

Treatment of Immune-Mediated Hemolytic Anemia with Individually Adjusted Heparin Dosing in Dogs

S.E. Helmond, D.J. Polzin, P.J. Armstrong, M. Finke, and S.A. Smith

Background: A major cause of death in dogs with immune-mediated hemolytic anemia (IMHA) is thromboembolism. Previous studies suggest unfractionated heparin (UH) is not effective in preventing thromboembolism in IMHA; however, subtherapeutic dosing could explain the seeming lack of efficacy.

Hypothesis: Providing therapeutic plasma concentration of UH by individually adjusting doses based on antifactor Xa activity would improve survival in IMHA.

Animals: Fifteen dogs with primary IMHA.

Methods: Randomized, prospective, controlled clinical trial. Dogs received standardized therapy for IMHA and either constant dose (CD) (150 U/kg SC) ($n = 7$) or individually adjusted dose (IAD) ($n = 8$) UH, monitored via an anti-Xa chromogenic assay, adjusted according to a nomogram. UH was administered every 6 hours until day 7, and every 8 hours thereafter. UH dose was adjusted daily in IAD dogs until day 7, weekly until day 28, then tapered over 1 week. Dogs were monitored for 180 days.

Results: At day 180, 7 dogs in the IAD group and 1 in the CD group were alive ($P = .01$). Median survival time for the IAD group was > 180 days, and 68 days for the CD group. Thromboembolic events occurred in 5 dogs in the CD group and 2 dogs in the IAD group. Doses of UH between 150 and 566 U/kg achieved therapeutic anti-Xa activity (0.35–0.7 U/mL).

Conclusions and Clinical Importance: This study suggests that IAD UH therapy using anti-Xa monitoring reduced case fatality rate in dogs with IMHA when compared with dogs receiving fixed low dose UH therapy.

Key words: Anti-Xa; Survival; Thromboembolism; Thrombosis.

Immune-mediated hemolytic anemia (IMHA) in dogs is a serious hematological disorder with a high case fatality rate.^{1–5} Venous thrombosis and pulmonary thromboembolism (PTE) are well-documented complications,^{2,5–13} with PTE an important cause of death, occurring in up to 80% of dogs that die of IMHA.^{2,10}

Hypercoagulability, as defined by hyperfibrinogenemia, antithrombin deficiency and increased fibrin degradation products (FDPs) and D-Dimers, occurs in dogs with IMHA.^{5,14} The precise underlying mechanisms have not been determined but several possible causes include increased activity of procoagulant factors, presence of free hemoglobin, decreased concentration of fibrinolytic or anticoagulant factors, vasculitis, and enhanced platelet reactivity.^{2,5,7} Development of an effective prophylactic approach for hypercoagulability could markedly decrease the case fatality rate in dogs with IMHA.

Unfractionated heparin (UH) decreases development of venous thrombosis and PTE in human patients^{15,16} but this has not been demonstrated in dogs with IMHA. Heparin therapy is frequently used to try to prevent TE in

Abbreviations:

aPTT	activated partial thromboplastin time
CD	constant dose
FDP	fibrin degradation product
IAD	individually adjusted dose
IMHA	immune-mediated hemolytic anemia
PT	prothrombin time
PTE	pulmonary thromboembolism
TE	thromboembolism
UH	unfractionated heparin

dogs,^{4,5,17–19} but evidence supporting efficacy of this treatment in dogs with IMHA is limited. Additionally, data validating recommendations for heparin dosing in dogs are scarce. Doses between 50 and 300 U/kg SC in dogs with IMHA have been reported^{4–6,11,19}; however, fixed low dose heparin therapy has not been shown to be effective in this population.⁴ While doses of 200–300 U/kg SC provide adequate anticoagulation in normal dogs,^{20,a} heparin pharmacokinetics might be different in dogs with IMHA. It is likely that fixed dose heparin protocols are inadequate because of differences in protein-binding, consumption, and excretion of the heparin molecules.^{21,22} Subtherapeutic heparin therapy is associated with an increased risk of recurrent thrombotic events in human patients with TE.^{23–25}

Based on extrapolation from human medicine, heparin therapy is often monitored in dogs using the activated partial thromboplastin time (aPTT) with the dose adjusted to achieve an aPTT prolongation of 1.5–2.5-fold.^{17,18} It has been shown, however, that the aPTT is not a consistent or reliable method of monitoring heparin therapy in dogs, especially those with inflammatory diseases.^{18,26,27}

The efficacy of heparin therapy can be improved by monitoring plasma heparin concentration.²³ In experimental models of thrombosis in dogs, plasma heparin concentrations of 0.35–0.7 U/mL (as measured by factor Xa

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inactivation assay [anti-Xa assay] correlate with decreased thrombus formation.²⁸ Maintaining plasma heparin concentration within this range is associated with improved clinical outcome in humans with venous thrombosis.^{6,16,29} Heparin monitoring via anti-Xa assay has been reported in some dogs with IMHA,^{6,18,19} but not in a controlled clinical trial.

We proposed that development of thrombosis in dogs with IMHA despite heparin therapy has been a consequence of inadequate dosing. We hypothesized that individual adjustment of heparin dose to achieve target therapeutic plasma concentrations based on anti-Xa activity would reduce thrombotic complications and improve survival.

Materials and Methods

Study Design

This study was a prospective, randomized, controlled clinical trial. The individually adjusted dose (IAD) group received adjusted dosing of UH to a target plasma concentration of 0.35–0.7 U/mL. The constant dose (CD) group received a standard dose of heparin similar to that reported previously in case series of dogs with IMHA. The study was originally designed to be double masked; however, unmasking of the primary investigators midstudy occurred because of logistical issues associated with preparation of the heparin doses.

