

STANDARD ARTICLE

The use of high-dose immunoglobulin M-enriched human immunoglobulin in dogs with immune-mediated hemolytic anemia

Jason P. Bestwick¹  | Mellora Sharman¹  | Nat T. Whitley² |
 Caroline Kisielewicz³ | Barbara J. Skelly⁴ | Simon Tappin⁵  |
 Lindsay Kellett-Gregory⁶ | Mayank Seth¹

¹Animal Health Trust, Suffolk, United Kingdom

²Davies Veterinary Specialists, Hertfordshire, United Kingdom

³Pride Veterinary Centre, Derby, United Kingdom

⁴Queen's Veterinary School, Cambridge, United Kingdom

⁵Dick White Referrals, Station Farm, Cambridgeshire, United Kingdom

⁶Queen Mother Hospital for Animals, The Royal Veterinary College, Hertfordshire, United Kingdom

Correspondence

Jason P. Bestwick, Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, CB3 0ES, United Kingdom. Email: jpb205@cam.ac.uk

Present address

Jason P. Bestwick, Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, CB3 0ES, United Kingdom

Mellora Sharman, VetCT, St John's Innovation Centre, Cowley Road, Cambridge, CB4 0WS, United Kingdom

Caroline Kisielewicz, Vet Oracle Telemedicine, CVS Group, Owen Road, Diss, Norfolk, IP22 4ER, United Kingdom

Lindsay Kellett-Gregory and Mayank Seth, Dick White Referrals, Station Farm, London Road, Six Mile Bottom, Cambridgeshire, CB8 0UH, United Kingdom

Abstract

Background: The IV use of human immunoglobulin (hIVIG) in dogs with primary immune-mediated hemolytic anemia (IMHA) has been described previously, but herein we describe the use of high-dose IgM-enriched hIVIG (Pentaglobin).

Hypothesis/Objectives: Dogs treated with high-dose Pentaglobin will experience shorter time to remission and hospital discharge and have decreased transfusion requirements compared to dogs receiving standard treatment alone.

Animals: Fourteen client-owned dogs diagnosed with primary IMHA at specialist referral hospitals in the United Kingdom.

Methods: All prospectively enrolled dogs received prednisolone, dexamethasone or both along with clopidogrel. Patients were randomized to receive Pentaglobin at 1 g/kg on up to 2 occasions, or to serve as controls. No additional immunosuppressive drugs were allowed within the first 7 days of treatment. Remission was defined as stable PCV for 24 hours followed by an increase in PCV.

Results: Ten of 11 dogs from the treatment group and 2 of 3 dogs from the control group achieved remission and survived until hospital discharge. Survival and time to remission were not significantly different between groups. The volume of packed red blood cells transfused, normalized for body weight, was not significantly different between groups. Potential adverse reactions to Pentaglobin occurred in 2 dogs, but their clinical signs may have been related to the underlying disease.

Conclusions and Clinical Importance: Treatment with high-dose Pentaglobin was well tolerated by dogs with primary IMHA but no significant advantage was found in this small study. Additional studies examining larger groups and subpopulations of dogs with primary IMHA associated with a poorer prognosis are warranted.

Abbreviations: BUN, blood urea nitrogen; DAT, direct antiglobulin test; hIVIG, human IV immunoglobulin; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, Immunoglobulin M; IMHA, immune-mediated hemolytic anemia; PCV, packed cell volume; PRBC, packed red blood cell; TED, thromboembolic disease.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. *Journal of Veterinary Internal Medicine* published by Wiley Periodicals LLC on behalf of American College of Veterinary Internal Medicine.

KEYWORDS

autoimmune, dog, hIVIG, IMHA, Pentaglobin

1 | INTRODUCTION

Human IV immunoglobulin (hIVIG) is created by purification of large volumes of donor plasma.¹ Depending on specific preparations, hIVIG typically contains immunoglobulin G (IgG) as its largest component with smaller and variable amounts of immunoglobulin A (IgA), immunoglobulin M (IgM), cluster of differentiation 4 (CD4), cluster of differentiation 8 (CD8), and human leukocyte antigen molecules.² In the United Kingdom, several hIVIG products are available, all of which contain IgG as the primary immunoglobulin component (typically >90%) along with a small amount of IgA and most have IgM as only a negligible or trace fraction of the total immunoglobulin (<0.1 mg/mL). One product, Pentaglobin, is unusual in this respect, being IgM-enriched and containing 50 mg/mL human plasma protein, of which immunoglobulin is at least 95%: 38 mg/mL IgG (76%), 6 mg/mL IgA (12%), and 6 mg/mL IgM (12%). In contrast to IgG that is associated with longer term humoral immunity, but provides more specific immunogenic functions to particular diseases, IgM is associated with polyreactivity, which allows B-lymphocytes that can produce IgM to rapidly respond to many antigens.³

In human medicine, hIVIG is used primarily to manage patients with immunodeficiencies, but it also is used to treat several inflammatory, infectious, and autoimmune diseases.^{1,4-6} Low doses are administered at regular intervals as replacement therapy for patients with immunodeficiencies whereas high-dose regimens more frequently are used for immunomodulatory treatment of patients with inflammatory or autoimmune diseases.⁷ Use of hIVIG in human medicine also has been reported to prevent hemolysis associated with sickle cell disease, and for blood transfusion in patients with lymphoma.¹ Similarly, in veterinary medicine hIVIG has been used for several diseases,^{2,8} including immune-mediated hemolytic anemia (IMHA),⁹⁻¹³ immune-mediated thrombocytopenia,¹⁴⁻¹⁶ cutaneous drug reactions,^{17,18} and myasthenia gravis.¹⁹

