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The effect of ε-aminocaproic acid on blood product requirement, outcome and thromboelastography parameters in severely thrombocytopenic dogs

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Abbreviations: EACA = ε-aminocaproic acid; FWB = fresh whole blood; HC = Historical control; ITP = immune-mediated thrombocytopenia; IVIG = intravenous immunoglobulin; MA = maximum amplitude; PAI-1 = plasminogen activator inhibitor 1; PRBC = packed red blood cellls; rTEG = rapid TEG; TEG = thromboelastography; tPA = tissue plasminogen activator; tPA-rTEG = tissue plasminogen activator stressed rapid thromboelastography; TP = total protein; TXA = tranexamic acid CRI = continuous-rate infusion

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Abstract

Background: No treatment beside platelet administration is known to protect against spontaneous hemorrhage in thrombocytopenic dogs.

Objectives: Primary: to determine if treatment with ε-aminocaproic acid (EACA) reduces the requirement for blood transfusions and improves outcome in dogs with severe thrombocytopenia. Secondary: to find evidence of hyperfibrinolysis and determine the effect EACA administration on rapid (rTEG) and tissue plasminogen activator-spiked (tPA-rTEG) thromboelastography variables.

Animals: Twenty-seven dogs with severe thrombocytopenia were treated with EACA, and data from an additional 33 were obtained from the hospital database as historical control (HC) cohort.

Methods: Single arm clinical trial with HCs. EACA group dogs received EACA (100 mg/kg IV followed by a CRI of 400mg/kg/24 hrs). Thromboelastography before and during EACA infusion, hospitalization days, number of transfusions, and mortality were compared.

Results: There was no difference in number of transfusions/dog (median, interquartile range, p value) (1, 0 - 2.5 vs .9, 0 - 2; p = .5) and hospitalization days (4, 4-6 vs 4.5, 3.75-6; p = .83) between HC and EACA groups, respectively, and no difference in survival determined by log-rank analysis (p = .15). Maximum amplitude on both rTEG and tPA-rTEG increased after EACA administration (rTEG baseline 23.6, 9.6-38.9; post-EACA: 27.3, 19.8-43.2, p < .001, tPA-rTEG baseline 23, 10.9-37.2, post-EACA: 24.7, 16.7-44.8, p < .002).

Conclusions and clinical importance: Although EACA increased clot strength, there was no effect on outcome. Treatment with EACA at this dose cannot be recommended as a routine treatment but may be considered for dogs with severe ongoing hemorrhage.

Introduction

Thrombocytopenia is a common finding in dogs presenting to veterinary referral hospitals; Grindem et al (1991) reported that over 5% of admissions had platelet counts lower than their reference range and 11% of those dogs had a platelet count < 50,000/ µl.[1] Until the primary disease process can be controlled, blood products are administered to treat clinically significant bleeding, adding significant cost to hospital care. O'Marra reported that 81% of dogs with immune thrombocytopenia (ITP) had spontaneous hemorrhage at the time of admission and 41% required blood transfusions during hospitalization, with some developing severe complications of acute hemorrhage.[2]

Epsilon-aminocaproic acid (EACA) and tranexamic acid (TXA) are antifibrinolytic agents. They are lysine analogues that reversibly bind to lysine receptor sites on plasminogen, blocking the binding of plasmin to fibrin and therefore decreasing lysis of newly formed clots. Indications for the use of antifibrinolytics in humans include the treatment of hemorrhage associated with hyperfibrinolysis (e.g., acute traumatic hemorrhage), post-partum bleeding, and intraoperative bleeding.[3-5] In veterinary medicine, studies in Greyhounds, a breed at risk of excessive fibrinolysis, suggest reduced postoperative bleeding in response to EACA.[6] Increased clot strength on viscoelastic testing was documented after the administration of EACA in another study. [7]

The use of antifibrinolytics as adjunctive treatment of bleeding of any origin, including hemorrhage secondary to thrombocytopenia, has become increasingly common in veterinary medicine.[8,9] Reasons for this may include low cost, low adverse effect profile, and the scarcity of point-of-care testing to screen for hyperfibrinolysis. These drugs have also been evaluated as a treatment to reduce spontaneous bleeding in severely thrombocytopenic humans[10], based on speculation that antifibrinolytics may stabilize fragile blood clots in thrombocytopenic patients and therefore reduce further hemorrhage.[11] some studies investigating humans with ITP and thrombocytopenia due to hematological malignancy suggest that antifibrinolytics may reduce clinical evidence of bleeding and need for platelet transfusions,

however, the evidence is limited.[10-12] A report describing the use of TXA in 4 dogs with ITP found no clinical benefit when compared to a contemporary control cohort of 6 dogs.[13]

A sensitive ex-vivo method of identifying a tendency towards systemic hyperfibrinolysis is the addition of tissue plasminogen activator (tPA) to a tissue factor or a kaolin and tissue factor activated thromboelastography. This technique of spiking the rTEG assay (tPA-rTEG) may detect reductions in fibrinolysis inhibitor activity that are not yet sufficient to cause overt hyperfibrinolysis but place patients at increased risk for decompensation to a hyperfibrinolytic state.[14] It appears to provide rapid assessment of the endogenous fibrinolytic potential of whole blood in people[15] and appears to be useful in dogs as well.[16,17] To our knowledge, the fibrinolytic potential of dogs with thrombocytopenia has not been characterized.