Dog Selection

Eligible study subjects were client-owned dogs suspected of having IMHA admitted to the Veterinary Medical Center (VMC) of the University of Minnesota (UMN) between November 2005 and August 2007. Informed client consent was obtained from all owners before enrollment and the study was approved by the university Institutional Animal Care and Use Committee. All eligible dogs were evaluated within 24 hours of admission with the following diagnostic tests: thoracic and abdominal radiographs, CBC, blood film evaluation, platelet count, serum biochemical profile, urinalysis, prothrombin time (PT),^b aPTT^b FDPs, and fibrinogen.

Inclusion Criteria

Dogs were eligible for inclusion if a diagnosis of IMHA was made based on the presence of regenerative anemia (hematocrit <30% with >60,000 reticulocytes/ μ L) and evidence of hemolysis (hyperbilirubinemia, hemoglobinemia, bilirubinuria, or hemoglobinuria). In addition, dogs must also have had one or more of the following: A positive saline agglutination test (direct saline agglutination test: 1 drop of EDTA blood mixed with 1 drop of PBS in a glass test tube; an aliquot of the mixture is poured onto a glass slide, covered with a glass coverslip, and examined under microscope for agglutination), spherocytosis (blood film examined under 100 \times oil immersion; reported as rare (<1), 1+ (1–8), 2+ (9–20), 3+ (21–50), or 4+ (>50) per field), or a positive Coombs test (canine direct Coombs test, developed by Dr Betsy Aird DVM, 1995, using commercially available goat anti-dog antisera; washed canine red cells incubated with antisera in microtiter plates diluted 1 : 2 to 1 : 2048).

Exclusion Criteria

Dogs with IMHA were excluded if any underlying cause for hemolytic anemia was identified as determined by history of hemolytic toxin or exposure to drugs or compounds known to cause hemolysis, thoracic radiographs, abdominal radiographs or abdominal ultrasound screening for neoplasia, heartworm antigen testing, examination of blood film for erythroparasites or antibody testing for tick-borne diseases (*Ehrlichia canis*, *Rickettsia rickettsii*, *Anaplasma phagocytophilum*,

Babesia gibsoni). Infectious disease and neoplasia screening was performed on all dogs. Because of the need for repeated sampling, dogs with body weight <5.0 kg were excluded. Additional exclusion criteria were severe thrombocytopenia (platelet count <40,000/ μ L), or possible preexisting hypocoagulability (PT or aPTT > 2 \times the upper end of the reference interval), because the safety of higher dose heparin therapy had yet to be confirmed, particularly in these dogs.

Dogs that had received any immunosuppressive drug in the 6 months before onset of disease, immunosuppressive drugs for the current episode for >3 days duration before admission, heparin therapy for longer than 24 hours before enrollment, or aspirin within 7 days of admission were also excluded.

Treatment

During the first 24 hours after examination at the UMN VMC, eligible dogs were managed using a standard treatment protocol. Dogs were admitted to the intensive care unit (ICU) and a silastic nonthrombogenic central catheter^d was placed in a lateral saphenous vein. All dogs then received 50 U/kg UH^e IV and 150 U/kg UH SC, and then 150 U/kg UH SC every 6 hours until day 3 of hospitalization (Fig 1). Therapy included immunosuppression, transfusions, crystalloid fluids IV, oxygen supplementation, antibiotics, gastroprotectants, and nutritional support according to a standard intervention protocol.

Heparin Therapy

Dogs were enrolled within 24 hours of admission and randomized to the 2 heparin groups with assignment group placed in sealed envelopes to be opened at the time of the 1st dose. Groups of 4 envelopes including 2 IAD and 2 CD were formed and a table of random numbers was used to randomize the envelopes. The envelopes were then numbered sequentially so that each entering dog was assigned without knowledge of treatment. Randomization and envelope preparation was performed by a person not associated with the study protocols. Randomization was preserved throughout the study period despite unmasking.

In both groups, UH was administered by SC injection every 6 hours until day 7, and every 8 hours thereafter until discontinuation. Dogs assigned to the CD protocol received a consistent dose of 150 U/kg UH SC until day 28. Dogs assigned to the IAD protocol initially received 150 U/kg UH SC every 6 hours until day 3.

Beginning on day 3 of hospitalization, plasma samples were collected from all dogs for measurement of peak (2 hours postdose) and trough (6 hours postdose) heparin concentration on days 3, 4, 5, 7, 14, 21, and 28 with a factor Xa inhibition assay. In the IAD group, the UH dose was adjusted on day 3, beginning with the 2nd heparin dose, then on days 4, 5, 7, 14, 21, and 28, using a nomogram (Appendix 1) in an attempt to maintain heparin plasma concentration within the therapeutic range (0.35–0.7 U/mL).

For both groups, the dosage was reduced daily after day 28 by 20% of the final therapeutic dose until discontinuation on day 35. Owners were asked to complete treatment logs in order to monitor compliance during the at-home phase of therapy.

Sampling

Except for arterial blood gas (ABG) samples, all samples during hospitalization were drawn from the central catheter. The catheter was first flushed with 5 mL of heparin-free saline then 5 mL of blood was withdrawn into a heparinized syringe (containing 1 mL of saline and 7 U of UH) and set aside. The blood sample to be analyzed was collected and the 5 mL of blood not used for sampling returned to the dog.