The mechanism of action of hIVIG is complex and incompletely understood. Elucidated mechanisms include binding to inhibitory and activating fragment crystallizable (Fc) receptors, downregulation of cytokine synthesis, eradication of autoantibodies, complement inhibition, and mediation of Fas-Fas ligand interactions.^{1,2} Preparations of IgM-enriched hIVIG had superior antiproliferative effects on T-lymphocytes compared to standard hIVIG preparations in an *in vitro* mouse model.²⁰ Likewise, in an *in vitro* human model, Pentaglobin was found to have more potent immunomodulatory capacity than standard hIVIG.²¹ There is ongoing interest in its use in people with sepsis, with a meta-analysis indicating that administration to adults with sepsis significantly decreased mortality rates.²² A concept paper describes its use along with a monoclonal antibody (rituximab) in humans with mucocutaneous blistering diseases²³ and the early use of IgM-enriched hIVIG (Pentaglobin) also appears to decrease the risk of exchange transfusion, as well as the duration of adjunctive phototherapy, in human patients with neonatal immune-mediated hemolytic anemia.²⁴

In dogs with IMHA, the destruction of erythrocytes is believed to represent a type 2 hypersensitivity reaction secondary to the binding of autoantibodies to cell membrane antigens.²⁵ This binding leads to destruction of the erythrocytes by the mononuclear phagocyte system (extravascular hemolysis) or through complement fixation (intravascular hemolysis).²⁶ An *in vitro* study indicated that hIVIG binds to canine lymphocytes and monocytes and inhibits Fc-mediated phagocytosis of autoantibody-opsonized erythrocytes.²⁷ Hence, in theory, hIVIG may assist in controlling hemolysis secondary to autoantibody opsonization in acute IMHA. Primed erythrocyte-reactive lymphocytes also have been implicated in the pathogenesis of IMHA.²⁸ Therefore, the aforementioned auxiliary effects of IgM-enriched hIVIG, which may at least in part be a consequence of its interaction with lymphocytes, also might be beneficial in dogs with IMHA.

Despite being a common hematological disorder in dogs,²⁹ beyond initial immunosuppressive treatment with glucocorticoids and the need for thromboprophylaxis, limited evidence supports other treatments.³⁰ This limited evidence, and associated high cost, has primarily restricted hIVIG to salvage treatment in dogs refractory to standard immunosuppressive protocols, and cost saving considerations often lead to administration at lower doses. Several studies have examined the use of hIVIG in dogs with IMHA, but no general agreement on its use has been reached.^{9,10,13,31} These studies have utilized lower doses of hIVIG than doses typically used in human patients where high-dose treatment is preferred for immunomodulation.

Our objective was to investigate whether the use of high-dose, IgM-enriched hIVIG would improve the initial response of dogs with IMHA as compared to standard treatment (glucocorticoids and thromboprophylaxis). We hypothesized that high-dose IgM-enriched hIVIG would be safe and effective, and that dogs treated with this product along with standard treatment would experience a shorter time to remission and hospital discharge and have decreased transfusion requirements compared to dogs receiving standard treatment alone.

2 | METHODS

One of the study centers (Animal Health Trust) received a donation of a large volume of IgM-enriched hIVIG that could not be utilized in the human medical field because of regulatory issues. At the time of receipt, only 5 months remained until product expiration. Ours was a prospective, randomized, open-label, controlled study.

2.1 | Animals

Fourteen client-owned dogs, diagnosed with nonassociative (primary) IMHA, were enrolled by participating referral hospitals, between

March 2017 and July 2017. Cases were enrolled at the following UK-based referral centers: Animal Health Trust, Dick White Referrals, The Queen's Veterinary School Hospital (University of Cambridge), Davies Veterinary Specialists and Pride Veterinary Centre. The study protocol was approved by the Animal Health Trust's Clinical Research and Ethical Approval Committee (project number: 66-2016). An Animal Test Certificate (Type S) was acquired for the experimental use of Pentaglobin from the Veterinary Medicines Directorate in the United Kingdom. Written consent was obtained from owners of included dogs. Included dogs underwent a minimum diagnostic investigation to establish a diagnosis of primary or nonassociative IMHA, including: complete history, physical examination, hematology (including reticulocyte count), serum biochemistry, thoracic and abdominal imaging (thoracic radiography and abdominal ultrasound, or computed tomography). Vector-borne disease testing was conducted at the discretion of the attending clinician, based on travel history and assessed risk of exposure. A diagnosis of IMHA was based on the following criteria: anemia (defined as PCV <35%) along with a positive saline agglutination test, positive direct antiglobulin test (DAT) or moderate to marked spherocytosis on a blood smear assessed by a board-certified clinical pathologist. Dogs were excluded if investigations showed evidence of an underlying disease that might lead to associative (secondary) IMHA, the dog had received glucocorticoids for >48 hours before study enrollment or if the dog had received any other systemic immunosuppressant treatment before study enrollment.

2.2 | Randomization

Enrolled dogs were randomly assigned to either the hIVIG treatment group or control group. Because of limited time before the study drug expiration and a desire to document the effects of the investigation treatment, a case allocation of 2 : 1 (hIVIG treatment group: control group) was utilized. Dogs were allocated by random selection of sealed envelopes that dictated group assignment.

