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

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**The effect of  $\epsilon$ -aminocaproic acid on blood product requirement, outcome and thromboelastography parameters in severely thrombocytopenic dogs**

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Manuscripts

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4 1 **The effect of  $\epsilon$ -aminocaproic acid on blood product requirement, outcome and thromboelastography**  
5  
6 2 **parameters in severely thrombocytopenic dogs**  
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26 **Keywords:** antifibrinolytics, hyperfibrinolysis, thrombocytopenia, platelets

27

28 **Abbreviations:** EACA =  $\epsilon$ -aminocaproic acid; FWB = fresh whole blood; HC = Historical control; ITP =

29

30 immune-mediated thrombocytopenia; IVIG = intravenous immunoglobulin; MA = maximum amplitude;

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32 PAI-1 = plasminogen activator inhibitor 1; PRBC = packed red blood cells; rTEG = rapid TEG; TEG =

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34 thromboelastography; tPA = tissue plasminogen activator; tPA-rTEG = tissue plasminogen activator

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36 stressed rapid thromboelastography; TP = total protein; TXA = tranexamic acid CRI = continuous-rate

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38 infusion

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6 41 North Carolina State University IACUC, ID# 18=065-O.  
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10 43 study.  
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For Peer Review

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4 60 **Abstract**

5  
6 61 **Background:** No treatment beside platelet administration is known to protect against spontaneous  
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9 62 hemorrhage in thrombocytopenic dogs.

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11 63 **Objectives:** Primary: to determine if treatment with  $\epsilon$ -aminocaproic acid (EACA) reduces the  
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13 64 requirement for blood transfusions and improves outcome in dogs with severe thrombocytopenia.  
14  
15 65 Secondary: to find evidence of hyperfibrinolysis and determine the effect EACA administration on rapid  
16  
17 66 (rTEG) and tissue plasminogen activator-spiked (tPA-rTEG) thromboelastography variables.

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20  
21 67 **Animals:** Twenty-seven dogs with severe thrombocytopenia were treated with EACA, and data from an  
22  
23 68 additional 33 were obtained from the hospital database as historical control (HC) cohort.

24  
25  
26 69 **Methods:** Single arm clinical trial with HCs. EACA group dogs received EACA (100 mg/kg IV followed by a  
27  
28 70 CRI of 400mg/kg/24 hrs). Thromboelastography before and during EACA infusion, hospitalization days,  
29  
30 71 number of transfusions, and mortality were compared.

31  
32 72 **Results:** There was no difference in number of transfusions/dog (median, interquartile range, p value)  
33  
34 73 (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  
35  
36 74 groups, respectively, and no difference in survival determined by log-rank analysis (p = .15). Maximum  
37  
38 75 amplitude on both rTEG and tPA-rTEG increased after EACA administration (rTEG baseline 23.6, 9.6-38.9;  
39  
40  
41 76 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 <  
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43 77 .002).

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46 78 **Conclusions and clinical importance:** Although EACA increased clot strength, there was no effect on  
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48 79 outcome. Treatment with EACA at this dose cannot be recommended as a routine treatment but may be  
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50 80 considered for dogs with severe ongoing hemorrhage.  
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## 81 Introduction

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

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

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

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4 105 however, the evidence is limited.<sup>10-12</sup> A report describing the use of TXA in 4 dogs with ITP found no clinical  
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6 106 benefit when compared to a contemporary control cohort of 6 dogs.<sup>13</sup>  
7

8 107 A sensitive ex-vivo method of identifying a tendency towards systemic hyperfibrinolysis is the  
9  
10 108 addition of tissue plasminogen activator (tPA) to a tissue factor or a kaolin and tissue factor activated  
11  
12 109 thromboelastography. This technique of spiking the rTEG assay (tPA-rTEG) may detect reductions in  
13  
14 110 fibrinolysis inhibitor activity that are not yet sufficient to cause overt hyperfibrinolysis but place patients  
15  
16 111 at increased risk for decompensation to a hyperfibrinolytic state.<sup>14</sup> It appears to provide rapid  
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18 112 assessment of the endogenous fibrinolytic potential of whole blood in people<sup>15</sup> and appears to be useful  
19  
20 113 in dogs as well.<sup>16,17</sup> To our knowledge, the fibrinolytic potential of dogs with thrombocytopenia has not  
21  
22 114 been characterized.  
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25  
26 115 We hypothesized that treatment with EACA will reduce spontaneous bleeding and transfusion  
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28 116 requirements in dogs with severe thrombocytopenia. Thus, the primary goals of the study reported here  
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30 117 were to determine if prophylactic treatment with EACA reduces the frequency and severity of  
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32 118 spontaneous hemorrhage, reduces the requirement for blood transfusions, and improves survival to  
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34 119 discharge in dogs with severe thrombocytopenia. A secondary goal of the study was to determine if  
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36 120 thrombocytopenic dogs have thromboelastographic evidence of hyperfibrinolysis and to determine the  
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38 121 effect of treatment with EACA on tPA-rTEG variables.  
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## 43 122 **Material and methods**

### 44 45 46 123 *Study design*

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48 124 This was a prospective single arm study using historical controls. The prospective arm was  
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50 125 approved by the institutional animal care and use committee, and informed owner consent was  
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52 126 obtained before enrollment of each dog (EACA group). Eligible dogs hospitalized for severe  
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54 127 thrombocytopenia between 2018 and 2020 were identified via manual platelet count within the  
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4 128 preceding 12 hours. Severe thrombocytopenia was defined as a platelet count < 30,000/ $\mu$ l, or <  
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6 129 50,000/ $\mu$ l if accompanied by evidence of bleeding consistent with thrombocytopenia (e.g. petechiae,  
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8 130 ecchymoses, epistaxis or melena).<sup>18</sup> Dogs weighing less than 2 kg (precluding safe blood collection for  
9  
10 131 the study) and dogs suspected of having a disorder of secondary hemostasis were excluded.