Antifactor Xa Chromogenic Assay

Quantitative measurement of UH was determined with a commercial chromogenic assay kit^f for antifactor Xa (anti-Xa) that has

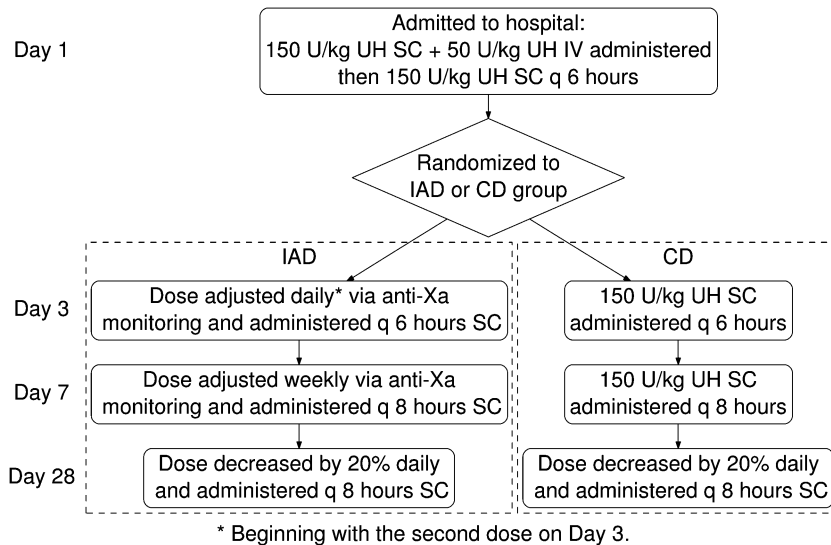


Fig 1. Heparin treatment protocol. Flow chart depicts heparin treatment during the 35-day treatment period for the 2 groups. IAD, individually adjusted dose group; CD, constant dose group; UH, unfractionated heparin.

been validated for use in dogs.³⁰ Briefly, 2.7 mL of dog blood was collected into a 3 mL 3.8% sodium citrate tube, to provide a 1:9 ratio of citrate:whole blood. The sample was centrifuged for 10 minute at $2,500 \times g$ at room temperature and the platelet-poor plasma was harvested. Plasma (1.2 mL) was diluted with 0.4 mL of Owren-Kohler buffer as recommended previously.³⁰ The diluted sample was then evaluated for anti-Xa activity by the UMN Fairview Acute Care Laboratory. To confirm accuracy of this dilution, and to validate the assay for use in dogs in the laboratory used, a series of tests was performed on normal dog plasma spiked *ex vivo* with known quantities of heparin. The correlation coefficient for this test by orthogonal regression was 0.98.

Standard Intervention Protocol

Packed red blood cells were administered at a dosage of 10 mL/kg if packed cell volume (PCV) was $<12\%$, there was an acute decrease in PCV to $<15\%$, or tachycardia or other clinical signs attributable to severe anemia were present. During hospitalization, PCV and vital signs were monitored and recorded every 6 hours. A physical examination was performed at least every 12 hours. Blood film examination for the presence of red cell morphological changes and autoagglutination was performed daily. CBCs were repeated at clinician discretion, and before dog discharge from the hospital. Thoracic radiographs and ABG analysis were performed if there was worsening of respiratory status.

Dogs remained hospitalized until their PCV was stable or increasing for >24 hours, and they did not require oxygen or other intensive supportive therapies. Follow-up examinations consisting of a history, physical examination, and CBC were performed on days 14, 21, 28, 60, 90, and 180. If owners consented, necropsies were performed at the UMN Diagnostic Laboratory on dogs that did not survive to the end of the study period.

Immunosuppressive Therapy

All dogs received standard immunosuppressive therapy. If no prednisolone had been administered before admission, 0.3 mg/kg IV of dexamethasone sodium phosphate^g was administered, followed by prednisolone 1–1.5 mg/kg PO every 12 hours (prednisone acetate 1.5 mg/kg IV every 12 hours if a dog was unable to tolerate oral

medications). Azathioprine^h (2 mg/kg PO once daily for 7 days followed by alternate day therapy) was added when the PCV had not stabilized or there was persistent autoagglutination by day 7. Cyclosporineⁱ (5 mg/kg PO once daily) was used either if azathioprine was not tolerated or additional immunosuppression was required (persistent hemolysis, autoagglutination, or spherocytosis and refractory anemia). Immunosuppressive therapy was tapered once the PCV was $>37\%$. The prednisolone dose was decreased by 20–25% every 2–4 weeks if the PCV was stable or increasing. Other immunosuppressive medication (azathioprine or cyclosporine) was tapered after prednisolone was discontinued.

Study Endpoints

The primary study endpoint was all-cause death at 180 days. Secondary endpoints were the occurrence of thrombosis and/or hemorrhage based on clinical, diagnostic imaging, and/or necropsy findings. Clinical findings considered to be consistent with PTE were acute onset of hypoxemia with hypocapnia, respiratory distress without associated radiographic findings supportive of other etiologies, or a supportive ventilation/perfusion scintigraphic scan.^{9,11} Findings considered to be consistent with thrombosis at other sites were ascites, otherwise unexplained organ failure, or acute onset neurologic signs. Hemorrhagic complications were indicated by clinical, necropsy findings, or both.

Statistical Analysis

For the primary endpoint (180-day all-cause death), the IAD protocol was compared with the CD protocol by Kaplan-Meier survival analysis, applying the log-rank test. Because of small numbers enrolled, we also applied Pearson's Chi-squared test to provide an exact *P*-value. Differences between the IAD and the CD groups on presentation were compared by Student's *t*-test for normally distributed continuous data, Mann-Whitney Rank-Sum test if not normally distributed, and by Fisher's exact test for categorical data. Difference in the length of ICU hospitalization was compared by Gehan-Wilcoxon analysis. Correlation between heparin dose and anti-Xa activity was performed by Pearson's product moment correlation. Statistical analyses were performed by a standard statistical software package^l and significance was set at $P < .05$.

Results

Characteristics of the Study Population

Sixteen dogs met the inclusion criteria; 1 dog was excluded because of severe thrombocytopenia. Eight dogs were enrolled into the IAD group and 7 into the CD group. Breeds represented were Shih-Tzu ($n = 3$), Border Collie ($n = 2$), and one each of the following breeds: Cocker Spaniel, German Shepherd Dog, Rat Terrier, Wire-haired Fox Terrier, Welsh Corgi, Standard Poodle, Cavalier King Charles Spaniel, Collie, Airedale Terrier, and Beagle. There was no difference between the treatment groups as to age or bodyweight. Females were overrepresented in the CD group. Two dogs in the CD group had a marked left shift (the censored dog and the sole survivor); however, there were no significant differences between groups when compared by possible indicators of severity of disease^{2,8,17} or coagulation status on admission (Table 1).

