Journal of Veterinary Emergency and Critical Care

Retrospective Study

Journal of Veterinary Emergency and Critical Care **00**(00) 2012, pp 1–9
doi: 10.1111/j.1476-4431.2012.00735.x

Retrospective evaluation of the effectiveness of epsilon aminocaproic acid for the prevention of postamputation bleeding in retired racing Greyhounds with appendicular bone tumors: 46 cases (2003–2008)

Liliana M. Marín, DVM, MSc; M. Cristina Iazbik, DVM; Sara Zaldivar-Lopez, DVM, MSc; Linda K. Lord, DVM, MSc, PhD; Nicole Stingle, RVT; Paulo Vilar, DVM, MSc; Ana Lara-Garcia, DVM, MSc, PhD, DACVIM, MRCVS; Francisco Alvarez, DVM, MSc, DACVIM; Kenji Hosoya, DVM, MSc, DACVR; Laura Nelson, DVM, DACVS; Antonio Pozzi, DVM, MS, DACVS; Edward Cooper, VMD, MSc, DACVECC; Mary A. McLoughlin, DVM, MSc, DACVS; Rebecca Ball, DVM, MSc, DACVS; William C. Kisseberth, DVM, MSc, PhD, DACVIM; Cheryl A. London, DVM, PhD, DACVIM; Robert Dudley, DVM, MS, DACVS; Jonathan Dyce, MA, VetMB, MRCVS, DSAO, DACVS; Melanie McMahon, DVM, MSc, DACVIM; Phillip Lerche, BVSc, PhD, DACVA; Richard Bednarski, VM, MSc, DACVA and C. Guillermo Couto, DVM, DACVIM

Abstract

Objectives – To determine the frequency of delayed postoperative bleeding in retired racing Greyhounds with appendicular bone tumors undergoing limb amputations. To identify if administration of epsilon-aminocaproic acid (EACA) was effective on the prevention of postoperative bleeding.
Design – Retrospective study from December 2003 to December 2008.
Setting – Veterinary university teaching hospital.
Animals – Forty-six retired racing Greyhounds (RRGs) diagnosed with primary appendicular bone tumors that underwent limb amputation were included in the study.
Interventions – None.
Measurements and Main Results – Thirteen of 46 RRGs (28%) included in the study had delayed postoperative bleeding starting 48–72 h after surgery. Bleeding episodes included cutaneous, subcutaneous, and external bleeding that extended from the area of the surgical site that became widespread within hours, and that required administration of blood components. A paired *t*-test suggests that there was a significant decrease in PCV postoperatively for both dogs that bled and dogs that did not bleed ($P < 0.0001$). Forty of 46 RRGs (86%) received either fresh frozen plasma (FFP) or EACA or both, for the prevention of postoperative bleeding. A

From the Department of Veterinary Clinical Sciences (Marin, Iazbik, Zaldivar-Lopez, Vilar, Lara-Garcia, Alzarez, Hosoya, Nelson, Pozzi, Cooper, McLoughlin, Ball, Kisseberth, London, Dudley, Dyce, McMahon, Lerche, Bednarski, Couto), the Veterinary Medical Center (Stingle, Couto), the Department of Veterinary Preventive Medicine (Lord), the Department of Veterinary Biosciences (London), and the College of Veterinary Medicine and Comprehensive Cancer Center (Kisseberth, London, Couto), The Ohio State University, Columbus, OH 43210.

Drs. Vilar's and Nelson's current address: College of Veterinary Medicine, Michigan State University, East Lansing, MI.

Dr. Lara-Garcia's current address: Department of Veterinary Clinical Sciences, The Royal Veterinary College, North Mymms, United Kingdom.

Dr. Alvarez's current address: Coral Springs Animal Hospital, University Drive Coral Springs, FL.

Dr. Hosoya's current address: Department of Veterinary Clinical Sciences, Hokkaido University, Sapporo, Hokkaido, Japan.

Dr. Pozzi's current address: Department of Small Animal Clinical Sciences University of Florida, Gainesville, FL.

Dr. Ball's current address: Indianapolis Veterinary Referral, Indianapolis, IN.

Dr. Dudley's current address: MedVet Medical and Cancer Center for Pets, Worthington, OH .

Dr. McMahon's current address: VCA Northwest Veterinary Specialists, Clackamas, OR.

Presented in part as an abstract at ACVIM Forum, Seattle, WA, June 6–9, 2007.

The authors declare no conflict of interest.

Address correspondence and reprint requests to
Dr. Liliana M. Marin, 601 Vernon L Tharp St, Columbus, OH 43210, USA.
Email: marin.25@osu.edu
Submitted December 8, 2010; Accepted March 11, 2012.

logistic regression model determined that dogs that did not receive EACA were 5.7 times more likely to bleed than dogs that did receive EACA, when controlling for whether or not they received FFP (95% CI: 1.02–32.15, $P = 0.047$).

Conclusion – This retrospective study suggests that preemptive postoperative administration of EACA appears to be efficacious in decreasing the frequency of bleeding in RRGs undergoing limb amputation; however, a prospective study is warranted to corroborate its effectiveness.