12  
13 132 For each dog enrolled in the EACA group, a kaolin and tissue factor-activated TEG (rapid TEG or  
14  
15 133 rTEG) and a tPA-modified rapid TEG (tPA stressed-rTEG or tPA-rTEG) were performed. Both rTEG and  
16  
17 134 tPA-rTEGs were performed prior to administration of EACA (T1), as described below. All assays were  
18  
19  
20 135 performed by the same two trained operators (JW or LR). After obtaining the assay samples, 100 mg/kg  
21  
22 136 EACA (Aminocaproic acid injection USP 5 g/20 mL, Hospira Inc, Lake Forest, Illinois) was administered  
23  
24 137 intravenously over 15 min, immediately followed by a constant-rate infusion of 400 mg/kg/24 hours.  
25  
26 138 The rTEG and tPA-rTEG assays were then repeated on blood samples collected prior to the end of the  
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29 139 infusion, sometime during hours 18 to 24 as determined by operator availability (T2). If the dog was  
30  
31 140 eating and judged to tolerate oral medication after T2 it was transitioned to oral administration of the  
32  
33 141 intravenous EACA formulation at a dose of 100 mg/kg every six hours.<sup>19</sup> Dogs that were hyporexic or  
34  
35 142 that had evidence of nausea or vomiting were continued on the intravenous constant-rate infusion and  
36  
37  
38 143 monitored for side effects. Aminocaproic acid treatment was continued in each dog until it was  
39  
40 144 discharged, its platelet count exceeded 50,000  $\mu$ l/ml with no evidence of active bleeding, or it died or  
41  
42 145 was euthanized. The number of days until the endpoints were reached were recorded. Day 1 was  
43  
44 146 defined as the day of admission, day 2 (and all subsequent days) began at 11 AM the following day.  
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46  
47 147 Manual platelets counts were obtained once daily, and packed cell volume (PCV) and total protein (TP)  
48  
49 148 were monitored every 12 hours or more frequently if clinically indicated. Bleeding events, defined as  
50  
51 149 observed hemorrhage including epistaxis, melena, hematuria, or a percent change reduction in PCV  
52  
53 150 and/or TP of >10%, were recorded. The more objective DOGibat scoring system was not used in this  
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56 151 study as the relevant information was not recorded for the control group.<sup>20</sup> All blood product  
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4 152 transfusions including packed red blood cells (pRBC) and fresh whole blood (FWB) were documented.  
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6 153 Approximately 10ml/kg pRBC or 20 ml/kg FWB were administered per transfusion to dogs in both  
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8 154 groups; thus depending on the size of the dog a single transfusion may have used > 1 unit of product. All  
9  
10 155 additional diagnostics and treatments were at the discretion of the primary clinician.  
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13 156 For the HC group, medical records between 2013 and 2017 were screened for dogs with severe  
14  
15 157 thrombocytopenia of any origin using the same inclusion and exclusion criteria as for the EACA group.  
16  
17 158 Three records from dogs that received EACA during the course of their treatment were found and were  
18  
19 159 not included in the HC group. Underlying etiology and additional treatments for both groups were  
20  
21 160 recorded to ensure both groups had comparable underlying causes and received similar treatment.  
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25 161 *TEG analysis*  
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28 162 For each dog enrolled in the EACA group, paired rTEG and tPA-rTEG were performed at T1 and  
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30 163 T2. Both channels of a dedicated TEG machine (TEG 5000, Haemoscope, Skokie, Ill) were used and for  
31  
32 164 each subject the channel assignment to each assay (rTEG and tPA-rTEG) was randomly assigned in blocks  
33  
34 165 of 10. Blood samples from each dog were collected from either a central venous catheter (Mila Small  
35  
36 166 Animal Guidewire Catheter Kit, Mila International, Florence, KY USA) freshly placed in the lateral  
37  
38 167 saphenous vein or via direct cephalic or lateral saphenous venipuncture with a winged needle catheter  
39  
40 168 during initial blood sampling at T1. All samples were collected from the central venous catheter at T2.  
41  
42 169 For either technique, a purge of 1.2 ml (winged needle) or 3 mL (central venous catheter) was collected  
43  
44 170 prior to diagnostic sample collection. The diagnostic sample of 1.4 ml of blood was collected directly into  
45  
46 171 vacuum citrate tubes and the tubes were then mixed 5 times by inversion and then allowed to rest for  
47  
48 172 30 min at room temperature. While the samples rested, a prediluted frozen tPA vial containing 25 IU of  
49  
50 173 tPA in 10 µL of solution was retrieved and thawed at room temperature. The tPA solution was made in  
51  
52 174 advance by serial dilutions of stock product (Cathflo™ Activase® [Alteplase], Genentech, South San  
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4 175 Francisco, CA 2 mg/mL) in BSA buffer solution to a final concentration of 25 IU/10  $\mu$ L. The tPA solution  
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6 176 was made fresh every 6 months and stored at -80° F (-62° C) prior to use. The tubes were numbered to  
7  
8 177 ensure a standardized sequence of use and each tube was assigned to either the left or right channel of  
9  
10 178 the TEG analyzer to ensure an equal distribution of channel assignment for the stressed rTEG assays.  
11  
12 179 The rTEG vials containing tissue factor activator and kaolin (RapidTEG™, Haemonetics®, Skokie, Ill) were  
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14 180 reconstituted with 20ul distilled water and gently swirled, then allowed to rest. Immediately prior to  
15  
16 181 assay, 10  $\mu$ L of rTEG mixture and 20  $\mu$ L of calcium chloride solution were added to each TEG cup. For the  
17  
18 182 rTEG assays, the citrated tube was gently inverted 5 times and 340  $\mu$ L of blood was pipetted directly into  
19  
20 183 the test cup and gently mixed by pipetting the reagent-blood mixture in and out of the cup 3 times. The  
21  
22 184 analyzer carriage was raised to start the test. For the tPA-rTEG assay, a 490  $\mu$ L aliquot of citrated blood  
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24 185 was pipetted into the tPA vial and was also gently mixed, yielding a final concentration of tPA in blood of  
25  
26 186 50 IU/mL. Then 340  $\mu$ L of the blood/tPA mixture were added to the test cup assigned to the tPA-rTEG.  
27  
28 187 This mixture was also pipetted out of and into the cup 3 times to mix all components prior to beginning  
29  
30 188 the assay. The following values were recorded at T1 and T2: R (reaction) time, K (clot formation time),  $\alpha$ -  
31  
32 189 angle (a measure of the rapidity of clot formation), MA (maximum amplitude), and LY30 (clot lysis at 30  
33  
34 190 minutes).