Survival

All dogs were monitored for 180 days, or until death. One dog in the CD group was withdrawn at day 10 at the owner's request and consequently censored in the survival analysis. Seven out of 8 dogs in the IAD group were alive at day 180; only 1 dog (out of 6) in the CD group was alive at day 180 ($P = .01$). Median survival time was >180 days for the IAD group, and 68 days for the CD group. Kaplan-Meier survival curves (Fig 2) for the 2 groups were significantly different ($P = .01$) by the log-rank test. Because of small numbers enrolled, we also applied Pearson's Chi-squared test. Survival at 180 days was significantly different using the exact distribution for this test ($P = .03$).

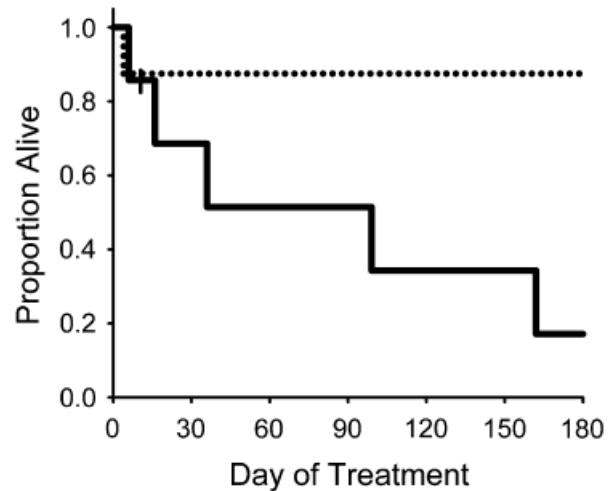


Fig 2. Kaplan-Meier survival curves. Curves depict survival fractions for the individually adjusted dose group (dashed line) and the constant dose group (solid line) over the 180-day observation period. Censoring is indicated by the hatch mark. Median survival times were >180 days for the individually adjusted group and 68 days for the constant dose group. The curves were significantly ($P = .01$) different by log rank test.

Thrombosis and Cause of Death

Documented thrombotic events occurred in 5 out of 6 dogs in the CD group and included fatal portal vein thrombosis ($n = 1$ on day 5, euthanized on day 36), fatal multiple organ microthrombi ($n = 1$, euthanized on day 6), fatal acute PTE ($n = 1$ on day 16), nonfatal PTE ($n = 1$ on day 3), based on ventilation/perfusion scan, and nonfatal PTE ($n = 1$ on day 13) based on ABG, clinical, and radiographic findings. Of the 2 dogs with nonfatal thrombotic events, the former dog died of unknown causes on day 162.

Table 1. Comparison of patient characteristics and indicators of disease severity at admission between treatment groups.

Parameter	IAD (n = 8)	CD (n = 7)	Reference Range	P-Value
Age (year)	6.2 (1.2–12.9)	5.9 (2.4–9.2)		.59
Sex	7 MN, 1 FS	2 MN, 5 FS		.04
Body weight (kg)	12.1 (6.25–32)	17.4 (4–39.5)		.66
Rectal temperature (°F)	102.1 (100.9–104.7)	101.8 (100–104.5)	99.5–102.5	.80
Neutrophil segs ($10^3/\mu\text{L}$)	11.01 (6.7–32.3)	13.69 (9.79–32.88)	2.1–11.2	.28
Neutrophil bands ($10^3/\mu\text{L}$)	0.22 (0–0.97)	0.68 (0.14–3.61)	0–0.13	.06
Hematocrit (%)	15.8 (10.3–27.9)	11.5 (8–22)	38.5–56.7	.29
Serum bilirubin (mg/dL)	0.7 (0.3–4)	1.5 (0.6–3.5)	0–0.3	.40
Auto-agglutination+	7 dogs	7 dogs		1.0
Coombs+	1/1 dogs tested	1/2 dogs tested		ND
Spherocytosis	8 dogs	6 dogs		.47
aPTT (s)	11.9 (11.4–14.6)	13.1 (9.5–15.5)	9.8–14.6	.43
PT (s)	7.2 (6.7–8.1)	7.5 (7.3–8.0)	6.2–7.7	.09
Platelets ($\times 10^3/\mu\text{L}$)	166 (95–474)	129 (75–384)	160–425	.28
FDPs ($\mu\text{g}/\text{mL}$)	3 dogs > 20	3 dogs > 20	< 5	1.0
Fibrinogen (mg/dL)	500 (300–800)	600 (400–700)	200–400	.2

Values reported as median (range).

P-value determined by Student's *t*-test for continuous data when normally distributed, Mann-Whitney rank-sum test if not normally distributed, and Fisher's exact test for categorical data.

IAD, individually adjusted heparin dose group; CD, constant dose heparin group; aPTT, activated partial thromboplastin time; PT, prothrombin time; FDPs, fibrin degradation products; ND, P-value not determined due to low numbers.

and the latter dog (which was euthanized on day 99) had thrombi in multiple organs on necropsy examination.

Three of the dogs that died in the CD group died after the UH therapy period. Two of these dogs had documented thrombosis early in the treatment period (day 5 for 1 dog, day 13 for the other) and were euthanized because of ongoing illness from these thrombotic events.

There was 1 confirmed and 1 suspected thrombotic event in the IAD group. The sole nonsurvivor in the IAD group died of PTE on day 4. Another dog developed unexplained pleural and peritoneal effusion (modified transudate without evidence of thrombi on abdominal, thoracic, or cardiac ultrasound) on day 8, which resolved without any specific therapy.

Hemorrhagic Complications

Despite some heparin concentrations being well above the therapeutic targets, no dog in either group had any evidence of hemorrhagic complications.