We hypothesized that treatment with EACA will reduce spontaneous bleeding and transfusion requirements in dogs with severe thrombocytopenia. Thus, the primary goals of the study reported here were to determine if prophylactic treatment with EACA reduces the frequency and severity of spontaneous hemorrhage, reduces the requirement for blood transfusions, and improves survival to discharge in dogs with severe thrombocytopenia. A secondary goal of the study was to determine if thrombocytopenic dogs have thromboelastographic evidence of hyperfibrinolysis and to determine the effect of treatment with EACA on tPA-rTEG variables.

Material and methods

Study design

This was a prospective single arm study using historical controls. The prospective arm was approved by the institutional animal care and use committee, and informed owner consent was obtained before enrollment of each dog (EACA group). Eligible dogs hospitalized for severe thrombocytopenia between 2018 and 2020 were identified via manual platelet count within the

preceding 12 hours. Severe thrombocytopenia was defined as a platelet count < 30,000/µl, or < 50,000/µl if accompanied by evidence of bleeding consistent with thrombocytopenia (e.g. petechiae, ecchymoses, epistaxis or melena).[18] Dogs weighing less than 2 kg (precluding safe blood collection for the study) and dogs suspected of having a disorder of secondary hemostasis were excluded.

For each dog enrolled in the EACA group, a kaolin and tissue factor-activated TEG (rapid TEG or rTEG) and a tPA-modified rapid TEG (tPA stressed-rTEG or tPA-rTEG) were performed. Both rTEG and tPA-rTEGs were performed prior to administration of EACA (T1), as described below. All assays were performed by the same two trained operators (JW or LR). After obtaining the assay samples, 100 mg/kg EACA (Aminocaproic acid injection USP 5 g/20 mL, Hospira Inc, Lake Forest, Illinois) was administered intravenously over 15 min, immediately followed by a constant-rate infusion of 400 mg/kg/24 hours. The rTEG and tPA-rTEG assays were then repeated on blood samples collected prior to the end of the infusion, sometime during hours 18 to 24 as determined by operator availability (T2). If the dog was eating and judged to tolerate oral medication after T2 it was transitioned to oral administration of the intravenous EACA formulation at a dose of 100 mg/kg every six hours.[19] Dogs that were hyporexic or that had evidence of nausea or vomiting were continued on the intravenous constant-rate infusion and monitored for side effects. Aminocaproic acid treatment was continued in each dog until it was discharged, its platelet count exceeded 50,000 µl/ml with no evidence of active bleeding, or it died or was euthanized. The number of days until the endpoints were reached were recorded. Day 1 was defined as the day of admission, day 2 (and all subsequent days) began at 11 AM the following day. Manual platelets counts were obtained once daily, and packed cell volume (PCV) and total protein (TP) were monitored every 12 hours or more frequently if clinically indicated. Bleeding events, defined as observed hemorrhage including epistaxis, melena, hematuria, or a percent change reduction in PCV and/or TP of >10%, were recorded. The more objective DOGIbat scoring system was not used in this study as the relevant information was not recorded for the control group.[20] All blood product

transfusions including packed red blood cells (pRBC) and fresh whole blood (FWB) were documented. Approximately 10ml/kg pRBC or 20 ml/kg FWB were administered per transfusion to dogs in both groups; thus depending on the size of the dog a single transfusion may have used > 1 unit of product. All additional diagnostics and treatments were at the discretion of the primary clinician.

For the HC group, medical records between 2013 and 2017 were screened for dogs with severe thrombocytopenia of any origin using the same inclusion and exclusion criteria as for the EACA group. Three records from dogs that received EACA during the course of their treatment were found and were not included in the HC group. Underlying etiology and additional treatments for both groups were recorded to ensure both groups had comparable underlying causes and received similar treatment.

TEG analysis

For each dog enrolled in the EACA group, paired rTEG and tPA-rTEG were performed at T1 and T2. Both channels of a dedicated TEG machine (TEG 5000, Haemoscope, Skokie, Ill) were used and for each subject the channel assignment to each assay (rTEG and tPA-rTEG) was randomly assigned in blocks of 10. Blood samples from each dog were collected from either a central venous catheter (Mila Small Animal Guidewire Catheter Kit, Mila International, Florence, KY USA) freshly placed in the lateral saphenous vein or via direct cephalic or lateral saphenous venipuncture with a winged needle catheter during initial blood sampling at T1. All samples were collected from the central venous catheter at T2. For either technique, a purge of 1.2 ml (winged needle) or 3 mL (central venous catheter) was collected prior to diagnostic sample collection. The diagnostic sample of 1.4 ml of blood was collected directly into vacuum citrate tubes and the tubes were then mixed 5 times by inversion and then allowed to rest for 30 min at room temperature. While the samples rested, a prediluted frozen tPA vial containing 25 IU of tPA in 10 µL of solution was retrieved and thawed at room temperature. The tPA solution was made in advance by serial dilutions of stock product (Cathflo™ Activase® [Alteplase], Genentech, South San