#### 191 *Statistical analysis*

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

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4 198 episodes/day at an alpha error rate of 5% and a beta error level of 20%. Data including study days,  
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6 199 mortality, and number of transfusions were compared between the HC and the EACA groups with the  
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8 200 Mann Whitney U test for continuous data and the Pearson Chi Square test for categorical data. A  
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10 201 Kaplan-Meier survival curve and log rank analysis was performed to compare fatality rates. The analysis  
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12 202 was then repeated including dogs with ITP only. In the EACA group, PCV, the rTEG and tPA-rTEG  
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14 203 variables R, K, MA, and LY30% at T1 and T2 were compared using the Wilcoxon signed-rank test. The  
15  
16 204 correlation between the platelet count on admission and platelet count on day2, between rTEG MA T1  
17  
18 205 and rTEG MA T2 and between tPA-TEG MA T1 and tPA-TEG MA T2 were determined with Pearson  
19  
20 206 correlation. A p-value of less than .05 was used as the criterion for statistical significance. Analyzing the  
21  
22 207 influence of different adjunctive drugs was not attempted due to the high variability of different  
23  
24 208 treatment protocols. Data analysis was conducted using commercial software (IBM SPSS Statistics for  
25  
26 209 Windows, Version 25.0. Armonk, NY: IBM Corp)

## 31 **Results**

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34 211 Records from 33 dogs were abstracted for the HC group, and 28 dogs were enrolled in the EACA  
35  
36 212 group. Our initial enrollment criteria excluded dogs with PT or aPTT values that exceeded the laboratory  
37  
38 213 reference interval. After excluding several dogs with slight elevations of their aPTT but no other clinical  
39  
40 214 evidence of causes of secondary coagulation disorders, we modified the criteria to include dogs with  
41  
42 215 values that were within 10% of the upper limit for either assay. The value of 10% was chosen because  
43  
44 216 many dogs with no physical evidence of coagulopathy at the time of the assay fell within this range, and  
45  
46 217 this figure is consistent with the magnitude of inter-assay variations between a point-of-care device and  
47  
48 218 laboratory assay results observed by us and others.<sup>21</sup> None of the dogs had any other evidence of  
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50 219 disorders of secondary hemostasis, and all had normal reaction times on thromboelastography.  
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220 Six dogs did not complete the study. In one of these, the intravenous catheter was lost after day  
221 1 and could not be replaced, therefore serial data collection was not possible. Two dogs developed  
222 urethral obstructions secondary to blood clot formation in their urinary bladders and EACA  
223 administration was therefore discontinued. A fourth dog was discharged 24 hours after admission per  
224 the owner's request. EACA was discontinued on day 5 in the fifth dog in response to persistent  
225 regurgitation. Where paired data was available, these dogs were included in the TEG analysis but not  
226 outcome analysis. A sixth dog was completely removed from the study after developing hemolysis and  
227 coagulation abnormalities consistent with disseminated intravascular coagulation the day after  
228 enrollment. Data for one rTEG T1 were unavailable due to an analyzer malfunction, and another dog  
229 died before T2. Thus, clinical outcome including transfusion data was evaluated for 22 EACA group dogs,  
230 paired rTEG data was available from 23, and paired tPA-rTEG data was available from 24 of those dogs.

231 The majority of thrombocytopenic dogs in both the HC (30/33, 91%,) and the EACA group  
232 (19/22, 86.4%) were diagnosed with primary ITP. A summary of all diseases represented can be found in  
233 Table 1. Therapy used to treat the underlying cause of the thrombocytopenia was variable, and several  
234 distinct combinations of immunosuppressive and adjunctive therapy were used (Table 2). Nine dogs in  
235 the control group received intravenous immunoglobulin (IVIG) versus none in the EACA group. One dog,  
236 which was pancytopenic secondary to a suspected infectious cause did not receive any  
237 immunosuppressants. To assess for an effect of IVIG treatment on study days and transfusion  
238 dependency, statistics were repeated excluding the dogs in the HC that received IVIG, and no difference  
239 in results were found. The influence of treatments other than EACA and IVIG on study endpoints was not  
240 analyzed due to the large number of treatment combinations used for dogs in both groups. Similarly, a  
241 repeated analysis was performed including only dogs with ITP and no difference in results could be  
242 found.

### 243 *PCV and transfusions*

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

250 Dogs in the HC group received a median of 1.33 transfusion/per dog of either pRBCs (24  
251 transfusions) or FWB (20 transfusions). The EACA group dogs received a median of 1.32 transfusions/per  
252 dog of either pRBCs (19 transfusions) or FWB (10 transfusions) Additional results related to transfusions  
253 administered are shown in Figure 1. There was no significant group difference in the number of any  
254 transfusions (pRBC or FWB) per dog ( $p = .5$ ) or in the proportion of dogs receiving a FWB transfusion ( $p =$   
255  $.57$ ). Although the proportion of dogs in the EACA group that did not need any blood products (59.1%)  
256 was larger than in the HC group (45.5%), the difference was not statistically significant ( $p = .32$ ). Some  
257 EACA dogs that required transfusions to treat hypovolemic hemorrhage did not have reductions in PCV  
258 of  $>10\%$  and therefore did not fulfill that diagnostic criterion for a bleeding event, despite having  
259 clinically important bleeding. There was no difference in the frequency of bleeding events, defined as  
260 acute reductions of PCV/TP, between groups ( $p = .31$ ). Because observed episodes of hemorrhage were  
261 not reliably recorded in the EACA group, we assumed that the same problem existed in the HC group  
262 and consequently dropped observed bleeding events from our primary goal.

### 263 *Outcome*

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

266 Two were euthanized, one failing to improve after 6 days of intensive treatment and the second one  
267 after developing severe respiratory distress and neurological signs. One dog died due to causes not  
268 related to thrombocytopenia. Seventeen (77%) dogs were discharged in the EACA group and 5 did not  
269 survive to discharge. Three patients died and two were euthanized (one after 3 and one after 6 days of  
270 treatment) due to refractory thrombocytopenia. There was no significant difference in fatality rate ( $p =$   
271  $.08$ ), and log-rank analysis identified no significant difference ( $p = .15$ ) in the survival time between the  
272 groups demonstrated on a Kaplan-Meier survival plot (Figure 2).

### 273 *TEG data*

274 Only one dog was mildly hyperfibrinolytic on the tPA-TEG at T1 (LY30% 23.4; reference range for  
275 our institution is  $< 20\%$ ), and this resolved during administration of EACA. This dog was diagnosed with  
276 ITP, was not anemic on presentation, and did not require any blood products. There was a significant T1-  
277 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  
278 (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  
279 on both TEGs prior to EACA and 7 had a MA within reference post EACA. No other TEG parameters were  
280 different between T1 and T2. A summary of the TEG data including institutional reference ranges is  
281 presented in Table 3.