Hospitalization and Therapy Administered

No dogs had any immunosuppressive or anticoagulant therapy before admission. All dogs were treated with prednisolone. Dogs received additional immunosuppression with azathioprine (5 out of 8 dogs in IAD group, 3 out of 6 dogs in CD group) or cyclosporine ($n = 2$ in each group) according to the protocol. In each group, cyclosporine was used concurrently with azathioprine (because of persistent spherocytosis and anemia) in 1 dog, and was used to replace azathioprine (because of adverse effects) in 1 dog.

Dogs were hospitalized in the ICU for a median duration of 74 hours (range 14–210 hours) in the IAD group and a median of 135 hours (range 69–206 hours) in the CD group ($P = .07$) (Fig 3). Oxygen therapy was provided for 1 dog in the IAD group and 2 dogs in the CD group. In the IAD group 1 dog received no transfusions, 5 dogs received 1 transfusion, and 2 dogs received 2 transfusions. In the CD group, 2 dogs received 1 transfusion, 3 dogs received 2 transfusions, and 2 dogs received 3 transfusions. There was no statistical difference between the 2 groups with respect to number of transfusions ($P = .336$) or immunosuppressive therapy.

Heparin Doses and Plasma Concentrations

In the IAD group, the dose of heparin administered varied between 150 and 712 U/kg (Fig 4). The dose associated with therapeutic anti-Xa activity varied between 150 and 566 U/kg, with a median of 360 U/kg. After day 3 (initial dose adjustment), 35% of the peak anti-Xa activity measurements in the IAD group were within the therapeutic range during the treatment period and 23% were above (Fig 5A). Fewer than 5% of trough measurements for the IAD dogs were within therapeutic range (Fig 5B). There was substantial inter- and intraindividual variation.

One dog in the CD group was presumptively diagnosed with PTE on day 13 and, in a deviation from protocol, was treated for the PTE with 370 U/kg of heparin between days 14 and 28.

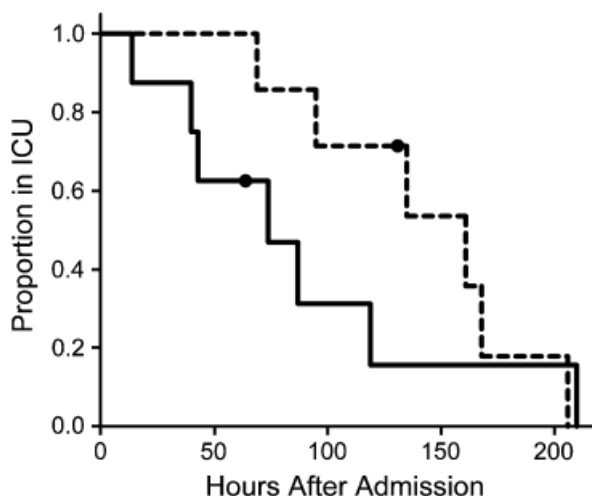


Fig 3. Kaplan-Meier hospitalization curves. Curves depict the fractions remaining hospitalized in the intensive care unit (ICU) for the individually adjusted dose group (solid line) and the constant dose group (dashed line). There was a trend toward statistical difference between the curves ($P = .07$) by Gehan-Wilcoxon test. Dogs that died while in ICU were censored from analysis. Censoring points are indicated by filled circles.

Of the CD group, only 1 dog (the sole survivor in this group) had plasma anti-Xa activity approaching the therapeutic range (mean anti-Xa level 0.1 U/mL) (Fig 6).

Plasma heparin level at peak was weakly ($r = .42$) but significantly ($P = .002$) positively correlated with dose administered for dogs in the IAD group (Fig 7). When data were individually analyzed for the 7 dogs for which >2 data points were available, peak heparin level was strongly and significantly positively correlated with dose in 3 dogs (range of r values: 0.90–0.93, range of P -values: .003–.006), strongly but nonsignificantly correlated in 2 dogs (range of r values: 0.70–0.73, range of P -values: .06–.08) and did not correlate with dose in 2 dogs.

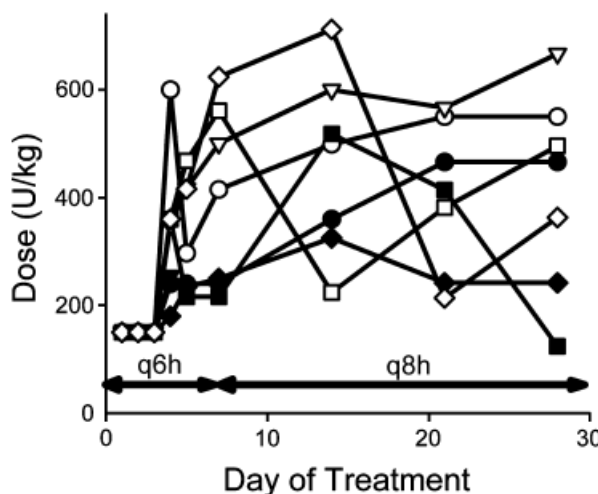


Fig 4. Unfractionated heparin dosing. The dose of unfractionated heparin administered to dogs in the individually adjusted group varied markedly. Heparin was administered every 6 hours for the first 7 days, and then every 8 hours thereafter. Each symbol indicates an individual dog in the individually adjusted dose group.