(J Vet Emerg Crit Care 2012; 00(00): 1–9) doi: 10.1111/j.1476-4431.2012.00735.x

Keywords: coagulation, dog, osteosarcoma, postoperative complications

Abbreviations

OSA	osteosarcoma
RRGs	retired racing Greyhounds
FFP	fresh frozen plasma
EACA	epsilon aminocaproic acid
pRBCs	packed red blood cells
cTnI	Troponin I concentration
OHE	ovariohysterectomy
OSPT	one-stage prothrombin time
APTT	activated partial thromboplastin time
FIB	fibrinogen concentration
KCL	potassium chloride
TPP	total plasma protein
HELLP	syndrome – Hemolysis, Elevated Liver enzyme Low Platelet count syndrome
CK	creatine kinase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
NSAIDs	nonsteroidal anti-inflammatory drugs
BP	blood pressure
DIC	disseminated intravascular coagulation
TAFI	thrombin activatable fibrinolysis inhibitor
T-TM	thrombin–thrombomodulin
PAI-1	plasminogen activator inhibitor-1
t-PA	tissue plasminogen activator
CRYO	cryoprecipitate
VEGF	vascular endothelial growth factor

Introduction

Greyhounds and other sighthounds have unique physiological traits that distinguish them from other breeds, including high PCV, hemoglobin concentration, and whole blood viscosity, low white blood cell, neutrophil and platelet counts,[1–4] low total serum protein concentration,[5] and acute phase protein concentrations[5,6] among others.

Canine appendicular osteosarcoma (OSA) is the most prevalent form of cancer reported in RRGs, with a prevalence of 45% for dogs with cancer (42 of 94 RRGs).[9] OSA is also the most common cause of death or euthanasia, accounting for approximately 25% of the deaths in the study period (28 of 113 RRGs).[9] Limb amputation followed by chemotherapy is frequently recommended for the treatment of OSA.[10] The etiology of canine OSA is generally unknown. Bone microtrauma, metallic implants, gonadal status,[11] and genetic abnormalities have all been proposed as possible risk factors.[12] Although we estimate that as many as 4,000 RRG/year will develop OSA,[9] the actual number may be higher, since not all cases are reported nor confirmed.

In a recent study, we demonstrated that 26% of RRGs developed delayed postoperative bleeding after routine gonadectomy (48–72 h).[18] Affected dogs had normal results of preoperative hemostasis assays but significantly lower activities of antiplasmin and antithrombin than dogs that did not bleed, suggesting that the excessive postoperative bleeding in RRGs may be due to abnormalities in clot maintenance or the fibrinolytic system, rather than to primary or secondary hemostatic defects.[18] This prevalence (26%) is considerably higher than previously reported after ovariohysterectomy (OHE) or orchiectomy in other dog breeds (0–2%).[19–21]

In some RRGs, the delayed postoperative bleeding may progress to a generalized bleeding disorder associated with clinical signs of illness, profuse widespread bruising, mild thrombocytopenia, and hemolysis, and increases in liver and muscle enzyme activities.[18] Thus, owners of RRGs with OSA who elect amputation can potentially face these complications and associated expenses related to blood component therapy and intensive care.[22] Providing a method to prevent or minimize the severity of postoperative bleeding in RRGs will not only have major economic impact but also will markedly decrease the associated complications for owners.

Fibrinolytic inhibitors are a pharmacologic option to manage postoperative bleeding and have proven to be effective in human patients and horses undergoing surgery.[28,29] Epsilon aminocaproic acid (EACA) is a potent inhibitor of fibrinolysis.[30,31] EACA prevents activation of plasminogen into plasmin on the surface of the fibrin clot,[44] by preventing the binding of

plasminogen to C-terminal lysine residues on partially degraded fibrin, thus blocking reversibly the plasminogen-binding site (Figure 1), which is essential for efficient plasmin formation.[42] EACA can either block enhanced fibrinolytic activity, or rapidly restore hyperfibrinolytic states to normal, thus EACA impedes the dissolution of fibrin clots.[36,38,46–48]

EACA has a wide therapeutic index; no relevant adverse effects were reported in toxicologic studies in dogs, rabbits, and rats, with doses as high as 0.5 g/kg,[31,49,50] a 100-fold dosage from that used in this study. Other potential uses of EACA in veterinary medicine include treatment of dogs diagnosed with degenerative myelopathy[51] and as a topical treatment of persistent corneal epithelial defects.[52] To our knowledge, there are no studies on the effects of EACA in spontaneously occurring hemostatic abnormalities in dogs.

The objective of this study was to determine the frequency of delayed postoperative bleeding in RRGs with appendicular bone tumors undergoing limb amputations and to determine if administration of EACA was effective on the prevention of postoperative bleeding. We hypothesized that the administration of a prohemostatic agent, such as EACA would prevent or minimize bleeding after amputation in RRGs.

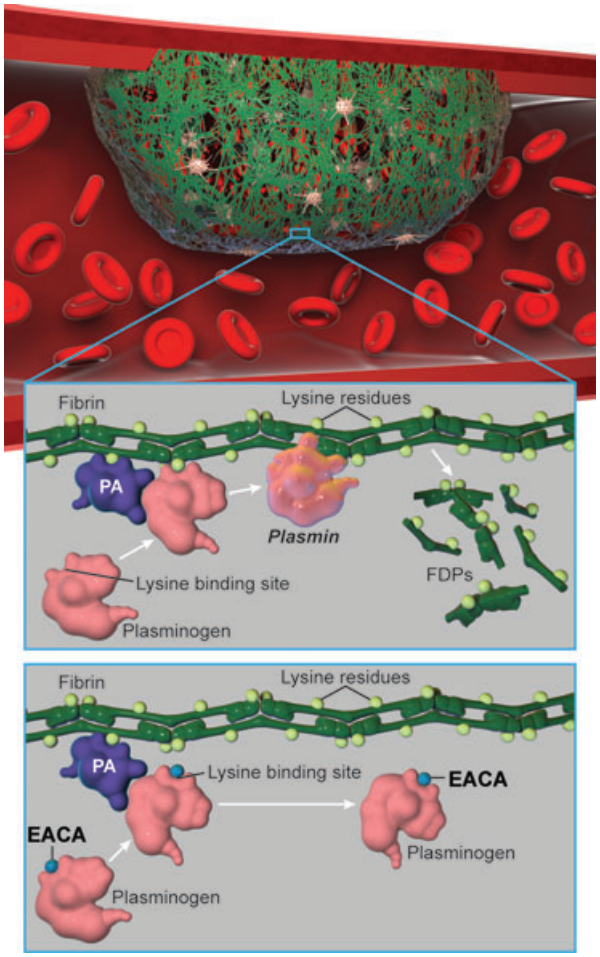

Figure 1: Schematic representation of the fibrinolytic system and the mechanism of action of epsilon aminocaproic acid (EACA). The upper figure shows how plasminogen activator and plasminogen bind to fibrin at the lysine residues, thus forming plasmin, which degrades the clot into fibrin degradation products. The lower figure shows how EACA prevents activation of plasminogen into plasmin on the surface of the fibrin clot, by blocking the lysine-binding site of plasminogen.