Francisco, CA 2 mg/mL) in BSA buffer solution to a final concentration of 25 IU/10 µL. The tPA solution was made fresh every 6 months and stored at -80° F (-62° C) prior to use. The tubes were numbered to ensure a standardized sequence of use and each tube was assigned to either the left or right channel of the TEG analyzer to ensure an equal distribution of channel assignment for the stressed rTEG assays. The rTEG vials containing tissue factor activator and kaolin (RapidTEG™, Haemonetics®, Skokie, Ill) were reconstituted with 20ul distilled water and gently swirled, then allowed to rest. Immediately prior to assay, 10 µl of rTEG mixture and 20 µL of calcium chloride solution were added to each TEG cup. For the rTEG assays, the citrated tube was gently inverted 5 times and 340 µl of blood was pipetted directly into the test cup and gently mixed by pipetting the reagent-blood mixture in and out of the cup 3 times. The analyzer carriage was raised to start the test. For the tPA-rTEG assay, a 490 µl aliquot of citrated blood was pipetted into the tPA vial and was also gently mixed, yielding a final concentration of tPA in blood of 50 IU/mL. Then 340 µl of the blood/tPA mixture were added to the test cup assigned to the tPA-rTEG. This mixture was also pipetted out of and into the cup 3 times to mix all components prior to beginning the assay. The following values were recorded at T1 and T2: R (reaction) time, K (clot formation time), α-angle (a measure of the rapidity of clot formation), MA (maximum amplitude), and LY30 (clot lysis at 30 minutes).

Statistical analysis

Feasibility of the study was determined with a power analysis assuming a fixed number of thrombocytopenia cases would be enrolled in the 18 months available for recruitment. We used reductions in PCV to define bleeding events in HC group dogs, and based on contemporary hospital admissions we estimated that we would be able to enroll at least 16 dogs. Setting a threshold definition for bleeding as a fall in PCV by 10%, the HC group bleeding event rate was 0.44 +/-0.08 episodes/day. Based on this HC group data, 16 EACA group dogs would allow us to detect a reduction to 0.37

episodes/day at an alpha error rate of 5% and a beta error level of 20%. Data including study days, mortality, and number of transfusions were compared between the HC and the EACA groups with the Mann Whitney U test for continuous data and the Pearson Chi Square test for categorical data. A Kaplan-Meier survival curve and log rank analysis was performed to compare fatality rates. The analysis was then repeated including dogs with ITP only. In the EACA group, PCV, the rTEG and tPA-rTEG variables R, K, MA, and LY30% at T1 and T2 were compared using the Wilcoxon signed-rank test**.** The correlation between the platelet count on admission and platelet count on day2, between rTEG MA T1 and rTEG MA T2 and between tPA-TEG MA T1 and tPA-TEG MA T2 were determined with Pearson correlation. A p-value of less than .05 was used as the criterion for statistical significance. Analyzing the influence of different adjunctive drugs was not attempted due to the high variability of different treatment protocols. Data analysis was conducted using commercial software (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp)

Results

Records from 33 dogs were abstracted for the HC group, and 28 dogs were enrolled in the EACA group. Our initial enrollment criteria excluded dogs with PT or aPTT values that exceeded the laboratory reference interval. After excluding several dogs with slight elevations of their aPTT but no other clinical evidence of causes of secondary coagulation disorders, we modified the criteria to include dogs with values that were within 10% of the upper limit for either assay. The value of 10% was chosen because many dogs with no physical evidence of coagulopathy at the time of the assay fell within this range, and this figure is consistent with the magnitude of inter-assay variations between a point-of-care device and laboratory assay results observed by us and others.[21] None of the dogs had any other evidence of disorders of secondary hemostasis, and all had normal reaction times on thromboelastography.

Six dogs did not complete the study. In one of these, the intravenous catheter was lost after day 1 and could not be replaced, therefore serial data collection was not possible. Two dogs developed urethral obstructions secondary to blood clot formation in their urinary bladders and EACA administration was therefore discontinued. A fourth dog was discharged 24 hours after admission per the owner's request. EACA was discontinued on day 5 in the fifth dog in response to persistent regurgitation. Where paired data was available, these dogs were included in the TEG analysis but not outcome analysis. A sixth dog was completely removed from the study after developing hemolysis and coagulation abnormalities consistent with disseminated intravascular coagulation the day after enrollment. Data for one rTEG T1 were unavailable due to an analyzer malfunction, and another dog died before T2. Thus, clinical outcome including transfusion data was evaluated for 22 EACA group dogs, paired rTEG data was available from 23, and paired tPA-rTEG data was available from 24 of those dogs.

The majority of thrombocytopenic dogs in both the HC (30/33, 91%,) and the EACA group (19/22, 86.4%) were diagnosed with primary ITP. A summary of all diseases represented can be found in Table 1. Therapy used to treat the underlying cause of the thrombocytopenia was variable, and several distinct combinations of immunosuppressive and adjunctive therapy were used (Table 2). Nine dogs in the control group received intravenous immunoglobulin (IVIG) versus none in the EACA group. One dog, which was pancytopenic secondary to a suspected infectious cause did not receive any immunosuppressants. To assess for an effect of IVIG treatment on study days and transfusion dependency, statistics were repeated excluding the dogs in the HC that received IVIG, and no difference in results were found. The influence of treatments other than EACA and IVIG on study endpoints was not analyzed due to the large number of treatment combinations used for dogs in both groups. Similarly, a repeated analysis was performed including only dogs with ITP and no difference in results could be found.