2.3 | Treatment

All enrolled dogs, in both the control and hIVIG treatment groups, were treated with immunosuppressive dosages of glucocorticoids (either prednisolone [1-3 mg/kg PO q24h] or dexamethasone [0.15-0.3 mg/kg IV q24h]) and antiplatelet treatment (clopidogrel at a loading dosages of 10 mg/kg PO on the first day followed by a dosage of 2-4 mg/kg PO q24h). During hospitalization, daily monitoring of PCV and total plasma protein concentration was performed as a minimal monitoring requirement.

Dogs in the hIVIG treatment group were treated with IgM-enriched hIVIG (Pentaglobin, Biotest Pharma, 63 303 Dreieich, Germany) at a dosage of 1 g/kg IV at a fixed infusion rate of 1.7 mL/kg/hour using a syringe driver. During administration of hIVIG, patients were monitored (heart rate, respiratory rate, temperature,

and blood pressure) and no other drugs or infusions were administered concurrently.

The day of recruitment (also the day of diagnosis) was considered study day 1 for the control and hIVIG treatment groups.

When a dog did not achieve stabilization of PCV within 24 hours of the completion of the hIVIG infusion, the same dose was administered on 1 additional occasion. However, if the patient received a blood transfusion immediately after hIVIG infusion, then PCV stability (for the purposes of justifying a second hIVIG infusion) was assessed 24 hours after the end of the blood transfusion.

The hIVIG preparation was stored at 4 to 6°C and brought to room temperature before administration.

For either treatment group, administration of other immunosuppressive drugs was not allowed within the first 7 days of treatment. The use of additional supportive treatments (eg, IV fluids, blood transfusions, analgesia, gastroprotectants, or antibiotics), however, was allowed at the discretion of attending clinicians. Remission was defined, for the purpose of the study, as stabilization of PCV for 24 hours followed by subsequent increases and lack of requirement for additional blood transfusions. After 7 days, if remission was not achieved, other immunosuppressants could be utilized as deemed appropriate by the attending clinician.

2.4 | Data collection

Data recorded during the study period included time to remission, number, and volume of packed red blood cell (PRBC) transfusions, duration of hospitalization, patient survival to discharge, 90-day follow-up and information on any suspected adverse reactions, and cause of death (if applicable).

2.5 | Statistical analysis

Data were collated and summarized using Microsoft Excel 2011. A commercial statistics package (MedCalc 19.2.0, MedCalc Software) was used for statistical analyses. Variables between the control group and hIVIG treatment group were compared using nonparametric tests: Fisher's exact test for datasets with categorical variables and Mann-Whitney *U* test for datasets with continuous variables. A *P* value of <.05 was considered significant.

3 | RESULTS

Fourteen dogs with nonassociative IMHA were enrolled into the study. Three were assigned to the control group and 11 to the hIVIG treatment group. No enrolled dogs were excluded from the study. No significant difference was found between the control group and hIVIG treatment group in weight, age, sex, or PCV at presentation (Table 1). Of previously proposed prognostic indicators (increased blood urea nitrogen [BUN] and total bilirubin concentration, decreased platelet

TABLE 1 Comparison of variables, at the time of diagnosis, between dogs within the control group and hVIG treatment group

Parameter	hVIG treatment group	Control group	P-value
Weight (kg)	15.2 (9.7-22.0)	17.2 (12.6-38.0)	.44
Age	7.0 (0.6-13.0)	4.3 (2.8-5)	.31
Sex	5 male (3 neutered, 2 entire)/6 female (5 neutered, 1 entire)	1 male (neutered)/2 female (both neutered)	1
PCV (%) at presentation	16 (9-24)	17 (10-26)	.7
Positive DAT	2/5	Not performed in any dog	-
Positive saline agglutination test	8/11	2/2	-
Spherocytosis	10/11	3/3	-
Presence of petechiae	0/11	0/11	1
Platelet count ($\times 10^9/L$)	207 (11-734)	512 (236-571)	.13
Band neutrophils ($\times 10^9/L$)	1.00 (0.21-1.90)	0.10 (0.00-6.68)	.46
Total bilirubin (mg/dL) at presentation	0.9 (0.1-26.4)	0.8 (0.3-0.9)	.44
BUN (mg/dL) at presentation	18.2 (11.8-45.7)	13.2 (7.0-14.3)	.05
Presence of hemoglobinemia (visual assessment of plasma)	6/11	2/3	1
Presence of hemoglobinuria	0/3	1/2	.4

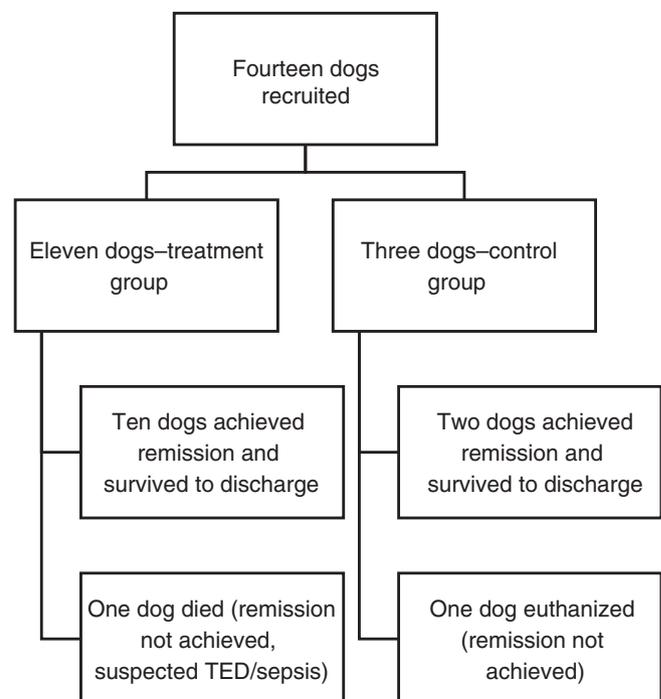
Note: Median and (range) displayed; or, number of affected or positive dogs/number of dogs or tests performed. Abbreviations: BUN, blood urea nitrogen; DAT, direct antiglobulin test; hVIG, human IV immunoglobulin.