Materials and Methods

Medical records from the Hospital for Companion Animals at The Ohio State University Veterinary Medical Center were searched for RRGs that underwent limb amputation for primary appendicular bone tumors from December 2003 to December 2008. Forty-six medical records were reviewed retrospectively and information was collected on the results of physical examinations, anesthesia and analgesia protocols, surgery, postoperative changes in PCV and total plasma protein (TPP), postoperative complications, management of complications (including the use of FFP or EACA), length of hospitalization, and total costs. Due to the fact that we observed postoperative signs compatible with rhabdomyolysis (ie, pigmenturia and high muscle enzyme activities) in some RRGs, postoperative changes in muscle enzyme activities were also evaluated.

Due to the retrospective nature of the present study, the anesthesia, analgesia, and antimicrobial protocols were not fully standardized and had minor variations. The dogs were monitored during surgery with ECG, pulse oximetry, respirometry, capnography, measurement of peripheral arterial blood pressure, and body temperature.

Postoperative bleeding was defined as moderate to severe delayed postoperative cutaneous, subcutaneous, or external bleeding originating in the surgical site that required administration of packed red blood cells (pRBCs), the latter was determined by the attending clinician. Potential variables in the bleeding status included association of bleeding and nonsteroidal anti-inflammatory drugs (NSAIDs), gender and front versus rear. In our clinical experience, rear limb amputations appeared to be associated with more severe bleeding and longer hospital stays than the front limb amputations, thus the location of the tumor was included as a variable.

Statistical analysis

Descriptive statistics were performed for all the variables measured. The D'Agostino and Pearson omnibus

test was used to evaluate for data normality. Variables that were normally distributed were compared using an independent samples *t*-test, and those that were not normally distributed were compared using nonparametric analysis (Mann-Whitney test); results are reported as mean ± SD when normally distributed or median (interquartile range) when data were not normally distributed.

Parameters from before and after surgery were compared using a paired *t*-test or a Wilcoxon rank sum test as appropriate for the distribution of the data, a nonparametric test (Mann-Whitney test) was used to compare length of hospitalization and the medical expenses between dogs with front and rear limb amputations, and between Group 1 and Group 2. A commercially available statistical software [a–f] was used for statistical analysis. For all analyses, values of $P < 0.05$ were considered significant. A Holm's procedure was used to adjust for the type 1 error as a result of performing multiple comparisons.

Logistic regression was used to evaluate potential predictors of whether or not bleeding occurred. The primary variable of interest was whether or not a dog received EACA so this variable was forced in the model. Other potential variables in the bleeding status included association of bleeding and NSAIDs, gender, and front versus rear. Variables with values of $P \leq 0.25$ in the initial bivariate analyses were included in the multivariate logistic regression analysis. Variables were removed from the full multivariate model on the basis of results of the Wald test. Standard statistical software was used.[g,h]

Results

Clinical features

Forty-six RRGs underwent amputation due to primary bone tumors. The RRGs included in this study were divided into two groups; Group 1 included the dogs that developed delayed postoperative bleeding within 48–72 h, Group 2 included the dogs that did not bleed postoperatively. Thirteen dogs (28%) were included in Group 1; there were eight neutered males and five spayed females, and their ages ranged from 4 to 13 years (median, 8 years). The median weight was 31.3 kg (range 21.3–43 kg). Eleven dogs had a histopathologic diagnosis of appendicular OSA (84.6%), one had hemangiosarcoma (HSA) (7.6%), and one had a histiocytic sarcoma (7.6%). Thirty-three dogs (71%) were included in Group 2, there were 21 neutered males and 12 spayed females, and their ages ranged from 5 to 11 years (median, 8 years). The median weight was 32.4 kg (range 23.5–43.3 kg). Thirty-two dogs had a histopathologic diagnosis of appendicular OSA (96.6%), one had hemangiosarcoma (HSA) (3.4%).

Preoperative clinicopathologic evaluation

Forty-one RRGs had blood sampled preoperatively for a CBC and serum biochemical profile; the remaining five dogs had a CBC and serum chemistry profile done by the referring veterinarian; 39 of 46 dogs had complete hemostasis panels (ie, one-stage prothrombin time [OSPT], activated partial thromboplastin time [APTT], and fibrinogen concentration [FIB]) only one of the seven dogs that did not have hemostasis panels developed postoperative bleeding, the remaining six dogs were included in Group 2. All the dogs had PCV, TPP, and systolic blood pressure measured prior to and after surgery. The results of the PCV, TPP, CBC, serum biochemical profile, hemostasis panels, and systolic blood pressure were within reference intervals for the breed in all dogs.

The results of the CBC and serum biochemical profile done by the referring veterinarians were within reference intervals for the respective laboratories, but the data were not available for statistical evaluation. No dogs had comorbidities at the time of surgery.