282 There was a strong positive correlation between MA at T1 and T2 for both rapid ( $r = .904$ ,  $p <$   
283  $.001$ ), and tPA-TEGs ( $r = .942$ ,  $p < .001$ ), and the MA increased at T2 for 20/23 rTEG and 19/24 tPA-rTEG  
284 assays (Figure 3B and Figure 3C). There was only a weak correlation for both TEG types between T1  
285 platelet count and MA (rTEG  $r = 0.351$ ,  $p = .07$  and tPA-TEG  $r = .36$ ,  $p = .06$ ) and T2 platelet count and  
286 MA (rTEG  $r = .27$ ,  $p = .22$  and tPA-TEG  $r = .34$ ,  $p = .11$ ). The MA in one or both TEG assays increased from T1  
287 to T2 in 10/12 dogs that experienced a concurrent reduction in platelet count. No statistically significant

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4 288 difference was found between the PCV at both timepoints (T1: 39, 23-45, T2: 32.5, 26.25 – 37.25; p =  
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6 289 .06)  
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## 8 9 290 **Discussion**

10  
11 291 Prophylactic treatment with EACA resulted in a statistically significant increased clot strength (as  
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13  
14 292 measured by the MA) in severely thrombocytopenic dogs. However, this increase did not seem to be  
15  
16 293 large enough to have significant effect on clinically relevant parameters including hospitalization time,  
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18 294 mortality, acute reductions of PCV or TP, and number of transfusions required.

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20  
21 295 There are only a small number of published reports of studies investigating the efficacy of  
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23 296 antifibrinolytics in dogs. To the authors' knowledge, the present report is the first prospective study  
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25  
26 297 investigating the use of EACA in thrombocytopenic dogs. A recent small study compared a group of dogs  
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28 298 with ITP (n=4) receiving TXA with a control group (n=6) and did not find a clinical benefit of TXA.<sup>13</sup> In a  
29  
30 299 retrospective study of the effect of TXA in dogs with acquired bleeding disorders, 15.7% of the study  
31  
32 300 population was thrombocytopenic. Dogs that were treated with TXA in that study required fewer  
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34 301 transfusions and had a lower mortality rate than dogs that did not receive TXA; however,  
35  
36 302 thrombocytopenic dogs were not analyzed separately and therefore it is not possible to draw any  
37  
38 303 conclusions regarding the efficacy of TXA specifically in thrombocytopenic dogs from that report.<sup>8</sup>

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40  
41 304 The majority of dogs in the present study were diagnosed with primary ITP. Most reported studies of  
42  
43 305 antifibrinolytic treatment of humans with thrombocytopenia investigated their use in patients with  
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45 306 hematologic malignancies rather than ITP. A 2016 Cochrane review concluded that there is a lack of high-  
46  
47 307 quality studies and there is insufficient evidence to justify the use of antifibrinolytics in thrombocytopenic  
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49 308 patients secondary to hematological neoplasia.<sup>11</sup> Since then, a large randomized double-blind placebo-  
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51 309 controlled trial reported in 2022 did not identify a significant reduction in transfusions, platelet  
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4 310 administration, or bleeding events in patients with hematologic malignancies treated prophylactically  
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6 311 with TXA.<sup>22</sup>  
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8 312 We are aware of only two case series describing the efficacy of antifibrinolytics in human ITP  
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10 313 patients with ongoing hemorrhage. In 12 patients that were treated with TXA as adjunctive treatment for  
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12 314 active bleeding, high success rates for hemorrhage control were reported <sup>12</sup>. In a second series of 17  
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14 315 thrombocytopenic patients (15 with ITP) with uncontrolled bleeding, treatment with EACA appeared to  
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16 316 facilitate discontinuation of platelet products in all 17.<sup>23</sup> We have not found any reports concerning the  
17  
18 317 efficacy of prophylactic use of antifibrinolytic agents in ITP. Based on the lack of high-quality data, current  
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20 318 guidelines on treatment of ITP in human medicine recommend against the routine use of EACA or TXA for  
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22 319 ITP. However, in patients with ongoing bleeding, these agents may be considered.<sup>24,25</sup>  
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#### 27 320 *Side effects and complications*