count, presence of petechiae and increased band neutrophils),^{32,33} none were statistically different between the 2 groups (Table 1). The presence of hemoglobinemia and hemoglobinuria, as markers of intravascular hemolysis, also was not statistically different between groups (Table 1).

Infectious disease testing was performed in 10/11 dogs in the hVIG treatment group and in 2/3 dogs in the control group. Most dogs had serology performed for *Borrelia burgdorferi*, *Ehrlichia canis*, *Ehrlichia ewingii*, *Anaplasma phagocytophilum*, *Anaplasma platys*, and *Dirofilaria immitis* (SNAP 4Dx Plus Test, IDEXX Laboratories Inc, Westbrook, Maine; 2/3 dogs in the control group; 8/11 dogs in the hVIG treatment group). Some dogs also underwent serological testing for *Angiostrongylus vasorum* (Angio Detect, IDEXX Laboratories Inc, Westbrook, Maine; 0/3 dogs in the control group and 2/11 dogs in the hVIG treatment group). Other infectious disease screening included PCR testing for *Babesia* spp. (0/3 dogs in the control group and 6/11 dogs in the hVIG treatment group), hemotropic *Mycoplasma* spp. (0/3 dogs in the control group and 2/11 dogs in the hVIG treatment group), and *Anaplasma* spp. and *Ehrlichia* spp. (0/3 dogs in the control group and 2/11 dogs in the hVIG treatment group). All infectious disease screening performed was negative.

Figure 1 summarizes the outcomes of the 14 dogs enrolled in the study. Between the control and hVIG treatment groups, no significant difference was found as to whether remission or hospital discharge was achieved, nor in the duration of time to remission or hospital discharge (Table 2).

All dogs in the control group (3/3) and 8 of 11 dogs in the hVIG treatment group received PRBC transfusions. No significant difference was found in the amount of blood product received between the 2 groups (Table 2). In all dogs that developed stable PCV for 24 hours,

**FIGURE 1** Flow diagram summarizing the outcomes of dogs enrolled in the current study. TED, thromboembolic disease

PCV subsequently increased and the dogs were discharged without need for additional blood transfusions.

In the control group, 2 of 3 dogs achieved remission and survived to hospital discharge (Table 2). The dog that did not achieve remission was euthanized in the hospital on study day 4. Euthanasia was as a result of a decreasing PCV, with additional treatment declined by the

TABLE 2 Comparison of treatment and outcome variables between dogs within the control group and hVIG treatment group

Parameter	hVIG treatment group	Control group	P-value
Initial glucocorticoid treatment and dose	Dexamethasone IV: 5/11; 0.3 mg/kg/day (0.23-0.3 mg/kg/day) Prednisolone PO: 6/11; 2 mg/kg/day (1.6-2 mg/kg/day)	Dexamethasone IV: 3/3; 0.3 mg/kg/day (0.3-0.4 mg/kg/day) Prednisolone PO: 0/3	Dexamethasone dose: .17 Prednisolone dose: -
PRBC transfusion(s) administered?	8/11	3/3	.55
PRBC transfusion volume (mL/kg)	20.0 (0-35.4)	17.2 (16.4-55.9)	.53
Remission/hospital discharge achieved?	10/11	2/3	.4
Day of remission	3 (2-17)	6 (2-10)	1
Day of discharge	6 (4-28)	8.5 (4-13)	.91
Day-90 status	7 alive/2 dead (2 lost to follow-up)	2 alive/1 dead	1

Note: Number of treated dogs/total number of dogs; median and (range) displayed; or, number of dogs alive/number of dogs dead.

Abbreviation: PRBC, packed red blood cells.

owners. The 2 dogs in the control group that survived to hospital discharge were still alive, receiving ongoing medical management, and in remission on study day 90.

In the hVIG treatment group, 10 of 11 patients achieved remission and survived to hospital discharge (Table 2). The dog that did not achieve remission deteriorated and died on study day 4. The cause of death was suspected to be sepsis and thromboembolic disease (TED) but these diagnoses were not confirmed and a necropsy was not performed. Of the 10 dogs that achieved remission and hospital discharge, 7 were alive and still in remission with ongoing medical treatment at study day 90. Of the remaining 3 dogs, 2 were lost to follow-up, but at the time of last follow-up (study day 60 for 1 dog and 49 for the other) the dogs were reported to be in remission and still receiving medical treatment. The remaining dog was euthanized on study day 18 at the owners' request at the primary veterinary practice. Based upon assessment of the available medical record at that time, euthanasia was requested despite continued remission with medical treatment, and because of potential steroid-related adverse effects. Enlarged submandibular lymph nodes had been identified on examination at this time, but cytology findings were consistent with reactive lymphoid hyperplasia.