Anesthesia, fluid therapy, antibiotic, and analgesic protocols

Most dogs were premedicated with acepromazine (0.025–0.05 mg/kg IM) and morphine[i] (0.2 mg/kg IM). Anesthesia was induced with propofol[j] (4–6 mg/kg IV); lidocaine[k] (50 μg/kg/min IV), and isoflurane[l] in oxygen was used for maintenance (1–3% induction, 0.5–2.0% maintenance). All of the dogs received intraoperative fluid therapy consisting of a balance electrolyte solution at 5 mL/kg/h IV.

The postoperative analgesia/sedation protocols included continuous rate infusion of fentanyl[m] (1–8 μg/kg/h/IV), lidocaine[n] (25–50 μg/kg/min/IV), ketamine[o] (10–15 μg/kg/min/IV), morphine[p] (0.01–0.03 mg/kg/h IV), and hydromorphone[q] (0.008–0.03 mg/kg/h), singly or in combination. Pain control protocols also included tramadol[r] (1–4 mg/kg), deracoxib[s] (3–4 mg/kg), and carprofen[t] (1–2 mg/kg). Cefazolin sodium[u] (22 mg/kg IV) was used as prophylactic antimicrobial therapy in 27 dogs, at the discretion of the surgeon. Eight RRGs (61%) included in Group 1 and 21 RRGs (63%) included in Group 2 had received NSAIDs orally (eg, deracoxib, carprofen, meloxicam) prior to surgery. NSAIDs administration did not have a significant effect on bleeding ($P > 0.05$).

Surgery

Eight of 13 RRGs in Group 1 (61.5%) underwent coxofemoral disarticulation and five dogs (38.4%) underwent scapulohumeral amputation. Fifteen of 33 in Group 2 (45.4%) underwent coxofemoral disarticulation or midfemoral amputation (for tibial tumors), and

18 (54.5%) underwent scapulohumeral amputation. Eight dogs (61.5%) in Group 1 and 15 (45.4%) in Group 2 underwent right limb amputation, while five dogs in Group 1 (38.4%) and 18 dogs (54.5%) in Group 2 had left limb amputation. Front versus rear leg and right versus left leg amputation did not have a significant effect on bleeding ($P > 0.05$).

Postoperative complications

None of the dogs experienced intraoperative or immediate postoperative bleeding over a 5-year period; however, four of the first six RRGs (66.6%) that underwent limb amputation during the study period developed severe bleeding complications and required transfusion of blood components. These were the index patients where the problem was first identified; none of them had received prophylactic prohemostatics.

After identifying this bleeding complication, FFP was preemptively administered immediately after inducing anesthesia (10–15 mL/kg, IV, starting dose) in the following 15 RRGs that underwent amputation. Five of these 15 dogs (33%) developed postoperative bleeding, requiring additional administration of blood components.

In an attempt to prevent postoperative bleeding and decrease transfusion requirements and related costs, EACA was administered in 25 RRGs that underwent limb amputation during the last 3 years of this study. Four RRGs received both FFP and EACA, none of them developed the postoperative bleeding. The first dose of EACA[v] (500–1,000 mg, IV total dose or 15–40 mg/kg) was administered immediately after surgery (1 mL diluted in 15 mL of 0.9%NaCl over 30 min), followed by administration of EACA tablets[w] (500–1,000 mg of EACA PO, total dose, q8 h, for 5 days). FFP was administered at the clinician's discretion, if bleeding developed. Four of the 25 RRGs (16%) that received EACA developed bleeding complications; two of them had received both EACA and FFP preemptively. Only one of the four dogs required a pRBC transfusion.

The observed signs of bleeding consisted of cutaneous bruising that extended from the area of the surgical site, and became widespread within hours. There was no bleeding from mucosal surfaces or in areas distant from the surgical site (Figure 2). In total 13 of 46 RRGs (28%) had delayed postoperative bleeding starting 36–48 h after surgery, four of the 13 dogs in Group 1 (30%) developed postsurgical local infection and sepsis. Three of them (75%) had undergone hindlimb amputation, and one (25%) had forelimb amputation. In the study reported here none of the RRGs that received EACA had any adverse effect.

Nine of the 13 dogs in Group 1 had biochemistry profiles postoperatively; all of them had marked postoperative increases in creatinine kinase (CK), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities. A paired *t*-test demonstrated that the CK activity in Group 1 significantly increased after amputation from 320 (147–2,208) U/L to 12,600 (4,587–33,720) U/L, reference interval 76–254 U/L ($P = 0.0425$), ALT from 46.0 (33.0–57.0) to 220.0 (123.0–277.0) U/L, reference interval 28–82 U/L ($P = 0.012$), and AST from 42.0 (32.0–118.0) to 1,253 (601.0–2,168) U/L reference interval 24–57 U/L ($P = 0.012$) (Table 2).

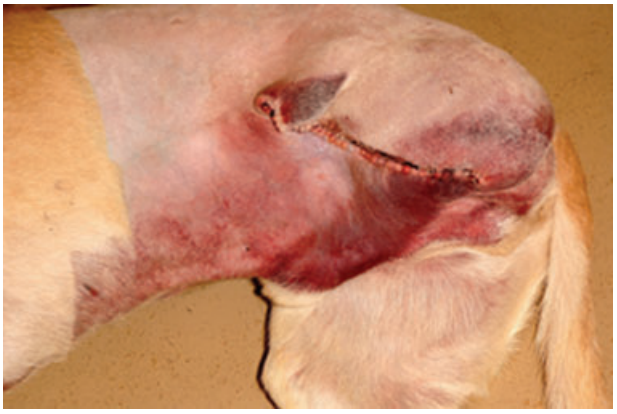

Figure 2: Postoperative bleeding in a RRG, 36 h after limb amputation.