PCV and transfusions

Anemia was defined as PCV below the lower limit of the reference range. The median PCV and incidence of anemia at the time of admission was 37% (IQR 21 - 43) and 14 of 33 dogs (42%), respectively, in the HC group, and 39% (24 - 45) and 7 of 22 (32%) dogs in the EACA group. These values were not significantly different between groups (p = .31 and .48, respectively). Similarly, no difference between median platelet count upon admission was found between the HC (2000/µl, 0 - 18000) and the EACA groups (1725/µl, 0 - 7500), p = .72).

Dogs in the HC group received a median of 1.33 transfusion/per dog of either pRBCs (24 transfusions) or FWB (20 transfusions). The EACA group dogs received a median of 1.32 transfusions/per dog of either pRBCs (19 transfusions) or FWB (10 transfusions) Additional results related to transfusions administered are shown in Figure 1. There was no significant group difference in the number of any transfusions (pRBC or FWB) per dog (p = .5) or in the proportion of dogs receiving a FWB transfusion (p = .57). Although the proportion of dogs in the EACA group that did not need any blood products (59.1%) was larger than in the HC group (45.5%), the difference was not statistically significant (p = .32). Some EACA dogs that required transfusions to treat hypovolemic hemorrhage did not have reductions in PCV of >10% and therefore did not fulfill that diagnostic criterion for a bleeding event, despite having clinically important bleeding. There was no difference in the frequency of bleeding events, defined as acute reductions of PCV/TP, between groups (p = .31). Because observed episodes of hemorrhage were not reliably recorded in the EACA group, we assumed that the same problem existed in the HC group and consequently dropped observed bleeding events from our primary goal.

Outcome

The median time from admission to the study end point was 4 days (IQR 4-6) in the HC group and 4.5 days (3.75-6) in the EACA group (p = .83). In the HC group, 30 (91%) dogs survived to discharge.

Two were euthanized, one failing to improve after 6 days of intensive treatment and the second one after developing severe respiratory distress and neurological signs. One dog died due to causes not related to thrombocytopenia. Seventeen (77%) dogs were discharged in the EACA group and 5 did not survive to discharge. Three patients died and two were euthanized (one after 3 and one after 6 days of treatment) due to refractory thrombocytopenia. There was no significant difference in fatality rate ($p = .08$), and log-rank analysis identified no significant difference ($p = .15$) in the survival time between the groups demonstrated on a Kaplan-Meier survival plot (Figure 2).

TEG data

Only one dog was mildly hyperfibrinolytic on the tPA-TEG at T1 (LY30% 23.4; reference range for our institution is < 20%), and this resolved during administration of EACA. This dog was diagnosed with ITP, was not anemic on presentation, and did not require any blood products. There was a significant T1-T2 difference in MA for both the rTEG (T1: 23.6, 9.6-38.9; T2: 27.3, 19.8-43.2, $p < 0.001$) and tPA-rTEG (T1: 23, 10.9-37.2, T2: 24.7, 16.7-44.8, $p < .002$) (Figure 3A). Five dogs had a MA within reference range on both TEGs prior to EACA and 7 had a MA within reference post EACA. No other TEG parameters were different between T1 and T2. A summary of the TEG data including institutional reference ranges is presented in Table 3.

There was a strong positive correlation between MA at T1 and T2 for both rapid ($r = .904$, $p < .001$), and tPA-TEGs ($r = .942$, $p < .001$), and the MA increased at T2 for 20/23 rTEG and 19/24 tPA-rTEG assays (Figure 3B and Figure 3C). There was only a weak correlation for both TEG types between T1 platelet count and MA (rTEG $r = 0.351$, $p = .07$ and tPA-TEG $r = .36$, $p = .06$) and T2 platelet count and MA (rTEG $r = .27$, $p = .22$ and tPA-TEG .34, $p = .11$). The MA in one or both TEG assays increased from T1 to T2 in 10/12 dogs that experienced a concurrent reduction in platelet count. No statistically significant

difference was found between the PCV at both timepoints (T1: 39, 23-45, T2: 32.5, 26.25 – 37.25; p = .06)

Discussion

Prophylactic treatment with EACA resulted in a statistically significant increased clot strength (as measured by the MA) in severely thrombocytopenic dogs. However, this increase did not seem to be large enough to have significant effect on clinically relevant parameters including hospitalization time, mortality, acute reductions of PCV or TP, and number of transfusions required.

There are only a small number of published reports of studies investigating the efficacy of antifibrinolytics in dogs. To the authors' knowledge, the present report is the first prospective study investigating the use of EACA in thrombocytopenic dogs. A recent small study compared a group of dogs with ITP (n=4) receiving TXA with a control group (n=6) and did not find a clinical benefit of TXA.[13] In a retrospective study of the effect of TXA in dogs with acquired bleeding disorders, 15.7% of the study population was thrombocytopenic. Dogs that were treated with TXA in that study required fewer transfusions and had a lower mortality rate than dogs that did not receive TXA; however, thrombocytopenic dogs were not analyzed separately and therefore it is not possible to draw any conclusions regarding the efficacy of TXA specifically in thrombocytopenic dogs from that report.[8]

The majority of dogs in the present study were diagnosed with primary ITP. Most reported studies of antifibrinolytic treatment of humans with thrombocytopenia investigated their use in patients with hematologic malignancies rather than ITP. A 2016 Cochrane review concluded that there is a lack of high-quality studies and there is insufficient evidence to justify the use of antifibrinolytics in thrombocytopenic patients secondary to hematological neoplasia.[11] Since then, a large randomized double-blind placebo-controlled trial reported in 2022 did not identify a significant reduction in transfusions, platelet

administration, or bleeding events in patients with hematologic malignancies treated prophylactically with TXA.[22]