In the hVIG treatment group, because of various factors, including time of presentation (ie, presentation out-of-hours as an emergency vs routine appointment) and the concurrent requirement for blood transfusion, patients in the hVIG group received their first infusion on a median of study day 2 (range, 1-2). Most dogs (7/11) received only 1 infusion of IgM-enriched hVIG; 4 dogs, that had a decrease in PCV within 24 hours after the first, received 2 hVIG infusions. These included the 2 dogs that experienced potential adverse reactions to the infusion of IgM-enriched hVIG. One of these dogs was the aforementioned patient that died on study day 4 because of suspected sepsis and TED. The other dog experienced transient tachypnea the day after a second infusion of hVIG. This dog had also received a PRBC transfusion in the 24 hours before the first hVIG infusion. Tachypnea resolved during hospitalization without further

investigation or treatment. This dog achieved remission on study day 5 and was alive on study day 90. The remaining 2 dogs that received 2 hVIG infusions also received additional second-line immunosuppressant agents, and were both alive on study day 90. For 1 of these dogs, mycophenolate mofetil was prescribed on study day 11 before remission was achieved on day 17. This dog survived to hospital discharge, but was lost to follow-up on study day 49, at which time remission was sustained on a decreased dose of prednisolone and concurrent ongoing management with mycophenolate mofetil. The second dog was prescribed azathioprine on study day 26 because of development of thrombocytopenia despite continued remission of the IMHA. This dog was a Cavalier King Charles Spaniel and had some macroplatelets observed on blood smear evaluation but, before the consistent findings of thrombocytopenia, normal platelet counts had been documented and, after the addition of azathioprine, thrombocytopenia resolved, leading to a presumptive diagnosis of immune-mediated thrombocytopenia. This patient achieved remission from IMHA (on study day 6) and hospital discharge, and was alive and in remission at study day 90.

4 | DISCUSSION

Our study aimed to evaluate the use of high-dose IgM-enriched hVIG in dogs with IMHA in a prospective, randomized, controlled manner. In this cohort, we found no significant difference between the treatment and control groups in terms of measured outcome variables.

Potential adverse effects to hVIG were reported in 2 of the 11 dogs that received the treatment. One dog died as a result of these potential complications (sepsis and TED) whereas the other experienced self-resolving tachypnea (suspected TED). However, definitive diagnosis of TED was not obtained in either case, and therefore it is difficult to be certain of this diagnosis. Furthermore, dogs with IMHA are known to already be at increased risk of TED,³⁴ and the timing of

events in both of these patients makes the relationship to treatment questionable. Adverse effects to hIVIG are reported to occur in 32% of people but are usually mild (fever being most common) and often can be managed by decreasing the infusion rate.³⁵ As in our study, adverse effects reported in other veterinary studies appear infrequent and include swelling at catheter sites, volume overload, erythema, and anaphylaxis.^{13,19} A hypercoagulable and a proinflammatory state, without clinical signs, also has reported in a small group of experimental dogs that received a single infusion of hIVIG.³⁶ This observation, along with the known increased risk of TED in dogs with IMHA, suggests that thromboprophylaxis might be particularly crucial in dogs with IMHA that receive hIVIG. In our study, clopidogrel was used on the basis of available literature, affordability, and ease of administration.³⁷ However, the American College of Veterinary Internal Medicine (ACVIM) consensus statement on the treatment of IMHA, published after the completion of our study, recommends the use of anticoagulant drugs in preference to antiplatelet treatment, but the strength of this recommendation is weak³⁰ and anticoagulants typically are more expensive, require additional monitoring and are less practical to administer over the long term, often requiring owners to give frequent injections. If antiplatelet drugs are used, clopidogrel is suggested over or along with aspirin, based on the available literature.^{30,37-39} No adverse effects were observed with the use of clopidogrel (including the loading dose) in our study.

Of interest in our study was the use of higher than previously utilized doses of hIVIG in dogs. In previous studies,^{9,13} smaller doses were used than in our study. One of these studies was a blinded randomized clinical trial that recruited 28 nonassociative IMHA cases in dogs (14 in the hIVIG treatment group and 14 in the placebo group).¹³ The dogs that received hIVIG had 0.5 g/kg administered over 6 hours on 3 consecutive days (1.5 g/kg total dose). The other study was retrospective and included 22 dogs (9 of which were treated with hIVIG) over a 6-year period from a single institution.⁹ The dogs that had hIVIG administered received a median dose of 0.35 g/kg (range, 0.19-0.68 g/kg). In both studies, no significant benefit was observed from treatment with hIVIG. In human medicine, doses of hIVIG are variable depending on the disease treated but higher doses, most frequently 2 g/kg/month, typically are recommended for the treatment of autoimmune diseases.¹ Therefore, our study allowed for the delivery of 1 g/kg of IgM-enriched hIVIG with a repeated dose if disease remission was not achieved within 24 hours (total dose of 2 g/kg). Four of the 11 dogs that received hIVIG in our study received 2 hIVIG transfusions and, therefore, the maximal dose. Two of these dogs experienced potential adverse reactions, but these effects may not have been related to hIVIG administration.