A multiple logistic regression model was used to predict factors associated with bleeding. After evaluating EACA administration, FFP administration, NSAID administration, gender, front versus rear leg amputation, and right versus left leg amputation, only EACA administration had a significant effect on bleeding. Dogs that did not receive EACA were 5.7 times more likely to bleed than dogs that received EACA, when controlling for whether or not they received FFP (95% CI: 1.02–32.15, $P = 0.047$). Interestingly, dogs that did not receive FFP were not significantly more likely to bleed than those that received FFP (95% CI: 0.51–15.7, $P = 0.230$).

Pre- and postoperative PCV, TPP, and platelet count

Forty-five dogs (98%) had postoperative PCV and TPP recorded 48–72 h after surgery. The PCV decreased significantly after surgery in both groups. In Group 1, the PCV decreased from 0.55 (0.50–0.60) to 0.33 (0.24–0.40) L/L; reference interval, 0.50–0.68 L/L, (55.0 [50.0–60.5] to 33.0 [24.5–40.0]%; reference interval 50–68%) ($P < 0.0014$; Table 1); in Group 2 the PCV also decreased from 0.52 (0.51–0.57) to 0.36 (0.34–0.43) L/L, reference interval 0.50–0.68 L/L, 52.0 [51.0–57.7] to 36.5 [34.2–43.2]%, reference interval 50–68% ($P < 0.0013$; Table 1). The postoperative PCV was not significantly different between the two groups ($P = 0.27$). There were no significant

Table 1: Results of preoperative and postoperative packed cell volume (PCV), total plasma protein (TPP), and systolic blood pressure (BP) in Group 1 (RRGs that bled) and Group 2 (RRGs that did not bleed)

Analyte (Greyhounds reference interval)	Group 1 Pre-Sx median (range) (*n*)	Group 1 Post-Sx median (range) (*n*)	*P* value	Group 2 Pre-Sx median (range) (*n*)	Group 2 Post-Sx median (range) (*n*)	*P* value
PCV (0.50–0.68 L/L)	0.55 (0.50–0.60)	0.33 (0.24–0.40)	0.0014	0.52 (0.51–0.57)	0.36 (0.34–0.43)	0.0013
[50—68%]	55.0 (50.0–60-5) (13)	33.0 (24.5–40.0) (13)		52.0 (51.0–57.7) (32)	36.5 (34.2–43.2) (32)	
TPP (48–63 g/L)	66.0 (61–69)	44.0 (39–52)	0.0012	65.0 (60.0–69.0)	42.5 (40.0–48.0)	0.0011
[4.8–6.3 g/dL]	6.6 (6.1–6.9) (13)	4.4 (3.9–5.2) (13)		6.5 (6.0–6.9) (32)	4.2 (4.0–4.8) (32)	
BP (mmHg)	115 (100–145) (12)	160 (127–177) (12)	0.0720	120 (110–140) (29)	146 (125–167) (29)	0.0344
Platelet Count (145–309 × 10^9/L)	217.0 (193.5–260.5) (9)	80.1 (58.5–91.8) (9)	0.0015	229.0 (202.5–263.0) (5)	198 (117.5–245.0) (5)	0.0224

Notes: Data are presented as median (interquartile range 25th–75th percentile). Sx, surgery.
Reference interval in parentheses next to analyte.

differences between the postoperative PCV of dogs that underwent rear versus front limb amputations ($P = 0.12$).

There was also a significant decrease in TPP after amputation in both groups; in Group 1 the TPP decreased from 66.0 (61–69) to 44.0 (39–52) g/L, reference interval 48–63 g/L (6.6 [6.1–6.9] to 4.4 [3.9–5.2] g/dL, reference interval 4.8–6.3 g/dL) ($P < 0.0012$) and in Group 2 the TPP also decreased from 65.0 (60.0–69.0) to 42.5 (40.0–48.0) g/L, reference interval 48–63 g/L, (6.5 [6.0–6.9] to 4.2 [4.0–4.8] g/dL, reference interval 4.8–6.3 g/dL) ($P < 0.0011$), but there was no difference in TPP postoperatively between groups ($P = 0.51$).

Nine RRGs in Group 1 and five in Group 2 had a complete CBC postoperatively, the platelet count in the nine dogs in Group 1 significantly decreased from 217.0 (193.5–260.5) to 80.1 (58.5–91.8) × 10^9/L, reference interval 145–309 × 10^9/L ($P < 0.0015$). There was no difference detected between the platelet count before and after surgery in the five dogs in Group 2 (229.0 (202.5–263.0) to 198 (117.5–245.0) × 10^9/L). There was a significant difference between platelet counts postoperatively between groups ($P = 0.0224$; Table 2).

Postoperative mortality, length of hospitalization, and total cost of hospitalization

None of the dogs died perioperatively. The length of hospitalization in Group 1 (median 7, range 3–14 days) was significantly longer than in the Group 2 (median 5, range 3–13 days) ($P = 0.009$). The hospital bill in Group 1 (median $4,775 USD; range $1,215–6,187 USD) was significantly higher than in Group 2 (median $2,687 USD; range $1,767–4,786 USD) ($P = 0.0002$).

Discussion

Over a 5-year period (2003–2008), 28% of the RRGs that underwent amputation due to primary bone tumors developed a delayed postoperative bleeding complication. Ninety-three percent of the Greyhounds included in this study had a histopathologic diagnosis of OSA, consistent with findings in previous studies.[53]

All the RRGs in Group 1 dogs had a normal platelet count before surgery. The significant decrease in their platelet count was concomitant with the decrease in PCV, which suggests that this degree of thrombocytopenia may be due to blood loss. In addition, a platelet count of 80,000 × 10^9/L is unlikely to result in spontaneous bleeding. The diagnosis of disseminated intravascular coagulation (DIC) in veterinary medicine is traditionally based on three or more abnormal hemostatic parameters, including OSPT, APTT, FIB, D-dimer concentration, platelet count, and erythrocyte morphology.[18] Both the postoperative OSPT and APTT values from dogs in Group 1 were within the reference interval; therefore,