We are aware of only two case series describing the efficacy of antifibrinolytics in human ITP patients with ongoing hemorrhage. In 12 patients that were treated with TXA as adjunctive treatment for active bleeding, high success rates for hemorrhage control were reported [12]. In a second series of 17 thrombocytopenic patients (15 with ITP) with uncontrolled bleeding, treatment with EACA appeared to facilitate discontinuation of platelet products in all 17.[23] We have not found any reports concerning the efficacy of prophylactic use of antifibrinolytic agents in ITP. Based on the lack of high-quality data, current guidelines on treatment of ITP in human medicine recommend against the routine use of EACA or TXA for ITP. However, in patients with ongoing bleeding, these agents may be considered.[24,25]

Side effects and complications

Aminocaproic acid appears to be well tolerated. In one report of a retrospective study of 122 dogs with hemorrhage secondary to neoplastic and non-neoplastic cause, only 3 dogs developed mild gastrointestinal signs possibly related to the EACA administration at doses of 14 – 24 mg/kg.[9] In the present study, higher doses of EACA (100 mg/kg every 6 hours orally or as continuous rate infusion) were administered based on recent evidence that increased doses are required to achieve more effective antifibrinolysis in dogs.[19] The high dose intravenous dose was chosen to maximize the potential to achieve constant therapeutic concentrations and identify any effect on thromboelastography at T2, since previously described characteristics of the drug in healthy dogs suggest that oral doses of 100 mg/kg are rapidly excreted and produce serum concentrations above the effective concentration of 25 µg/mL for only 3 hours.[19] We did not identify any significant side effects from this dose in the EACA group. One dog developed regurgitation during the study period and its oral EACA was stopped on day 5 as a precaution. It was not possible to determine if the regurgitation was more likely secondary due to the dog's primary

disease process or an adverse effect of EACA. However, as this study was not designed as a safety study, we cannot draw any conclusions with regards to safety of the EACA protocol used in this report.

Two dogs with significant hematuria developed clots causing urethral obstruction and EACA was discontinued in these dogs. Urinary tract obstruction secondary to blood clot formation has been previously reported in a dog with ITP[26] and any contribution of EACA to this complication is unknown. However, considering that antifibrinolytics stabilize blood clots, the use of TXA or EACA in dogs and cats with severe hematuria should be considered carefully.

TEG data

While the main reason for hemorrhage in dogs with thrombocytopenia is the lack of platelets, there might be mechanisms causing increased clot lysis to justify the specific use for antifibrinolytics in these dogs. One hypothesis regarding underlying mechanisms in thrombocytopenic patients is the reduced availability of plasminogen activator inhibitor 1 (PAI-1) released from granules on platelet surface, increasing susceptibility to fibrinolysis.[27,28] Additionally, dogs hemorrhaging severely may theoretically develop hyperfibrinolysis similar to traumatic coagulopathy or canine hemoperitoneum. [29] To screen for evidence of systemic hyperfibrinolysis and to investigate the effect of EACA on TEG tracings, we performed rTEGs and tPA-rTEGs in the present study. Only one dog had mild hyperfibrinolysis on tPA-rTEG, and this resolved after administration of EACA. This finding did not seem to have any clinical significance as the dog did not show any signs of hemorrhage had a normal PCV on presentation and did not require blood transfusions. Although it is possible that even the tPA-rTEG is not sensitive enough to detect endogenous hyperfibrinolysis[30], the normal fibrinolytic profile might explain the lack of response to prophylactic treatment with EACA in the dogs in this study. We found no other studies investigating hyperfibrinolysis in dogs with ITP. However, our findings are similar to data from thrombocytopenic human patients with hematologic malignancies. A study published in 2022 found that only 3 out of 115 thrombocytopenic humans showed a hyperfibrinolytic profile, median plasma PAI-I

concentration was normal, and there were no differences in plasma PAI-1 concentration between those who did or did not go on to develop significant hemorrhage.[28] Similarly, decreased plasma PAI-I concentration was not identified in humans with ITP in another study.[30] Although hyperfibrinolysis on a local level cannot be excluded, the findings of the present study and human data suggest that systemic fibrinolytic activation does not play a major role in the pathophysiology of hemorrhage in thrombocytopenic patients.

We identified a significant increase in MA for both TEG types after EACA administration, a change that was not attributable to an increase in platelet count as no relationship between platelet count and MA could be shown. Unfortunately, fibrinogen concentrations were not consistently available for all patients at both T1 and T2, which is a limitation of this study. Another parameter potentially increasing MA is a decreased PCV as suggested in previous publications. [31] In this study population the PCV at T2 was slightly lower (32.5%) compared to T1 (39%), which represents a clinical change. However, the difference was not statistically significant and the finding of previous studies that PCV influences MA could not be confirmed in a recent ex vivo study. [32]. Although the increase in MA in the tPA-rTEG assay in this study is consistent with a previous report showing an increase in MA using a tPA-modified TEG following administration of a single dose of EACA to dogs[19], the reason for the mild increase in rTEG MA is unclear. Because we have no TEG data on untreated control dogs, one possible explanation is that the MA increased with time in most dogs during the course of treatment with corticosteroids for their thrombocytopenia, independent of any effect by EACA or change in platelet numbers. [33] Another could be that a low level of fibrinolytic tendency is present in these dogs, reducing the MA without causing measurable increases in LY30, and this was inhibited by EACA. Regardless of the mechanism, this change was not associated with any identifiable clinical benefit in our dogs.