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30 321 Aminocaproic acid appears to be well tolerated. In one report of a retrospective study of 122 dogs  
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32 322 with hemorrhage secondary to neoplastic and non-neoplastic cause, only 3 dogs developed mild  
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34 323 gastrointestinal signs possibly related to the EACA administration at doses of 14 – 24 mg/kg.<sup>9</sup> In the  
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36 324 present study, higher doses of EACA (100 mg/kg every 6 hours orally or as continuous rate infusion) were  
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38 325 administered based on recent evidence that increased doses are required to achieve more effective  
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40 326 antifibrinolysis in dogs.<sup>19</sup> The high dose intravenous dose was chosen to maximize the potential to achieve  
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42 327 constant therapeutic concentrations and identify any effect on thromboelastography at T2, since  
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44 328 previously described characteristics of the drug in healthy dogs suggest that oral doses of 100 mg/kg are  
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46 329 rapidly excreted and produce serum concentrations above the effective concentration of 25 µg/mL for  
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48 330 only 3 hours.<sup>19</sup> We did not identify any significant side effects from this dose in the EACA group. One dog  
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50 331 developed regurgitation during the study period and its oral EACA was stopped on day 5 as a precaution.  
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52 332 It was not possible to determine if the regurgitation was more likely secondary due to the dog's primary  
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4 333 disease process or an adverse effect of EACA. However, as this study was not designed as a safety study,  
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6 334 we cannot draw any conclusions with regards to safety of the EACA protocol used in this report.  
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8 335 Two dogs with significant hematuria developed clots causing urethral obstruction and EACA was  
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10 336 discontinued in these dogs. Urinary tract obstruction secondary to blood clot formation has been  
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12 337 previously reported in a dog with ITP<sup>26</sup> and any contribution of EACA to this complication is unknown.  
13  
14 338 However, considering that antifibrinolytics stabilize blood clots, the use of TXA or EACA in dogs and cats  
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16 339 with severe hematuria should be considered carefully.  
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19 340 *TEG data*  
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22 341 While the main reason for hemorrhage in dogs with thrombocytopenia is the lack of platelets,  
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24 342 there might be mechanisms causing increased clot lysis to justify the specific use for antifibrinolytics in  
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26 343 these dogs. One hypothesis regarding underlying mechanisms in thrombocytopenic patients is the  
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28 344 reduced availability of plasminogen activator inhibitor 1 (PAI-1) released from granules on platelet  
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30 345 surface, increasing susceptibility to fibrinolysis.<sup>27,28</sup> Additionally, dogs hemorrhaging severely may  
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32 346 theoretically develop hyperfibrinolysis similar to traumatic coagulopathy or canine hemoperitoneum.<sup>29</sup>  
33  
34 347 To screen for evidence of systemic hyperfibrinolysis and to investigate the effect of EACA on TEG  
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36 348 tracings, we performed rTEGs and tPA-rTEGs in the present study. Only one dog had mild  
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38 349 hyperfibrinolysis on tPA-rTEG, and this resolved after administration of EACA. This finding did not seem  
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40 350 to have any clinical significance as the dog did not show any signs of hemorrhage had a normal PCV on  
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42 351 presentation and did not require blood transfusions. Although it is possible that even the tPA-rTEG is not  
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44 352 sensitive enough to detect endogenous hyperfibrinolysis<sup>30</sup>, the normal fibrinolytic profile might explain  
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46 353 the lack of response to prophylactic treatment with EACA in the dogs in this study. We found no other  
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48 354 studies investigating hyperfibrinolysis in dogs with ITP. However, our findings are similar to data from  
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50 355 thrombocytopenic human patients with hematologic malignancies. A study published in 2022 found that  
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52 356 only 3 out of 115 thrombocytopenic humans showed a hyperfibrinolytic profile, median plasma PAI-I  
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4 357 concentration was normal, and there were no differences in plasma PAI-1 concentration between those  
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6 358 who did or did not go on to develop significant hemorrhage.<sup>28</sup> Similarly, decreased plasma PAI-I  
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8 359 concentration was not identified in humans with ITP in another study.<sup>30</sup> Although hyperfibrinolysis on a  
9  
10 360 local level cannot be excluded, the findings of the present study and human data suggest that systemic  
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12 361 fibrinolytic activation does not play a major role in the pathophysiology of hemorrhage in  
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14 362 thrombocytopenic patients.  
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17 363 We identified a significant increase in MA for both TEG types after EACA administration, a  
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19 364 change that was not attributable to an increase in platelet count as no relationship between platelet  
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21 365 count and MA could be shown. Unfortunately, fibrinogen concentrations were not consistently available  
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23 366 for all patients at both T1 and T2, which is a limitation of this study. Another parameter potentially  
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25 367 increasing MA is a decreased PCV as suggested in previous publications.<sup>31</sup> In this study population the  
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27 368 PCV at T2 was slightly lower (32.5%) compared to T1 (39%), which represents a clinical change.  
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29 369 However, the difference was not statistically significant and the finding of previous studies that PCV  
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31 370 influences MA could not be confirmed in a recent ex vivo study.<sup>32</sup> Although the increase in MA in the  
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33 371 tPA-rTEG assay in this study is consistent with a previous report showing an increase in MA using a tPA-  
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35 372 modified TEG following administration of a single dose of EACA to dogs<sup>19</sup>, the reason for the mild  
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37 373 increase in rTEG MA is unclear. Because we have no TEG data on untreated control dogs, one possible  
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39 374 explanation is that the MA increased with time in most dogs during the course of treatment with  
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41 375 corticosteroids for their thrombocytopenia, independent of any effect by EACA or change in platelet  
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43 376 numbers.<sup>33</sup> Another could be that a low level of fibrinolytic tendency is present in these dogs, reducing  
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45 377 the MA without causing measurable increases in LY30, and this was inhibited by EACA. Regardless of the  
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47 378 mechanism, this change was not associated with any identifiable clinical benefit in our dogs.  
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379 Additionally, 7 dogs in this study had a MA within or just below reference range that normalized after  
380 EACA, which is an unexpected finding for thrombocytopenic dogs. Interestingly, only one of these dogs  
381 required a blood transfusion. Further studies investigating the potential use of viscoelastic testing to  
382 predict clinical bleeding and correlation with bleeding scores in dogs with ITP are required.

### 383 *Limitations*

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

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

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4 402 dogs had any other clinical evidence of a disorder of secondary hemostasis and their reaction times  
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6 403 were normal on T1 thromboelastography we cannot completely rule out subtle disorders of secondary  
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8 404 hemostasis. However, the impact of any such disorder is likely small.<sup>34</sup>  
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#### 10 11 405 *Conclusions*

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14 406 We found no evidence for hyperfibrinolysis in thrombocytopenic dogs or any survival benefit in  
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16 407 dogs treated prophylactically with EACA, which is similar to results in human studies. Based on these  
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18 408 results, routine treatment with EACA cannot be recommended in dogs with ITP. Nevertheless, EACA  
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20 409 administration might be considered for dogs with severe ongoing bleeding. A randomized controlled  
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22 410 trial with a concurrent control group that differs only in the administration of EACA is necessary to  
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24 411 further evaluate the question if antifibrinolytics are useful in dogs with ITP. However, our results  
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26 412 suggest that such a study would require a very large number of subjects to demonstrate a treatment  
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28 413 effect. Assays for hemostatic parameters like PAI-1 concentration might be of interest to investigate  
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30 414 possible reasons of underlying local hyperfibrinolysis justifying the use of antifibrinolytic agents.  
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#### 36 416 **References**

- 37  
38  
39 417 1. Grindem CB, Breitschwerdt EB, Corbett WT, et al. Epidemiologic survey of thrombocytopenia in dogs:  
40  
41 418 a report on 987 cases. *Vet Clin Pathol* 1991;20:38-43.  
42  
43  
44 419 2. O'Marra SK, Delaforcade AM, Shaw SP. Treatment and predictors of outcome in dogs with immune-  
45  
46 420 mediated thrombocytopenia. *J Am Vet Med Assoc* 2011;238:346-352.  
47  
48 421 3. Ker K, Roberts I, Shakur H, et al. Antifibrinolytic drugs for acute traumatic injury. *Cochrane Database*  
49  
50 422 *Syst Rev* 2015:CD004896.  
51  
52  
53 423 4. Henry DA, Carless PA, Moxey AJ, et al. Anti-fibrinolytic use for minimising perioperative allogeneic  
54  
55 424 blood transfusion. *Cochrane Database Syst Rev* 2011;2011:CD001886.  
56  
57  
58  
59  
60