Table 2: Results of preoperative and postoperative of creatine kinase (CK) activity, and bilirubin and creatinine concentrations in dogs in Group 1

Analyte (Greyhounds reference intervals)	Group 1 Pre-Sx median (range) (*n*)	Group 1 Post-Sx median (range) (*n*)	*P* value
CK (76–254 IU/L)	320 (147–2,208) (9)	12,600 (4,587–33,720) (9)	0.0425
ALT (28–82 IU/L)	46.0 (33.0–57.0) (9)	220.0 (123.0–277.0) (9)	0.012
AST (24–57 IU/L)	42.0 (32.0–118.0) (9)	1,253 (601.0–2,168) (9)	0.012

Notes: Data are presented as median (interquartile range 25th–75th percentile). Sx, surgery.
Reference interval in parentheses next to analyte.

DIC was an unlikely cause of bleeding in this patient population.

Although the analgesia protocol was not standardized and had minor variations, it is unlikely that the drugs administered perioperatively contributed to the bleeding outcomes. In this study, 52% of the RRGs had received NSAIDs prior to the surgery, but there was no association between the bleeding and administration of NSAIDs. Thus drug-associated platelet dysfunction is not likely the mechanism responsible of the postoperative complication in the RRGs. Moreover, platelet dysfunction typically results in intraoperative bleeding rather than delayed postoperative bleeding.

Independently of the use of FFP, administration of EACA was the only parameter that had a significant effect on the frequency of bleeding in the RRGs in this study, in people EACA has been used to manage postoperative bleeding since the 1960s after prostatectomy,[32] after abnormal hemorrhage in women with intrauterine devices,[33] and to treat hematuria and excessive bleeding after dental extractions in hemophiliacs.[34,35] Since then, EACA has been studied broadly for its efficacy in reducing perioperative blood loss in various types of surgery, including cardiovascular,[36,37] spinal,[38] acute trauma,[39] and orthopedic surgery.[40] The in vitro and in vivo actions of EACA in dogs were extensively studied in the 1950s and 1960s, but to our knowledge there are not any reports of the use of EACA in dogs since then. Interestingly, in dogs EACA neutralizes bleeding states created experimentally by infusion of plasmin or a plasminogen activator.[31,41–43] The dose of EACA used in this study was extrapolated from the dose used in people. None of the RRGs that received EACA had any adverse effect.

The delayed local postoperative bleeding in most of the RRGs in Group 1, progressed to a generalized bleeding disorder associated with profuse widespread bruising, mild thrombocytopenia, anemia, and increases in liver and muscle enzyme activities. The mechanism responsible for these changes may be similar to that observed in women with preeclampsia and HELPP syndrome, where massive endothelial dysfunction leads to similar clinicopathologic changes.[54] The use of specific plasma markers such as big endothelin 1, von Willebrand factor, vascular endothelial growth factor (VEGF), and hyaluronic acid will be needed to confirm the presence of endothelial dysfunction in the RRGs. The rhabdomyolysis could also have been the results of muscle hypoxia due to hypoperfusion or thromboembolism.

The standard of care for small animal patients with spontaneous bleeding is the administration of plasma components, such as FFP, cryoprecipitate, or cryopoor plasma. FFP is the most commonly used plasma component, since it contains all clotting factors and inhibitors such as antithrombin (AT) and protein C, which are used in patients with inherited deficiencies of these inhibitors. The cost of a transfusion of FFP for a Greyhound (average weight 32 kg) using 10–15 mL/kg or 320–480 mL (approximately 3–5 units) ranges from $330 to $550 USD, whereas a 5-day course of EACA costs approximately $45 USD, thus enhancing the appeal of this perioperative hemostatic agent in RRGs for the prevention of postamputation bleeding. The mean length of hospitalization and total hospital bill were significantly higher in Group 1 than in Group 2. Roughly, the length of hospitalization was 50% longer and the bill almost twice as high for the dogs in Group 1.

Platelet number and function abnormalities, intrinsic, extrinsic, and common pathway abnormalities, von Willebrand's disease and von Willebrand's syndrome, fibrin stabilization defects and fibrinogen activity have been ruled out as likely causes of the bleeding in RRGs.[18,55] In addition to the absence of abnormalities in the hemostasis profile, RRGs with delayed postoperative bleeding have lower antiplasmin activity than the nonbleeders.[18] Thus, we propose that the mechanism of the bleeding is likely a "clot maintenance disorder" (eg, enhanced fibrinolysis) or it is associated with endothelial dysfunction.[56]

Limitations of this study include its retrospective nonrandomized design, the lack of controlled variables, the limited sample size, and the fact that we did not account for variations on costs over time. Although we found a potential protective effect of EACA in Greyhounds with bleeding tendencies, our confidence intervals were wide, thus a prospective randomized study is needed to determine if RRGs that undergo other surgical procedures will benefit from administration of EACA.

In conclusion, this retrospective study suggests that preemptive postoperative administration of EACA appears to be efficacious in decreasing the frequency of bleeding in RRGs undergoing limb amputation. However, a prospective study is warranted to corroborate its effectiveness.

Acknowledgments

The authors would like to thank Tim Vojt for the production of the illustration.

Footnotes

[a] Gary Guccione, National Greyhound Association, personal communication.
[b] Cell-Dyn 3500 R, Abbott Laboratories, Abbott Park, IL.
[c] LaserCyte, IDEXX Laboratories, Westbrook, MD.
[d] Roche Laboratories, Indianapolis, IN.
[e] ACL® 7000, Beckman Coulter, Inc, IL, USA.
[f] Prism version 4.0, GraphPad Software Inc, San Diego, CA.
[g] Stata, version 10.0, StataCorp, College Station, TX.
[h] Aceproject, Butler Animal Health Supply, Dublin, OH.