An additional point of interest when examining both our study and the previous study¹³ is the overall good survival rates both for dogs treated with hIVIG and the placebo or control groups. This finding is in contrast to the high mortality rates of up to 70%⁴⁰ reported for dogs with IMHA in older veterinary literature. A more recent publication, based on data from a British multicenter online case registry, reported higher survival rates of dogs with primary IMHA, describing 25.7% mortality at discharge, and 30-day mortality of 32.6%.³²

However, these mortality rates still appear higher than those seen in our study where mortality was 14.3% at discharge and 21.4% at 30-days. Although our study represents a relatively small data set, this finding could suggest that dogs treated with a strict and consistent treatment and monitoring regimen, including thromboprophylaxis, might experience better outcomes. Additional credence might be added to this idea with future longitudinal studies following the recent publication of ACVIM consensus statements on the diagnosis and management of IMHA, which aim to rationalize and standardize treatment approaches.^{30,41} The latter of these consensus statements concludes that hIVIG should be considered as a salvage treatment for dogs unresponsive to 2 immunosuppressive drugs, and hIVIG is not currently recommended for routine treatment.³⁰

Our study was limited by small sample size and, therefore, restricted ability to perform statistical analysis and detect statistical differences in patient outcome. Unfortunately, this small sample size was unavoidable as a result of factors surrounding use of the IgM-enriched hIVIG preparation. The product (Pentaglobin) was donated to 1 of the institutions (Animal Health Trust) but with a limited shelf-life (an expiration date of July 2017) and therefore all cases were enrolled between March 2017 and July 2017. Several centers were recruited to maximize enrollment of cases within the available time. To ensure enrollment of an adequate number of cases to the hIVIG treatment group, a randomization allocation of 2 : 1 was used for the hIVIG treatment and control groups. However, this approach ultimately led to the recruitment of a minimal number of control dogs, which impacted the ability to detect significant differences between the control and hIVIG treatment groups. Continued enrollment of control cases was considered but not performed because of the possible introduction of selection bias. Post hoc power calculations indicate that the cases collected to that point only had a power of 25% to detect a difference in survival between treatment groups. Moreover, if the same survival rates could be extrapolated to a larger data set, several hundred control cases would need to be recruited to identify a significant survival benefit to the treatment with only 11 cases in that group, making it a futile endeavor. If there is a survival benefit in using hIVIG at presentation in dogs with IMHA, it is likely to be small and studies to identify it are quite possibly cost prohibitive. Future studies should be more targeted, evaluating only those cases for which the prognosis is thought to be poor such that any benefit is more readily identifiable.

The inclusion criteria for our study were less stringent than the diagnostic algorithm proposed by the recent ACVIM consensus statement for the diagnosis of IMHA in dogs and cats.⁴¹ However, retrospectively, all dogs in our study met the consensus statements' criteria (≥ 2 signs of immune-mediated destruction and ≥ 1 sign of hemolysis) to be considered diagnostic for IMHA. Two dogs did not undergo infectious disease screening. Although some infectious organisms may cause IMHA,⁴¹ these are uncommon in the United Kingdom, where the study was performed, and generally are not seen in dogs with an absence of travel history. Compared with other countries, where infectious disease screening is imperative for achieving a diagnosis of nonassociative IMHA, the decision to

undertake infectious disease testing, and the breadth of that testing, was decided on a case-by-case basis dictated by clinical history and index of suspicion.

Markers of intravascular hemolysis, hemoglobinemia, and hemoglobinuria were assessed where the information was available. However, measurement of cell-free hemoglobin was unavailable and assessment of hemoglobinemia was based on visual inspection of plasma, which could have been spurious secondary to sample collection (eg, traumatic venipuncture), handling or storage.⁴²⁻⁴⁴ Urinalysis and assessment of hemoglobinuria was only available for a small number of dogs. Therefore, it is difficult to draw conclusions about the number of dogs in our study with intravascular hemolysis. Future studies are warranted to assess if hIVIG treatment, and in particular IgM-enriched hIVIG, might preferentially benefit dogs with intravascular hemolysis.

Our study was nonblinded, which could have created bias. Blinding and the use of a placebo infusion was considered, but given that fluid overload was a possible risk considered for these IMHA patients, because of the combination of anemia, blood transfusions and potential IV fluid therapy, the addition of an additional nonessential IV transfusion (ie, placebo drug) was not considered safe, ethical, or legal in the United Kingdom. Additionally, remission was considered confirmed by identification of a stable PCV, an objective variable that should be less prone to bias than a subjective outcome.

5 | CONCLUSION

This multicenter prospective study suggested that high-dose IgM-enriched hIVIG, in addition to glucocorticoids, is well tolerated by patients but no significant benefits were found compared to glucocorticoids alone in this small cohort. Additional studies utilizing larger groups of dogs with IMHA using a blinded study design and a placebo-controlled group are warranted, in addition to studies examining subpopulations of dogs with IMHA associated with a poorer prognosis to determine if hIVIG treatments would benefit such cases.

ACKNOWLEDGMENT

No funding was received for this study. Presented as a research abstract at the 2018 ACVIM Forum, Seattle, WA. The authors are grateful to the staff of all contributing institutions that aided in the collection of clinical data. We would also like to thank colleagues who took the time to engage in the project but were unsuccessful in recruiting cases during the study period.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Specific off-label use of antimicrobials did not form part of this prospective study. However, off-label use of antimicrobials may have featured in the management of some of the cases included as deemed appropriate, by the attending clinician on a case-by-case basis.

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

Approved by the Ethics Committee of the Animal Health Trust (Project Number: 66-2016). An Animal Test Certificate for the use of the IgM-enriched hIVIG (Pentaglobin) in this study was obtained from the UK Veterinary Medicines Directorate. Informed written consent was obtained from the owners of enrolled patients.