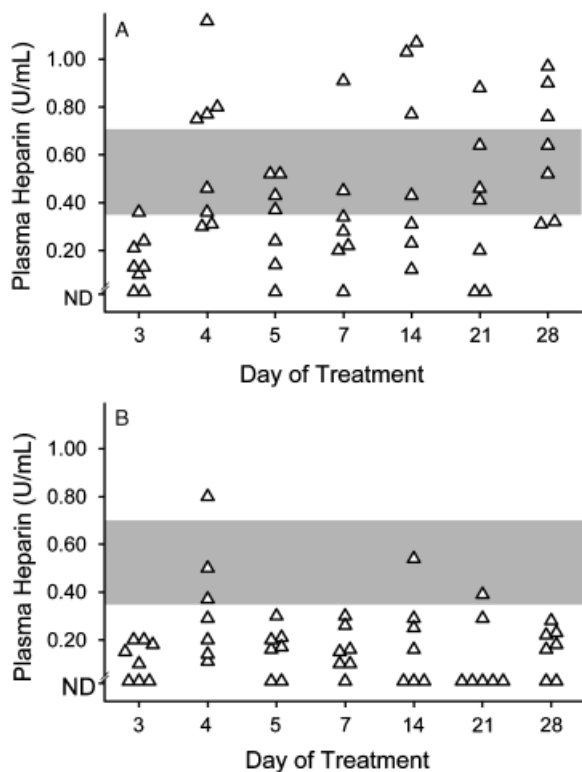


Fig 5. Plasma heparin concentration: individually adjusted dose group. Plasma heparin concentrations as measured by anti-Xa activity varied markedly over the treatment period of 28 days. Target therapeutic range was 0.35–0.7 U/mL (shaded area). ND, not detected, indicating that the value was below the lower limit of detection of anti-Xa assay (<0.1 U/mL). (A) Plasma heparin concentration at “peak” (2 hours postdose) for the individually adjusted group. The majority of samples were within or above target range. (B) Plasma heparin concentration at “trough” (6 hours postdose) for the individually adjusted group. The majority of samples were below target range.

Discussion

The results of this prospective study are consistent with the hypothesis that UH therapy adjusted to maintain plasma concentration within a target therapeutic range significantly reduces case fatality rate in dogs with IMHA when compared with a constant low-dose of 150 U/kg. While case fatality rates were markedly different between the 2 groups, it is not clear whether this finding represents a positive impact on survival by individual dose adjustment in the treatment group, a negative impact on survival by subtherapeutic doses in the control group, or a combination of the 2 events.

Direct comparison of survival between published studies of IMHA in dogs is complicated because of variability in study populations and in methods used to evaluate and report survival data. Survival rates range from 30 to 83%^{2,3,4} indicating that the IAD group in the current study was at or above the upper end of the expected survival rate. This suggests that an IAD approach might improve survival in dogs with IMHA. This interpretation is supported by a recent report, which also described

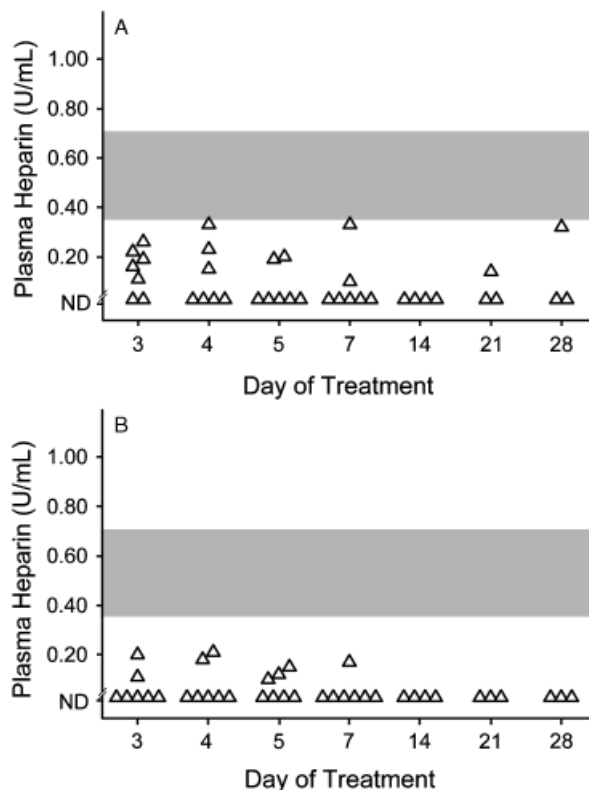


Fig 6. Plasma heparin concentration: constant dose (CD) group. Plasma heparin concentrations as measured by anti-Xa activity were subtherapeutic during the majority of the 28 days of measurement. Target therapeutic range was 0.35–0.7 U/mL (shaded area). ND, not detected, indicating that the value was below the lower limit of detection of anti-Xa assay (<0.1 U/mL). (A) Plasma heparin concentration at “peak” (2 hours postdose) for the constant dose group. The majority of samples were below target range. After day 10, only 1 dog in the CD group had detectable peak heparin concentration, all other dogs had nondetectable heparin concentration. (B) “Trough” (6 hours postdose) for the constant dose group. All values were below the target range.

individual adjustment of UH to achieve target anticoagulation and achieved high survival rates (13/18 alive at 1 month; 11/18 at 1 year).⁶

Survival in the CD group was low at 180 days, worse than rates generally reported for IMHA in dogs. The difference between survival rates in our 2 groups could have been partially because of an adverse effect on survival by subtherapeutic UH therapy. Survival rates are 16% at 60 days in dogs receiving 75–125 U/kg UH.⁴ It appears, therefore, that subtherapeutic doses of UH are at least not effective in treatment of IMHA in dogs, and might be detrimental. It is not clear why subtherapeutic heparin therapy would be harmful; however, consumption of antithrombin or noncoagulation protein-mediated effects of heparin have previously been suggested.^{19,21} Further research is required to confirm the adverse effect of subtherapeutic dose heparin and elucidate its mechanism. The number of thromboembolic events was lower in the IAD group. This finding is potentially more subjective as the antemortem confirmation of thrombosis can be difficult, often relying on interpretation of clinical and diagnostic findings.^{9,11} Although it is possible that the