Additionally. 7 dogs in this study had a MA within or just below reference range that normalized after EACA, which is an unexpected finding for thrombocytopenic dogs. Interestingly, only one of these dogs required a blood transfusion. Further studies investigating the potential use of viscoelastic testing to predict clinical bleeding and correlation with bleeding scores in dogs with ITP are required.

Limitations

The present study has several limitations including the small treatment group and variable individual treatment regimens. Although the treatment of primary ITP is immunosuppression, individual clinician preferences and variable need to escalate therapy make it very difficult to control for variations in treatment for all dogs in a small study. There were many different treatment protocols consisting of different combinations of immunosuppressants and adjunctive therapies, and there were group differences in the use of IVIG as described in Table 2. Because of the small group sizes and marked variation in treatment and the timing of escalating drug therapy, it was not possible to determine if there was a potential effect of these treatments on study endpoints.

We used historical controls in an effort to maximize case numbers for this single-site study, knowing that treatment for primary ITP at our institution has not changed substantially since 2013. The HC dogs were selected using the same inclusion and exclusion criteria, however, there may have been some impact of selection bias in the HC group from, for example, excluding dogs that were treated with EACA, which may have limited the HC group to dogs with a milder clinical course. More objective clinical severity scoring like the DOGIBat system [26] was not possible as the necessary information was not recorded for the HC group. Bleeding events were not reliably documented and therefore could not be used for analysis. Baseline coagulation testing (PT and aPTT) for both the HC and EACA group dogs was done using different assays including a point-of-care device, commercial laboratories, and our hospital clinical pathology laboratory, and several dogs had slight elevations of one or both values. Although no

dogs had any other clinical evidence of a disorder of secondary hemostasis and their reaction times were normal on T1 thromboelastography we cannot completely rule out subtle disorders of secondary hemostasis. However, the impact of any such disorder is likely small.[34]

Conclusions

We found no evidence for hyperfibrinolysis in thrombocytopenic dogs or any survival benefit in dogs treated prophylactically with EACA, which is similar to results in human studies. Based on these results, routine treatment with EACA cannot be recommended in dogs with ITP. Nevertheless, EACA administration might be considered for dogs with severe ongoing bleeding. A randomized controlled trial with a concurrent control group that differs only in the administration of EACA is necessary to further evaluate the question if antifibrinolytics are useful in dogs with ITP. However, our results suggest that such a study would require a very large number of subjects to demonstrate a treatment effect. Assays for hemostatic parameters like PAI-1 concentration might be of interest to investigate possible reasons of underlying local hyperfibrinolysis justifying the use of antifibrinolytic agents.

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Etiology of thrombocytopenia	**HC group** N (%)	**EACA group** N (%)
Primary ITP	30 (91)	19 (86.4)
Pancytopenia	1 (3)	1 (4.5)
Tick-borne (suspected)	0	1 (4.5)
Lymphoma (suspected)	0	1 (4.5)
Acute leukemia	1 (3)	0
ITP secondary to neoplasia	1 (3)	0

Table 1: Underlying etiologies of thrombocytopenia in the control and EACA group.

Immunosuppressants	**HC group** N (%)	**EACA group** N (%)
GC* only	6 (18.2)	8 (36.4)
GC + azathioprine	10 (30.3)	4 (18.2)
GC + cyclosporine	5 (15.2)	4 (18.2)
GC + mycophenolate	8 (24.2)	5 (22.7)
GC + 2 second agents	3 (9.1)	1 (4.5)
No immunosuppressants	1 (3)	0
IVIG or vincristine	**HC group** N (%)	**EACA group** N (%)
Vincristine	20 (60.6)	15 (68.2
IVIG	3 (9.1)	0
IVIG + vincristine	6 (18.2)	0
No IVIG or vincristine	4 (12.1)	7 (31.8)

Table 2. Overview of adjunctive treatment for thrombocytopenia for the HC and EACA groups. GC*: glucocorticoid

	Reference range rTEG	rTEG T1	rTEG T2	Reference range tPA-rTEG	tPA-rTEG T1	tPA-rTEG T2
R	0 -1.26	.2 (.2 - .4)	.3 (.2 - .4)	0-1.4	.3 (.2 - .3)	.3 (.2 - .4)
α-angle	50 - 78	75.3 (58.6 – 79.5)	78.1 (65.6 – 80)	41 - 83	77.2 (67 – 80.4)	77.4 (65 – 79.9)
K	0.4 - 4.7	1.2 (.8 – 2.8)	1.2 (.8 – 2.95)	0.3 - 5	.85 (.8 – 3.8)	1.2 (1.2 – 4.2)
MA	40 - 67	23.6 (9.6 – 38.9)	27.3 (19.8 – 43.2)	38 - 65	23 (10.9 – 37.2)	24.7 (16.7 – 44.8)
LY30%	< 3.8%	0	0	< 20%	0 (0 - .35)	0

Table 3. Summary of rapid and tPA stressed rapid TEG results including institutional reference ranges