- 1  
2  
3  
4 425 5. Shakur H RI, Fawole B, Chaudhri R, El-Sheikh M, Akintan A, Qureshi, Z. Effect of early tranexamic acid  
5  
6 426 administration on mortality, hysterectomy, and other morbidities in women with post-partum  
7  
8 427 haemorrhage (WOMAN): an international, randomised, double-blind, placebo-controlled trial. *Lancet*  
9  
10 428 2017;389:2105-2116.
- 11  
12 429 6. Marin LM, Iazbik MC, Zaldivar-Lopez S, et al. Retrospective evaluation of the effectiveness of epsilon  
13  
14 430 aminocaproic acid for the prevention of postamputation bleeding in retired racing Greyhounds with  
15  
16 431 appendicular bone tumors: 46 cases (2003-2008). *J Vet Emerg Crit Care* 2012;22:332-340.
- 17  
18 432 7. Marin LM, Iazbik MC, Zaldivar-Lopez S, et al. Epsilon aminocaproic acid for the prevention of delayed  
19  
20 433 postoperative bleeding in retired racing greyhounds undergoing gonadectomy. *Vet Surg* 2012;41:594-  
21  
22 434 603.
- 23  
24 435 8. Kelmer E, Marer, K., Bruchim, Y., Klainbart, S., Aroch, I., Segev, G. Retrospective evaluation of the  
25  
26 436 safety and efficacy of tranexamic acid (Hexakapron®) for the treatment of bleeding disorders in dogs.  
27  
28 437 *Israel J Vet Med* 2013;68:94 - 100.
- 29  
30 438 9. Davis M, Bracker K. Retrospective study of 122 dogs that were treated with the antifibrinolytic drug  
31  
32 439 aminocaproic acid: 2010-2012. *J Am Anim Hosp Assoc* 2016;52:144-148.
- 33  
34 440 10. Antun AG, Gleason S, Arellano M, et al. Epsilon aminocaproic acid prevents bleeding in severely  
35  
36 441 thrombocytopenic patients with hematological malignancies. *Cancer* 2013;119:3784-3787.
- 37  
38 442 11. Estcourt LJ, Desborough M, Brunskill SJ, et al. Antifibrinolytics (lysine analogues) for the prevention  
39  
40 443 of bleeding in people with haematological disorders. *Cochrane Database Syst Rev* 2016;3:CD009733.
- 41  
42 444 12. Mayer B, Salama A. Successful treatment of bleeding with tranexamic acid in a series of 12 patients  
43  
44 445 with immune thrombocytopenia. *Vox Sang* 2017;112:767-772.
- 45  
46 446 13. Olivares G, Sharman M, Miller R, et al. Use of tranexamic acid in dogs with primary immune  
47  
48 447 thrombocytopenia: A feasibility study. *Front Vet Sci* 2023;10:946127.  
49  
50  
51  
52  
53  
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55  
56  
57  
58  
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2  
3  
4 448 14. Kupesiz A, Rajpurkar M, Warriar I, et al. Tissue plasminogen activator induced fibrinolysis:  
5  
6 449 standardization of method using thromboelastography. *Blood Coagul Fibrinolysis* 2010;21:320-324.  
7  
8 450 15. Moore HB, Moore EE, Chapman MP, et al. Viscoelastic tissue plasminogen activator challenge  
9  
10 451 predicts massive transfusion in 15 minutes. *J Am Coll Surg* 2017;225:138-147.  
11  
12 452 16. Osekavage KE, Brainard BM, Lane SL, et al. Pharmacokinetics of tranexamic acid in healthy dogs and  
13  
14 453 assessment of its antifibrinolytic properties in canine blood. *Am J Vet Res* 2018;79:1057-1063.  
15  
16  
17 454 17. Spodsberg EH, Wiinberg B, Jessen LR, et al. Endogenous fibrinolytic potential in tissue-plasminogen  
18  
19 455 activator-modified thromboelastography analysis is significantly decreased in dogs suffering from  
20  
21 456 diseases predisposing to thrombosis. *Vet Clin Pathol* 2013;42:281-290.  
22  
23  
24 457 18. Putsche JC, Kohn B. Primary immune-mediated thrombocytopenia in 30 dogs (1997-2003). *J Am*  
25  
26 458 *Anim Hosp Assoc* 2008;44:250-257.  
27  
28 459 19. Brown JC, Brainard BM, Fletcher DJ, et al. Effect of aminocaproic acid on clot strength and clot lysis  
29  
30 460 of canine blood determined by use of an in vitro model of hyperfibrinolysis. *Am J Vet Res* 2016;77:1258-  
31  
32 461 1265.  
33  
34  
35 462 20. Makielski KM, Brooks MB, Wang C, et al. Development and implementation of a novel immune  
36  
37 463 thrombocytopenia bleeding score for dogs. *J Vet Intern Med* 2018;32:1041-1050.  
38  
39 464 21. Yang W, Hosgood G, Luobikis K, et al. Agreement of point-of-care prothrombin and activated partial  
40  
41 465 thromboplastin time in dogs with a reference laboratory. *Aust Vet J* 2018;96:379-384.  
42  
43  
44 466 22. Gernsheimer TB, Brown SP, Triulzi DJ, et al. Prophylactic tranexamic acid in patients with  
45  
46 467 hematologic malignancy: a placebo-controlled, randomized clinical trial. *Blood* 2022;140:1254-1262.  
47  
48 468 23. Bartholomew JR, Salgia R, Bell WR. Control of bleeding in patients with immune and nonimmune  
49  
50 469 thrombocytopenia with aminocaproic acid. *Arch Intern Med* 1989;149:1959-1961.  
51  
52  
53 470 24. Zitek T, Weber L, Pinzon D, et al. Assessment and management of immune thrombocytopenia (ITP)  
54  
55 471 in the emergency department: current perspectives. *Open Access Emerg Med* 2022;14:25-34.  
56  
57  
58  
59  
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- 1  
2  
3  
4 472 25. Provan D, Arnold DM, Bussel JB, et al. Updated international consensus report on the investigation  
5  
6 473 and management of primary immune thrombocytopenia. *Blood Adv* 2019;3:3780-3817.  
7  
8 474 26. Hooi KS, Lemetayer JD. The use of intravesicular alteplase for thrombolysis in a dog with urinary  
9  
10 475 bladder thrombi. *J Vet Emerg Crit Care* 2017;27:590-595.  
11  
12 476 27. Heubel-Moenen F, Henskens YMC, Verhezen PWM, et al. Fibrinolysis in patients with  
13  
14 477 chemotherapy-induced thrombocytopenia and the effect of platelet transfusion. *J Thromb Haemost*  
15  
16 478 2019;17:1073-1084.  
17  
18  
19 479 28. Ilich A, Gernsheimer TB, Triulzi DJ, et al. Absence of hyperfibrinolysis may explain lack of efficacy of  
20  
21 480 tranexamic acid in hypoproliferative thrombocytopenia. *Blood Adv* 2022.  
22  
23  
24 481 29. Fletcher DJ, Rozanski EA, Brainard BM, et al. Assessment of the relationships among coagulopathy,  
25  
26 482 hyperfibrinolysis, plasma lactate, and protein C in dogs with spontaneous hemoperitoneum. *J Vet Emerg*  
27  
28 483 *Crit Care (San Antonio)* 2016;26:41-51.  
29  
30 484 30. Garabet L, Ghanima W, Monceyron Jonassen C, et al. Effect of thrombopoietin receptor agonists on  
31  
32 485 markers of coagulation and P-selectin in patients with immune thrombocytopenia. *Platelets*  
33  
34 486 2019;30:206-212.  
35  
36  
37 487 31. Smith SA, McMichael MA, Gilor S, et al. Correlation of hematocrit, platelet concentration, and  
38  
39 488 plasma coagulation factors with results of thromboelastometry in canine whole blood samples. *Am J Vet*  
40  
41 489 *Res* 2012;73:789-798.  
42  
43  
44 490 32. Lynch AM, Ruterbories L, Jack J, et al. The influence of packed cell volume versus plasma proteins on  
45  
46 491 thromboelastographic variables in canine blood. *J Vet Emerg Crit Care (San Antonio)* 2020;30:418-425.  
47  
48 492 33. Rose LJ, Dunn ME, Allegret V, et al. Effect of prednisone administration on coagulation variables in  
49  
50 493 healthy Beagle dogs. *Vet Clin Pathol* 2011;40:426-434.  
51  
52  
53 494 34. Poitout-Belissent F. Nonclinical Evaluation of Compound-Related Alterations in Hemostasis. In:  
54  
55 495 *Schalm's Veterinary Hematology* 2022:108-115.  
56  
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<b>Etiology of thrombocytopenia</b>	<b>HC group</b>	<b>EACA group</b>
	N (%)	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