[i] Duramorpho, Baxter, Deerfield, IL.
[j] Propoflo, Abbott Laboratories.
[k] Lidocaine, Butler, Columbus, OH
[l] Isosol, Vedco Inc, St Joseph, MO.
[m] Fentanyl, Hospira Inc, Lake Forest, IL.
[n] Lidocaine, Sparhawk Laboratories, Inc, Lenexa, KS.
[o] Ketaset, Fort Dodge Animal Health, Fort Dodge, IA.
[p] Duramorpho, Baxter.
[q] Hydromorphone, Baxter Healthcare Corporation.
[r] Tramadol hydrochloride, Mylan Pharmaceuticals Inc, Morgantown, WV.
[s] Deramax, Novartis Animal Health Inc, US.
[t] Rymadil, Pfizer Inc, New York City, NY.
[u] Cephazolin sodium, Sandoz Inc, Princetown, NJ.
[v] Aminocaproic acid, Hospira Inc.
[w] Xanodyne Pharmaceuticals, Inc, Newport, KY.

References

1. Neuhaus D, Fedde MR, Gaehtgens P. Changes in hemorheology in the racing Greyhound as related to oxygen delivery. Eur J Appl Physiol 1992;65:278–285.
2. Steiss J, Brewer W, Welles E, et al. Hematologic and serum biochemical reference values in retired Greyhounds. Comp Cont Educ Pract 2000;22:243–248.
3. Couto CG, Lara A, Iazbik MC, et al. Evaluation of platelet aggregation using a point-of-care instrument in retired racing Greyhounds. J Vet Intern Med 2006;20:365–370.
4. Campora C, Freeman KP, Lewis FI, et al. Determination of haematological reference intervals in healthy adult Greyhounds. J Small Anim Pract 2011;52:301–309.
5. Fayos M, Couto CG, Iazbik MC, et al. Serum protein electrophoresis in retired racing Greyhounds. Vet Clin Path 2005;34:397–400.
6. Couto C, Cerón J, Parra M, et al. Acute phase protein concentrations in retired racing Greyhounds. Vet Clin Pathol 2009;38:219–223.
7. Gaughan K, Bruyette D. Thyroid function testing in Greyhounds. Am J Vet Res 2001;62:1130–1133.
8. Shiel R, Brennan S, Omodo-Eluk A, et al. Thyroid hormone concentrations in young, healthy, pretraining Greyhounds. Vet Rec 2007;161:616–619.
9. Lord LK, Yaissle JE, Marin L, et al. Results of a web-based health survey of retired racing Greyhounds. J Vet Intern Med 2007;21:1243–1250.
10. Straw RC, Powers BE. Management of canine appendicular osteosarcoma. Vet Clin North Am Small Anim Pract 1990;20:1141–1161.
11. Cooley DM, Beranek BC, Schlittler DL, et al. Endogenous gonadal hormone exposure and bone sarcoma risk. Cancer Epidemiol Biomarkers Prev 2002;11:1434–1440.
12. Mueller F, Fuchs B, Kaser-Hotz B. Comparative biology of human and canine osteosarcoma. Anticancer Res 2007;27(1A):155–164.
13. Straw RC, Withrow SJ. Limb-sparing surgery versus amputation for dogs with bone tumors. Vet Clin N Am Small 1996;26:135–143.
14. Liptak JM, Dernell WS, Ehrhart N, et al. Canine appendicular osteosarcoma: curative-intent treatment. Comp Cont Educ Pract 2004;26:186–197.
15. Adams GL, Manson RJ, Turner I, et al. The balance of thrombosis and hemorrhage in surgery. Hematol Oncol Clin N 2007;21:13–24.
16. Kirpensteijn J, Van den Bos R, Endenburg N. Adaptation of dogs to the amputation of a limb and their owners' satisfaction with the procedure. Vet Rec 1999;144:115–118.
17. Knapp DW, Tomlinson JL, Constantinescu GM. Pelvic limb removal by coxo-femoral disarticulation in 13 dogs. J Small Anim Pract 1990;31:561–567.
18. Lara-Garcia A, Couto CG, Iazbik MC, et al. Postoperative bleeding in retired racing Greyhounds. J Vet Intern Med 2008;22:525–533.
19. Berzon JL. Complications of elective ovariohysterectomies in the dog and cat at a teaching institution: clinical review of 853 cases. Vet Surg 1979;8:89–91.
20. Pollari F, Bonnett B, Bamsey S, et al. Postoperative complications of elective surgeries in dogs and cats determined by examining electronic and paper medical records. J Am Vet Med Assoc 1996;208:1882–1886.
21. Burrow R, Batchelor D, Cripps P. Complications observed during and after ovariohysterectomy of 142 bitches at a veterinary teaching hospital. Vet Rec 2005;157:829–833.
22. Marín L CC, Iazbik MC, Lara A, et al. Hemostatic complications after limb amputation in retired racing Greyhounds. In: proceedings of the 25th ACVIM forum, Seattle, WA, 2007, p. 573.
23. Logan JC, Callan MB, Drew K, et al. Clinical indications for use of fresh frozen plasma in dogs: 74 dogs (October through December 1999). J Am Vet Med Assoc 2001;218:1449–1455.
24. Knowler C, Giger U, Dodds WJ, et al. Factor-Xi deficiency in Kerry-Blue terriers. J Am Vet Med Assoc 1994;205:1557–1561.
25. Stokol T, Parry B. Efficacy of fresh-frozen plasma and cryoprecipitate in dogs with von Willebrand's disease or hemophilia A. J Vet Intern Med 1998;12:84–92.
26. Feldman DG, Brooks MB, Dodds WJ. Hemophilia B (factor IX deficiency) in a family of German shepherd dogs. J Am Vet Med Assoc 1995;206:1901–1905.
27. Carpenter SL, Mathew P. Alpha(2)-antiplasmin and its deficiency: fibrinolysis out of balance. Haemophilia 2008;14:1250–1254.
28. Ross J, Dallap BL, Dolente BA, et al. Pharmacokinetics and pharmacodynamics of epsilon-aminocaproic acid in horses. Am J Vet Res 2007;68:1016–1021.
29. Mannucci PM. Hemostatic drugs. N Engl J Med 1998;339:245–253.
30. Okamoto S. Plasmin and antiplasmin. Their pathologic physiology. Keio J Med 1959;8:211–217.
31. Sherry S, Fletcher A, Alkjaerisg N, et al. E-amino-caproic acid. "A potent antifibrinolytic agent." Trans Assoc Am Physicians 1959;72:62–70.
32. Fetter TR, Bowman WD, Cottone RN, et al. Effect of epsilon aminocaproic acid on bleeding after prostatectomy. J Urol 1961;85:970–972.
33. Rueda Gonzalez R. Use of epsilon aminocaproic acid in abnormal hemorrhage using intrauterine contraceptive devices. Rev Colomb Obstet Ginecol 1969;20:273–278.
34. Steiger B, White JG, Krivit W. Epsilon-aminocaproic acid for hematuria in hemophilia. J Lancet 1962;82:421–429.
35. Reid WO, Francisc J, Lucas ON, et al. Use of epsilon-aminocaproic acid in management of dental extractions in hemophiliac. Am J Med Sci 1964;248:184–188.
36. Koster A, Schirmer U. Re-evaluation of the role of antifibrinolytic therapy with lysine analogs during cardiac surgery in the post aprotinin era. Curr Opin Anesthesio 2011;24:92–97.
37. Martin K, Gertler R, Sterner A, et al. Comparison of blood-sparing efficacy of epsilon-aminocaproic acid and tranexamic acid in newborns undergoing cardiac surgery. Thorac Cardiovasc Surg 2011;59:276–280.
38. Berenholtz SM, Pham JC, Garrett-Mayer E, et al. Effect of epsilon aminocaproic acid on red-cell transfusion requirements in major spinal surgery. Spine 2009;34:2096–2103.
39. Roberts I, Shakur H, Ker K, et al. Antifibrinolytic drugs for acute traumatic injury. Cochrane Database Syst Rev 2011;Jan 19;(1):CD004896.
40. Eubanks JD. Antifibrinolytics in major orthopaedic surgery. J Am Acad Orthop Surg 2010;18:132–138.
41. Okamoto S, Nakajima T, Okamoto U, et al. A suppressing effect of e-amino-n-caproic acid on the bleeding of dogs, produced with the activation of plasmin in the circulatory blood. Keio J Med 1959;8:247–266.
42. Belko JS, Warren R, Regan EE, et al. Induced fibrinolytic activity and hypofibrinogenemia – effect of epsilon-amino-caproic acid. Arch Surg 1963;86:396–401.
43. Okamoto S, Oshiba S, Mihara H, et al. Synthetic inhibitors of fibrinolysis – in vitro and in vivo mode of action. Ann N Y Acad Sci 1968;146(A2):414–429.
44. Hoylaerts M, Lijnen HR, Collen D. Studies on the mechanism of the antifibrinolytic action of tranexamic acid. Biochim Biophys Acta 1981;673:75–85.
45. Bouma BN, Mosnier LO. Thrombin activatable fibrinolysis inhibitor (TAFI) – how does thrombin regulate fibrinolysis? Ann Med 2006;38:378–388.