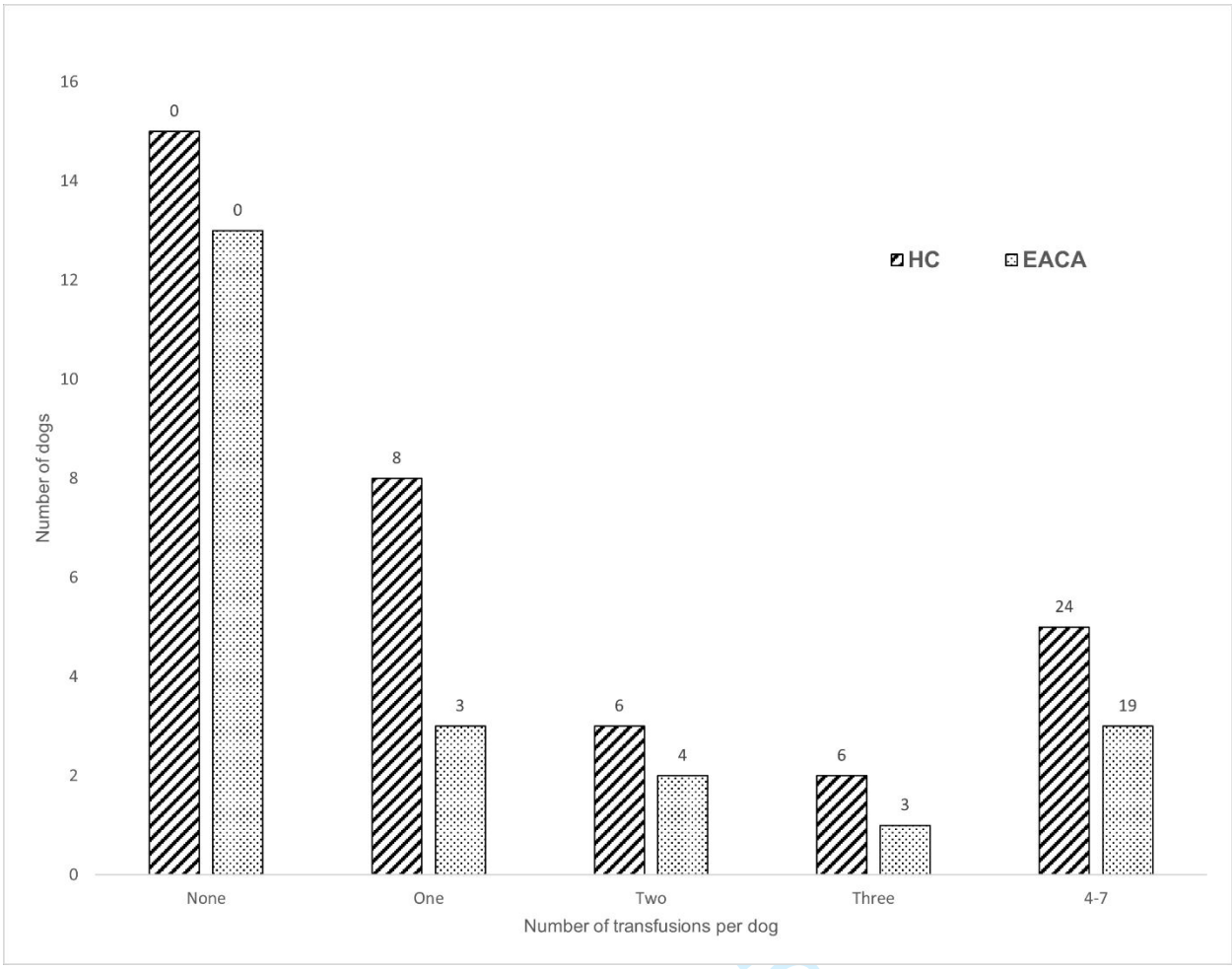


Figure 1. Bar chart showing the number of blood transfusions received per dog in both groups. Numbers at the top of each bar indicate the total number of transfusions administered to the control and EACA groups