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

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<b>Immunosuppressants</b>	<b>HC group N (%)</b>	<b>EACA group N (%)</b>
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
<b>IVIg or vincristine</b>	<b>HC group N (%)</b>	<b>EACA group N (%)</b>
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

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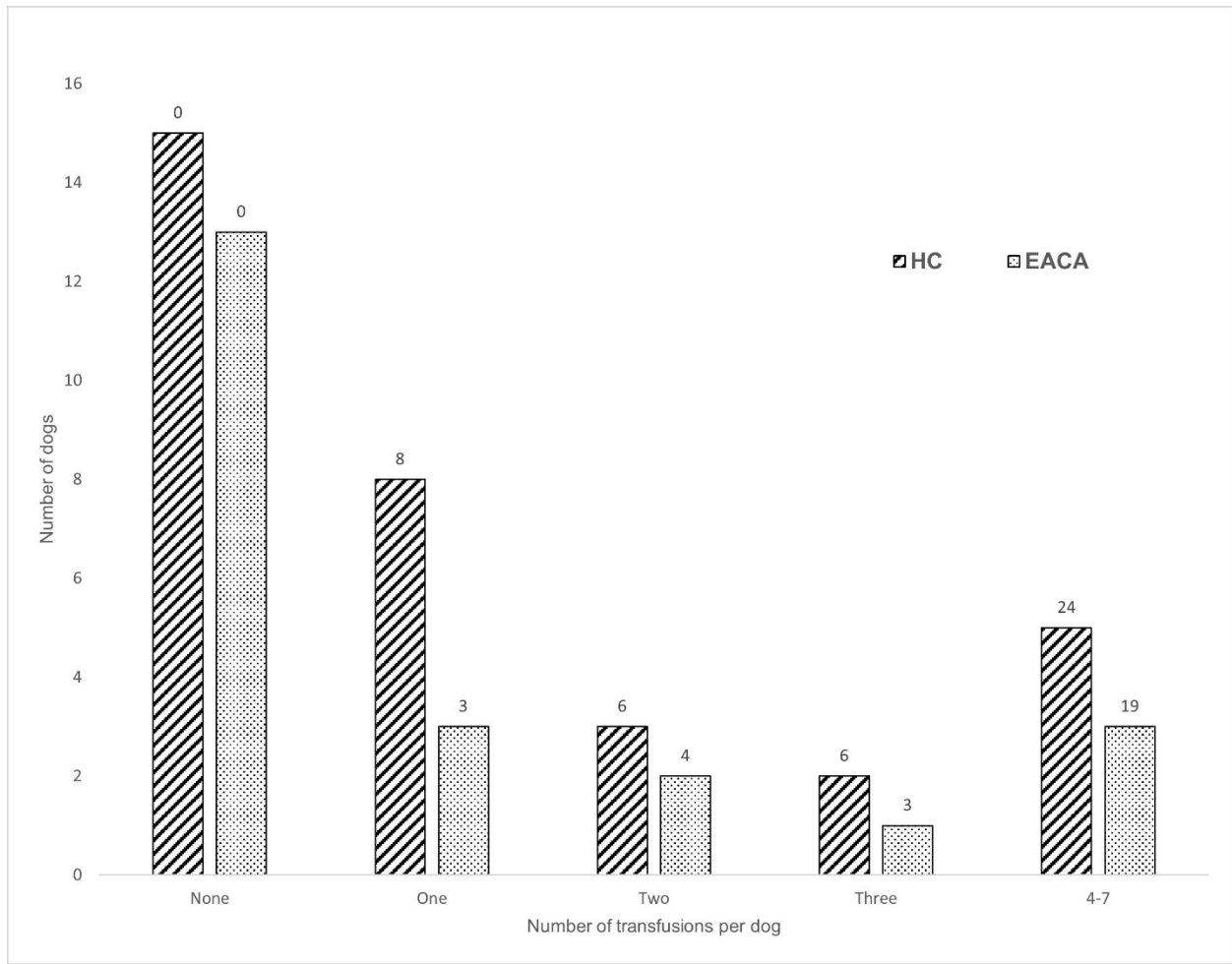
	Reference range rTEG	rTEG T1	rTEG T2	Reference range tPA-rTEG	tPA-rTEG T1	tPA-rTEG T2
<b>R</b>	0 - 1.26	.2 (.2 - .4)	.3 (.2 - .4)	0 - 1.4	.3 (.2 - .3)	.3 (.2 - .4)
<b><math>\alpha</math>-angle</b>	50 - 78	75.3 (58.6 - 79.5)	78.1 (65.6 - 80)	41 - 83	77.2 (67 - 80.4)	77.4 (65 - 79.9)
<b>K</b>	0.4 - 4.7	1.2 (.8 - 2.8)	1.2 (.8 - 2.95)	0.3 - 5	.85 (.8 - 3.8)	1.2 (1.2 - 4.2)
<b>MA</b>	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)
<b>LY30%</b>	< 3.8%	0	0	< 20%	0 (0 - .35)	0