46. Henry DA, Carless PA, Moxey AJ, et al. Anti-fibrinolytic use for minimising perioperative allogeneic blood transfusion. Cochrane Database Syst Rev 2007:CD001886.
47. Cliffton EE. I. Evidence that a hypercoagulable state precedes massive oozing during and after major surgery. 2. Effect of epsilon-aminocaproic acid (EACA) in control of surgical oozing. Bibl Haematol 1965; (23P5):1264–1277.
48. Jordan D, Delphin E, Rose E. Prophylactic epsilon-aminocaproic acid (EACA) administration minimizes blood replacement therapy during cardiac-surgery. Anesth Analg 1995;80:827–829.
49. Pokorny F. Toxicological experiments with cyclohexamine oxine, e caprolactein and e aminocaproic acid. Mutual biological comparison. Sb Lek 1952;54:28.
50. Lang K, Bitz H. Metabolism of e aminocaproic acid. Biochem Z 1954;324(7):495–498.
51. Polizopoulou ZS, Koutinas AF, Patsikas MN, Soubasis N. Evaluation of a proposed therapeutic protocol in 12 dogs with tentative degenerative myelopathy. Acta Vet Hung 2008;56:293–301.
52. Regnier A, Cazalot G, Cantaloube B. Topical treatment of non-healing corneal epithelial ulcers in dogs with aminocaproic acid. Vet Rec 2005;157:510–513.
53. Rosenberger JA, Pablo NV, Crawford PC. Prevalence of and intrinsic risk factors for appendicular osteosarcorna in dogs: 179 cases (1996–2005). JAVMAn 2007;231:1076–1080.
54. Mihu D, Costin N, Mihu C, et al. HELLP syndrome–a multisystemic disorder. J Gastrointest Liver Dis 2007;16:419–424.
55. Hoffman M, Monroe DM. Coagulation 2006: a modern view of hemostasis. Hematol Oncol Clin North Am 2007;21:1–11.
56. Kraft P, Schwarz T, Meijers JCM, et al. Thrombin-activatable fibrinolysis inhibitor (TAFI) deficient mice are susceptible to intracerebral thrombosis and ischemic stroke. Plos One 2010;19;5(7):e11658.