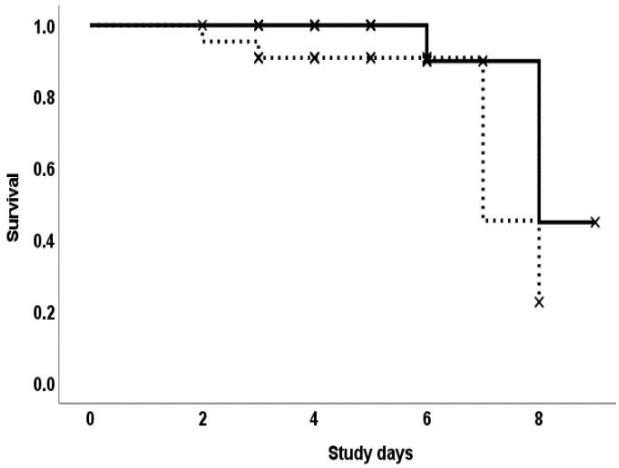
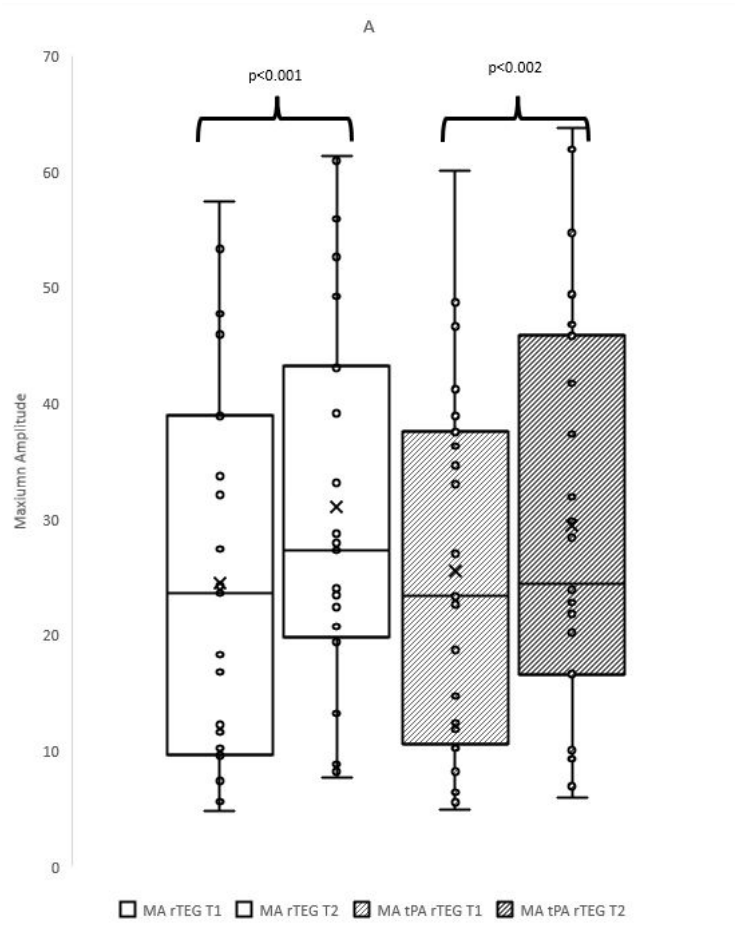
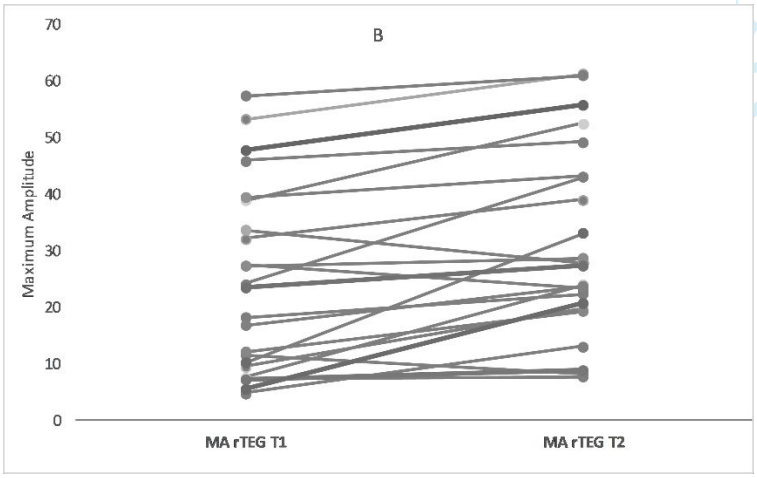


Figure 2: Kaplan-Meier Curve comparing survival between the EACA (dotted line) and HC (solid line) groups. X indicate censored events. P - value = .15


A
p<0.001
p<0.002
Maxiumn Amplitude
70
60
50
40
30
20
10
0
MA rTEG T1
MA rTEG T2
MA tPA rTEG T1
MA tPA rTEG T2

566


B
Maximum Amplitude
70
60
50
40
30
20
10
0
MA rTEG T1
MA rTEG T2

567

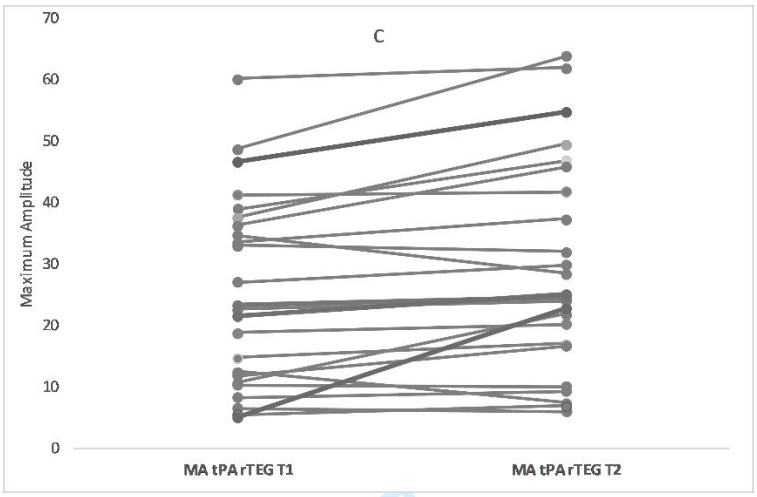


Figure 3. MA data. A: rTEG and tPA rTEG bar-and-whisker plots at baseline (T1) and next day (T2). Bars represent the range between the 1st and 3rd quartiles, the horizontal lines represent median values, and the x marks represent average values. B: Slope graph showing the changes in rTEG MA values for individual dogs between T1 and T2. C: Slope graph showing the changes in tPA-rTEG MA values for individual dogs between T1 and T2.