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

For Peer Review

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

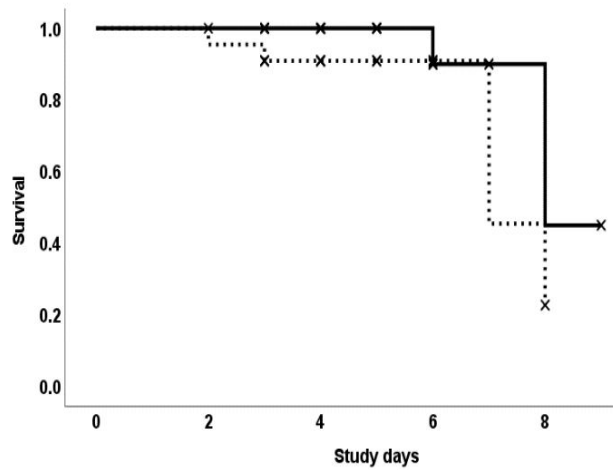
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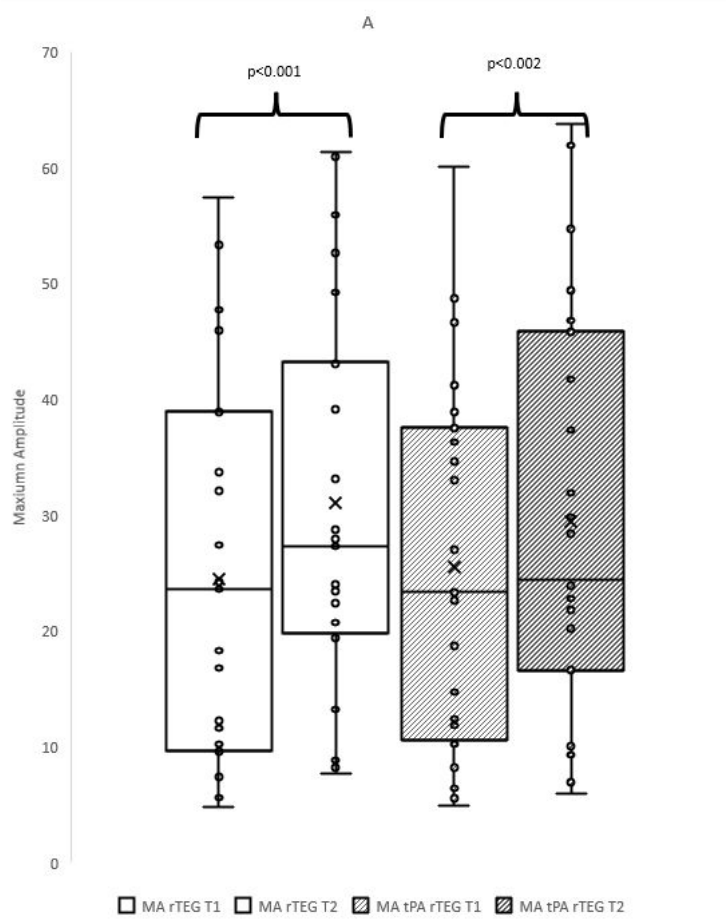
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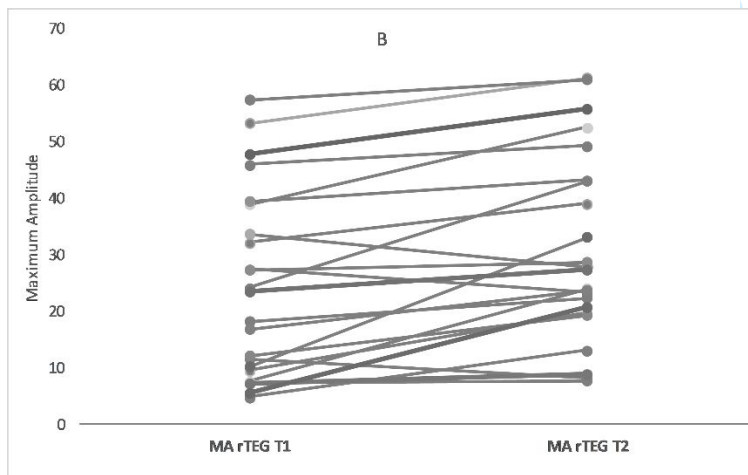


**Figure 2:** Kaplan-Meier Curve comparing survival between the EACA (dotted line) and HC (solid line)

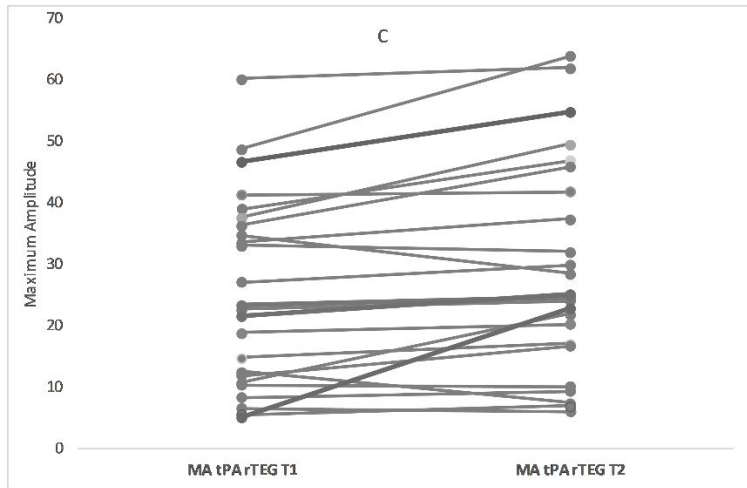
groups. X indicate censored events. P - value = .15



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569 **Figure 3.** MA data. A: rTEG and tPA rTEG bar-and-whisker plots at baseline (T1) and next day (T2). Bars

570 represent the range between the 1<sup>st</sup> and 3<sup>rd</sup> quartiles, the horizontal lines represent median values, and

571 the x marks represent average values. B: Slope graph showing the changes in rTEG MA values for

572 individual dogs between T1 and T2. C: Slope graph showing the changes in tPA-rTEG MA values for

573 individual dogs between T1 and T2.

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