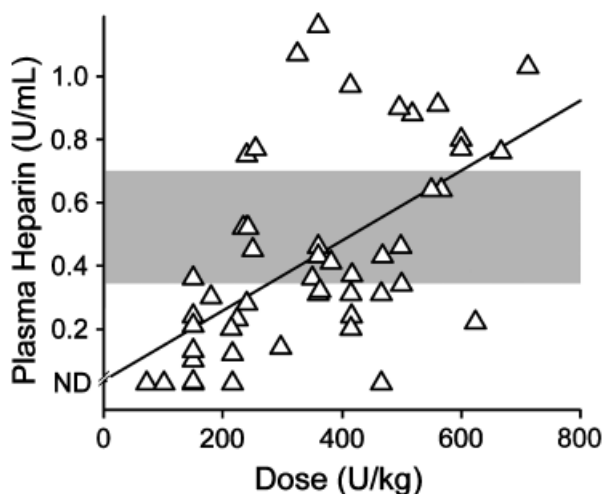


Fig 7. Plasma heparin concentration versus heparin dose. There was a weak ($r = .422$) but statistically significantly ($P = .002$) positive correlation between dose of unfractionated heparin administered and peak plasma heparin concentration for the individually adjusted group. Target therapeutic range was 0.35–0.7 U/mL (shaded area). ND, not detected, indicating that the value was below the lower limit of detection of anti-Xa assay (<0.1 U/mL).

difference in thrombosis rates was affected by lack of clinician masking, this seems unlikely as in all cases where thrombosis was suspected in a dog that subsequently died, necropsy confirmed the presence of thrombi.

In this study 3 dogs died after the UH therapy period, with 2 of them euthanized because of ongoing illness from earlier thrombotic events. Thrombotic complications later in the disease course have been documented in other studies⁶ and further investigation into the appropriate duration of anticoagulation therapy is warranted.

One possible explanation for the low case fatality rate in the IAD group is that our population of dogs had less severe disease than in previous reports. This appears unlikely as, based on negative prognostic indicators, our population consisted primarily of severely affected dogs referred for intensive care. The observed differences in outcome between our treatment groups could have been because of discrepancies in severity of disease, despite randomization. The only significant differences between the 2 groups was sex. Sex has not been recognized as a prognostic factor, but the presence of circulating band-form neutrophils is associated with a worse prognosis.^{3,8} As only 2 dogs in the CD group had a marked left shift (the censored case and the sole survivor), this factor does not explain the significant difference in survival.

Part of the beneficial effect of heparin in this study could have been unrelated to coagulation. In addition to its anticoagulant effect, heparin reduces endothelial-leukocyte interactions, inhibits leukocyte migration, protects against free-radicals, and reduces the production of endothelin-1, a potent vasoconstrictor.³¹ In vitro studies have shown that heparin can inhibit and regulate complement activation and has been used to inhibit complement-mediated hemolysis in humans with hemolytic anemia.³²

Although our study was not specifically designed to evaluate the pharmacokinetics of UH therapy in dogs with

IMHA, the results provide useful information regarding dosing approaches. We evaluated plasma heparin concentrations at “peak” (2 hours postdose) and “trough” (immediately before the next dose). We elected to use 2 hours postdose as a measure of peak concentration based on published studies of pharmacokinetics of single and repeated dose SC UH in normal dogs, where peak plasma concentrations generally occurred 2–4 hours after administration.^{20,a} The increase in acute phase reactants (present in dogs with IMHA^{33,34}) and inflammation can decrease the bioavailability of SC administered heparin in human patients.^{16,21,22} It is therefore possible that absorption of SC UH in dogs with IMHA is different than in normal dogs. Regardless of whether or not the 2-hour postdose samples truly represented peak plasma concentration, they were markedly different between the 2 groups over the course of monitoring.

None of the dogs in the CD group had peak plasma concentrations within the therapeutic target range, whereas most samples for the IAD group were either within or above therapeutic target range. Trough concentrations for the IAD group were mostly subtherapeutic, whether dosed every 6 or 8 hours, indicating a shorter than anticipated duration of effect. In normal dogs every 6–8 hours dosing can be used to achieve concentrations that cycle within the therapeutic range.^{20,35,a} Dogs with IMHA appear to require more frequent dosing. Nonetheless, subtherapeutic trough concentrations did not appear to adversely affect survival.

There was wide interindividual variation in the dose required to obtain target peak plasma heparin concentration in the IAD group. These dogs often required markedly higher doses (up to 566 U/kg) than those reported previously to achieve therapeutic ranges in normal dogs (250–300 U/kg).^{20,28} There are 3 possible reasons for this increased dose requirement: decreased bioavailability, increased volume of distribution, or increased clearance. Heparin has been documented to bind to a variety of acute phase reactants.^{16,21,22} Based on data from humans and experimental animals, it is likely the increased dose is a function of decreased bioavailability of the SC administered drug. Analysis of the data points from our study, when compared with that of Kellerman and colleagues^a indicates that 5 of 6 of our IMHA dogs had peak values less than the mean values for normal dogs (at a particular dose), but the slopes (ie, half lives) are essentially identical. In this analysis, when the peak value is decreased, but the half-life remains the same, it suggests decreased bioavailability. This supports our hypothesis that reduced bioavailability is the main cause of the increased dose requirement.

While the positive correlation between dose and peak plasma heparin concentration was weak on a population basis, it was strong in some individual dogs in the IAD group. These findings suggest that the dose-response relationship may be predictable on an individual basis in some dogs but underscore the need for monitoring.^{26,35}

The optimal method of monitoring heparin therapy in dogs with IMHA has yet to be determined. We chose to use the previously validated anti-Xa assay in part because of previous reports of the high variability in the response of aPTT reagents to heparin concentrations in

both canine,^{26,27} and human^{23,29} plasma. Additionally, the aPTT may be inadequately prolonged by heparinization in the face of elevated fibrinogen and be affected by other inflammatory mediators, which are present in IMHA.³⁶ It appears that there is a strong correlation between anti-Xa activity and the natural log of aPTT in samples from IMHA dogs receiving heparin⁶; however, this finding cannot be extrapolated to other reagents and laboratories because of lack of consistency in response between reagents used in different institutions.²³

A disadvantage of utilizing the anti-Xa assay is obtaining results quickly enough to be clinically useful. The assay is available through the Cornell University Comparative Coagulation Laboratory, but not in commercial veterinary laboratories. Our approach of sending prediluted samples to the local human hospital laboratory may facilitate clinical application in some settings. Adjustment of the nomogram may be indicated to allow finer control of anti-Xa activity for future use, as the anti-Xa activities were not as tightly controlled as desired.