HUMAN ETHICS APPROVAL DECLARATION

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

ORCID

Jason P. Bestwick  <https://orcid.org/0000-0002-0138-0565>

Mellora Sharman  <https://orcid.org/0000-0002-0327-298X>

Simon Tappin  <https://orcid.org/0000-0002-8645-178X>

REFERENCES

- Jolles S, Sewell WAC, Misbah SA. Clinical uses of intravenous immunoglobulin. *Clin Exp Immunol*. 2005;142(1):1-11.
- Spurlock NK, Prittie JE. A review of current indications, adverse effects, and administration recommendations for intravenous immunoglobulin. *J Vet Emerg Crit Care*. 2011;21(5):471-483.
- Schroeder HW, Cavacini L. Structure and function of immunoglobulins. *J Allergy Clin Immunol*. 2010;125(2 suppl 2):S41-S52.
- Hughes RA, Swan AV, van Doorn PA. Intravenous immunoglobulin for Guillain-Barré syndrome. *Cochrane Database of Syst Rev*. 2014;2014(9):CD002063.
- Gharebaghi N, Nejadrahim R, Mousavi SJ, Sadat-Ebrahimi S-R, Hajizadeh R. The use of intravenous immunoglobulin gamma for the treatment of severe coronavirus disease 2019: a randomized placebo-controlled double-blind clinical trial. *BMC Infect Dis*. 2020;20(1):786-788.
- Pourpak Z, Aghamohammadi A, Sedighpour L, et al. Effect of regular intravenous immunoglobulin therapy on prevention of pneumonia in patients with common variable immunodeficiency. *J Microbiol Immunol Infect*. 2006;39(2):114-120.
- Jolles S. A review of high-dose intravenous immunoglobulin (hdIVIG) in the treatment of the autoimmune blistering disorders. *Clin Exp Dermatol*. 2001;26(2):127-131.
- Spurlock N, Prittie J. Use of human intravenous immunoglobulin in veterinary clinical practice. *Vet Clin North Am Small Anim Pract*. 2020;50(6):1371-1383.
- Gerber B, Steger A, Hässig M, Glaus TM. Use of human intravenous immunoglobulin in dogs with primary immune mediated hemolytic anemia. *Schweiz Arch Tierheilkd*. 2002;144(4):180-185.
- Kellerman DL, Bruyette DS. Intravenous human immunoglobulin for the treatment of immune-mediated hemolytic anemia in 13 dogs. *J Vet Intern Med*. 1997;11(6):327-332.
- Oggier D, Tomsa K, Mevissen M, Glaus T. Efficacy of the combination of glucocorticoids, mycophenolate-mofetil and human immunoglobulin for the therapy of immune mediated haemolytic anaemia in dogs. *Schweiz Arch Tierheilkd*. 2018;160(3):171-178.
- Park S-Y, Kim H, Kang B-T, Kang J-H, Yang M-P. Prognostic factors and efficacy of human intravenous immunoglobulin G in dogs with idiopathic immune-mediated hemolytic anemia: a retrospective study. *Korean J Vet Res*. 2016;56(3):139-145.
- Whelan MF, O'Toole TE, Chan DL, et al. Use of human immunoglobulin in addition to glucocorticoids for the initial treatment of dogs with immune-mediated hemolytic anemia. *J Vet Emerg Crit Care*. 2009;19(2):158-164.

