting factor in mycophenolate mofetil therapy (Barnoon *et al*. 2016). The limited gastrointestinal side effects noted in this study should be considered an important finding and serve as a basis for future dosing recommendations when using mycophenolate mofetil for immune-mediated diseases. Pharmacodynamics studies would be required to fully assess the validity of this claim. A previous pharmacodynamic study in a canine model found effective immunosuppression when doses of 20 to 40 mg/kg orally daily were used (Langman *et al*. 1996). Another pharmacodynamic study found that oral mycophenolate mofetil did not have detectable immunosuppressive effects when dosed at 10 mg/kg every 12 hours (Mackin *et al*. 2016). Based on the results of these two studies, the doses of mycophenolate mofetil used in the current study should not have provided any appreciable immunosuppressive effects, therefore implying that the corticosteroids were the

Table 4. Comparison of outcomes after treatment

	HD	DS	s30	s60	
CC group					
1	3	+	+	+	Anaemia
2	5	+	+	+	Diarrhoea
3	9	+	+	+	Haematuria
4	7	–	–	–	Diarrhoea
5	2	+	+	+	–
6	0	+	+	+	Panting
7	4	+	+	+	Lick granuloma
8	6	+	+	+	–
9	0	+	+	+	Diarrhoea
10	3	+	+	+	Septicaemia
11	2	+	+	–	Anaemia
12	2	+	+	+	Anaemia
13	0	+	–	–	–
14	0	+	+	+	–
15	3	+	+	+	–
16	6	+	+	+	–
17	3	+	+	+	–
MC group					
1	3	+	+	+	Relapse 24 months
2	0	+	+	+	Relapse 12 months
3	6	+	+	+	–
4	4	+	+	+	Anaemia
5	0	+	–	–	–
6	0	+	+	+	Relapse 7 months
7	0	+	+	+	Haematochezia
8	3	+	+	+	–
9	4	+	+	–	–
10	0	+	+	+	–
11	5	–	–	–	–
12	0	+	+	+	
13	3	+	+	+	Haematochezia
14	3	+	+	+	–
15	5	+	**Unknown**	**Unknown**	–
16	0	+	+	**Unknown**	–
17	2	+	+	+	–
18	4	+	+	+	–
19	5	–	–	–	
20	0	+	+	+	–

HD hospitalisation duration, DS discharge status, s30 survival to 30 days, s60 survival to 60 days, MC mofetil and corticosteroids, CC cyclosporine and corticosteroids

primary contributor in achieving remission in these dogs. A possible explanation for the positive response in the MC group may be that when used in conjunction with corticosteroids, the immunosuppressive effects may be additive. Future pharmacodynamic studies examining the immunosuppressive effects of oral mycophenolate mofetil with and without concurrent corticosteroid administration would be needed to validate this claim.

The anaemia, haematuria and haematochezia were likely a direct result of the existing thrombocytopenia and the predisposition to bleeding seen in IMTP. Panting was most likely due to the corticosteroids (Stroup *et al.* 2006). Septicaemia may have been secondary to immunosuppression (Seibel *et al.* 1989).

Remission times in the three dogs that relapsed in the MC group ranged from 7 to 24 months, with a median of 12 months. All dogs had discontinued immunosuppressive medications at the time relapse had occurred. A recent study of dogs with presumptive IMTP undergoing mycophenolate therapy revealed no haematologic or biochemical changes for a period of 5 to 32 months (Yau & Bianco 2014). There were no dogs in the CC group that showed any evidence of a relapse of clinical signs after starting therapy.

Primary IMTP is one of the most common causes of thrombocytopenia (Grindem *et al.* 1991, Botsch *et al.* 2009); however, the consistent use of mycophenolate mofetil and other immunosuppressive drugs as a first-line therapy is generally not advocated. Corticosteroids remain the first-line treatment in immune-mediated diseases despite the frequently reported adverse effects of polyuria, polydipsia, polyphagia, weight gain, ligament rupture, diabetes mellitus, dermatologic changes and haematologic and biochemical abnormalities (Stroup *et al.* 2006). For this reason, other immunosuppressive agents are often used in conjunction with corticosteroids to minimise the need for higher doses and mitigate potential adverse effects. Although there are previous reports of mycophenolate mofetil being used in veterinary medicine to treat various immune-mediated diseases and possibly having steroid-sparing effects, reports are sparse and sample populations are small (Abelson *et al.* 2009, Ginel *et al.* 2010, Whitley & Day 2011, Segev *et al.* 2013). Historically, this may have been due to the lack of familiarity with the drug, cost and availability. More recently, the cost of mycophenolate mofetil has decreased, thus making it less cost-prohibitive to owners when compared to cyclosporine.

The main limitations of this study are related to its retrospective nature, including lack of randomisation, masking, standardised diagnostic investigation, standardised treatment protocols, standardised outcome measures and small number of enrolled dogs. Consequently, robust conclusions regarding the efficacy of mycophenolate mofetil compared to cyclosporine for the treatment of presumed primary IMTP in dogs cannot be drawn from the results of this study.

In conclusion, treatment with mycophenolate mofetil in addition to glucocorticoids should be considered an option in dogs with primary IMTP. This study showed that hospitalisation and survival times in dogs treated with the combination of mycophenolate and corticosteroids were similar when compared to treatment with CC. Pancreatitis has previously not been reported to be associated with primary IMTP; however, because of the findings in this study, clinicians should be aware of this disease when managing dogs with primary IMTP. Additionally, the frequency of adverse events with mycophenolate mofetil may be lower when dosed between 11 and 17 mg/kg/day and the cost of therapy is less when compared to cyclosporine. Further larger prospective, controlled, double-masked, outcome-based, multi-institutional studies are required before claims that combination of mycophenolate MC should be utilised as the treatment of choice in dogs with primary IMTP.

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Conflict of interest

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

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Supporting Information

The following supporting information is available for this article:

Table S1. Platelet ranges in MC and CC