The results of our study are striking but must be interpreted in light of some limitations. The study was limited to a small number of patients. Based on initial power calculations, planned enrollment was 40 cases. Unfortunately, after enrollment of 15 dogs, availability of heparin became restricted because of product recalls. Interim analysis suggested a significant difference in all-cause mortality between the 2 groups, and continuation of the study was considered unethical because of a potential for a detrimental effect on survival in the CD group. An additional possible limitation was the loss of clinician blinding during the course of study enrollment. Given that the primary endpoint was objective (all-cause death rate) it is unlikely that the loss of blinding markedly affected the results. In addition, randomization was maintained throughout the study and a treatment protocol was used to standardize therapy regardless of group assignment.

In conclusion, this study suggests that high dose, individually adjusted UH therapy may be a safe and effective means of improving survival in canine IMHA. Constant low dose (150 U/kg q6–8h) heparin does not appear to be effective, and potentially may adversely affect outcome. Future research needed includes pharmacokinetics of heparin in dogs with IMHA and controlled clinical trials comparing IAD heparin therapy to other anticoagulants or platelet inhibitors in a large group of dogs.

Footnotes

^a Kellerman DL, Lewis DC, Bruyette DS, Davenport DJ. Determination and monitoring of a therapeutic heparin dosage in the dog. *J Vet Intern Med* 1995; 9(3):187 (abstract)

^b PT/aPTT Coagulation Analyzer: STA compact; Diagnostica Stago, Parsippany, NJ

^c MP Biomedicals, Solon, OH

^d V-Cath PICO; HDC Corp, Milpitas, CA

^e Heparin Sodium 10,000 U/mL; Baxter Healthcare Corporation, Deerfield, IL

^f Rotachrom Hybred Heparin 2, University of Minnesota Medical Center Fairview, Acute Care Laboratory; Diagnostica Stago,

Asnieres-sur-Sein, France, distributed in the United States by American Bioproducts Company, Parsippany, NJ

^g Dexamethasone Sodium Phosphate Injection 4 mg/mL (generic); American Regent, Inc, Shirley, NY

^h Azathioprine Tablets; *Imuran*, Faro Pharmaceuticals, Bedminster, NJ

ⁱ Cyclosporine (Modified) Capsules; *Atopica*; Novartis Animal Health, Greensboro, NC

^j SPSS 16.0 for Windows; SPSS Inc, Chicago, IL

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Appendix 1. Nomogram used to adjust unfractionated heparin dose based on peak and trough heparin level (measured as anti-Xa activity in U/mL).

Peak Anti-Xa	Trough Anti-Xa	Multiply Current Dose by	Peak Anti-Xa	Trough Anti-Xa	Multiply Current Dose by
0–0.05		4	0.65–0.70	0.15–0.30	1.1
0.05–0.10		3		0.30–0.35	1
0.10–0.15		2.4		0.35–0.40	1
0.15–0.20		1.9		0.40–0.45	1
0.20–0.25		1.7		0.45–0.50	1
0.25–0.30		1.5		0.50–0.55	1
0.30–0.35	0–0.15	1.3		0.55–0.60	1
	0.15–0.30	1.2		0.60–0.65	1
	0.30–0.35	1.1		0.65–0.70	1
0.35–0.40	0–0.15	1.25	0.70–0.75	0.30–0.35	0.95
	0.15–0.30	1.2		0.35–0.40	0.95
	0.30–0.35	1.1		0.40–0.45	0.9
	0.35–0.40	1.05		0.45–0.50	0.9
0.40–0.45	0–0.15	1.3		0.50–0.55	0.9
	0.15–0.30	1.2		0.55–0.60	0.8
	0.30–0.35	1.1		0.60–0.65	0.8
	0.35–0.40	1.05		0.65–0.70	0.7
	0.40–0.45	1	0.75–0.80	0.30–0.35	0.95
0.45–0.50	0–0.15	1.3		0.35–0.40	0.9
	0.15–0.30	1.2		0.40–0.45	0.9
	0.30–0.35	1.1		0.45–0.50	0.9
	0.35–0.40	1.05		0.50–0.55	0.8
	0.40–0.45	1		0.55–0.60	0.8
	0.45–0.50	1		0.60–0.65	0.7
0.50–0.55	0–0.15	1.3		0.65–0.70	0.7
	0.15–0.30	1.2		0.75–0.80	0.6
	0.30–0.35	1.1	0.80–0.85	0.30–0.35	0.9
	0.35–0.40	1		0.35–0.40	0.9
	0.40–0.45	1		0.40–0.45	0.9
	0.45–0.50	1		0.45–0.50	0.8
	0.50–0.55	1		0.50–0.55	0.8
0.55–0.60	0–0.15	1.2		0.55–0.60	0.7
	0.15–0.30	1.1		0.60–0.65	0.7
	0.30–0.35	1		0.65–0.70	0.6
	0.35–0.40	1		0.75–0.80	0.6
	0.40–0.45	1		0.80–0.85	0.5
	0.45–0.50	1	0.85–0.90	0.30–0.35	0.9
	0.50–0.55	1		0.35–0.40	0.8
	0.55–0.60	1		0.40–0.45	0.8
0.60–0.65	0.15–0.30	1.2		0.45–0.50	0.7
	0.30–0.35	1.1		0.50–0.55	0.7
	0.35–0.40	1		0.55–0.60	0.6
	0.40–0.45	1		0.60–0.65	0.6
	0.45–0.50	1		0.65–0.70	0.6
	0.50–0.55	1		0.75–0.80	0.5
	0.55–0.60	1		0.80–0.85	0.5
	0.60–0.65	1	0.90–0.95		0.4
			0.95–1.00		0.3
			> 1.00		0.2

Adapted from Kellerman and Lewis, unpublished pilot data.