14. Bianco D, Armstrong PJ, Washabau RJ. A prospective, randomized, double-blinded, placebo-controlled study of human intravenous immunoglobulin for the acute management of presumptive primary immune-mediated thrombocytopenia in dogs. *J Vet Intern Med.* 2009;23(5):1071-1078.
15. Bianco D, Armstrong PJ, Washabau RJ. Treatment of severe immune-mediated thrombocytopenia with human IV immunoglobulin in 5 dogs. *J Vet Intern Med.* 2007;21(4):694-699.
16. Balog K, Huang AA, Sum SO, Moore GE, Thompson C, Scott-Moncrieff JC. A prospective randomized clinical trial of vincristine versus human intravenous immunoglobulin for acute adjunctive management of presumptive primary immune-mediated thrombocytopenia in dogs. *J Vet Intern Med.* 2013;27(3):536-541.
17. Trotman TK, Phillips H, Fordyce H, King LG, Morris DO, Giger U. Treatment of severe adverse cutaneous drug reactions with human intravenous immunoglobulin in two dogs. *J Am Anim Hosp Assoc.* 2006;42(4):312-320.
18. Ramos SJ, Beale VM, Langohr IM, Woodward MC. Erythema multiforme major in a dog treated with intravenous human immunoglobulin and immunosuppressive therapy. *J Am Anim Hosp Assoc.* 2020;56(2):133-138.
19. Abelson AL, Shelton GD, Whelan MF, Cornejo L, Shaw S, O'Toole TE. Use of mycophenolate mofetil as a rescue agent in the treatment of severe generalized myasthenia gravis in three dogs. *J Vet Emerg Crit Care.* 2009;19(4):369-374.
20. Vassilev T, Mihaylova N, Vovnova E, Nikolova M, Kazatchkine M, Kaveri S. IgM-enriched human intravenous immunoglobulin suppresses T lymphocyte functions in vitro and delays the activation of T lymphocytes in hu-SCID mice. *Clin Exp Immunol.* 2006;145(1):108-115.
21. Nachbaur D, Herold M, Gächter A, Niederwieser D. Modulation of alloimmune response in vitro by an IgM-enriched immunoglobulin preparation (Pentaglobin). *Immunology.* 1998;94(2):279-283.
22. Cui J, Wei X, Lv H, et al. The clinical efficacy of intravenous IgM-enriched immunoglobulin (Pentaglobin) in sepsis or septic shock: a meta-analysis with trial sequential analysis. *Ann Intensive Care.* 2019;9(1):27-14.
23. Ahmed AR, Kaveri S. Reversing autoimmunity combination of rituximab and intravenous immunoglobulin. *Front Immunol.* 2018;9:1189.
24. Aqrabawi HE. The use of Pentaglobulin in neonatal immune hemolytic anaemia. *Arch Dis Child.* 2012;97(suppl 2):A219-A220.
25. Hernandez DM, Goggs R, Behling-Kelly E. In vitro inhibition of canine complement-mediated hemolysis. *J Vet Intern Med.* 2017;32(1):142-146.
26. McCullough S. Immune-mediated hemolytic anemia: understanding the nemesis. *Vet Clin North Am Small Anim Pract.* 2003;33(6):1295-1315.
27. Reagan WJ, Scott-Moncrieff C, Christian J, Snyder P, Kelly K, Glickman L. Effects of human intravenous immunoglobulin on canine monocytes and lymphocytes. *Am J Vet Res.* 1998;59(12):1568-1574.
28. Corato A, Shen CR, Mazza G, Barker RN, Day MJ. Proliferative responses of peripheral blood mononuclear cells from normal dogs and dogs with autoimmune haemolytic anaemia to red blood cell antigens. *Vet Immunol Immunopathol.* 1997;59(3-4):191-204.
29. Swann JW, Skelly BJ. Systematic review of evidence relating to the treatment of immune-mediated hemolytic anemia in dogs. *J Vet Intern Med.* 2013;27(1):1-9.
30. Swann JW, Garden OA, Fellman CL, et al. ACVIM consensus statement on the treatment of immune-mediated hemolytic anemia in dogs. *J Vet Intern Med.* 2019;33(3):1141-1172.
31. Grundy SA, Barton C. Influence of drug treatment on survival of dogs with immune-mediated hemolytic anemia: 88 cases (1989-1999). *J Am Vet Med Assoc.* 2001;218(4):543-546.
32. Goggs R, Dennis SG, Di Bella A, et al. Predicting outcome in dogs with primary immune-mediated hemolytic anemia: results of a multicenter case registry. *J Vet Intern Med.* 2015;29(6):1603-1610.
33. Piek CJ, Junius G, Dekker A, Schrauwen E, Slappendel RJ, Teske E. Idiopathic immune-mediated hemolytic anemia: treatment outcome and prognostic factors in 149 dogs. *J Vet Intern Med.* 2008;22(2):366-373.
34. Kidd L, Mackman N. Prothrombotic mechanisms and anticoagulant therapy in dogs with immune-mediated hemolytic anemia. *J Vet Emerg Crit Care.* 2013;23(1):3-13.
35. Palabrica FRR, Kwong SL, Padua FR. Adverse events of intravenous immunoglobulin infusions: a ten-year retrospective study. *Asia Pac Allergy.* 2013;3(4):249-256.
36. Tsuchiya R, Akutsu Y, Ikegami A, et al. Prothrombotic and inflammatory effects of intravenous administration of human immunoglobulin G in dogs. *J Vet Intern Med.* 2009;23(6):1164-1169.
37. Mellett AM, Nakamura RK, Bianco D. A prospective study of clopidogrel therapy in dogs with primary immune-mediated hemolytic anemia. *J Vet Intern Med.* 2011;25(1):71-75.
38. Björkman J-A, Zachrisson H, Forsberg G-B, et al. High-dose aspirin in dogs increases vascular resistance with limited additional anti-platelet effect when combined with potent P2Y12 inhibition. *Thromb Res.* 2013;131(4):313-319.
39. Brainard BM, Kleine SA, Papich MG, Budsberg SC. Pharmacodynamic and pharmacokinetic evaluation of clopidogrel and the carboxylic acid metabolite SR 26334 in healthy dogs. *Am J Vet Res.* 2010;71(7):822-830.
40. Reimer ME, Troy GC, Warnick LD. Immune-mediated hemolytic anemia: 70 cases (1988-1996). *J Am Anim Hosp Assoc.* 1999;35(5):384-391.
41. Garden OA, Kidd L, Mexas AM, et al. ACVIM consensus statement on the diagnosis of immune-mediated hemolytic anemia in dogs and cats. *J Vet Intern Med.* 2019;33(2):313-334.
42. Grant MS. The effect of blood drawing techniques and equipment on the hemolysis of ED laboratory blood samples. *J Emerg Nurs.* 2003;29(2):116-121.
43. Fang L, Fang S-H, Chung Y-H, Chien S-T. Collecting factors related to the haemolysis of blood specimens. *J Clin Nurs.* 2008;17(17):2343-2351.
44. Kennedy C, Angermuller S, King R, et al. A comparison of hemolysis rates using intravenous catheters versus venipuncture tubes for obtaining blood samples. *J Emerg Nurs.* 1996;22(6):566-569.

How to cite this article: Bestwick JP, Sharman M, Whitley NT, et al. The use of high-dose immunoglobulin M-enriched human immunoglobulin in dogs with immune-mediated hemolytic anemia. *J Vet Intern Med.* 2021;1-8. doi:10.1111/jvim.16315