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Postoperative Bleeding in Retired Racing Greyhounds

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Background: Some retired racing Greyhounds (RRG) that undergo surgery bleed excessively.

Hypothesis: Greyhounds that bleed excessively will have one or more preoperative hemostatic abnormalities that can be used to predict the risk and severity of postoperative bleeding.

Animals: Eighty-eight RRG undergoing ovariohysterectomy or castration.

Methods: All dogs were evaluated preoperatively with a physical exam, CBC, platelet count, OSPT, APTT, platelet function with PFA-100[a]; fibrinogen, D-dimer, plasminogen (Plmg), antiplasmin (AP), antithrombin (AT), and vWF concentration (vWF:Ag); vWF collagen binding assay (vWF:CBA), and Factor XIII assay. Assays were repeated in the dogs that bled, and in an age- and sex-matched control group of RRG.

Results: Twenty-six percent of the dogs had bleeding 36–48 hours after surgery. AP ($P < .0001$) and AT concentration ($P = .007$) were significantly lower, and vWF:CBA ($P = .0284$) was higher preoperatively in the dogs with excessive hemorrhage. A lower platelet count ($P = .001$) and hematocrit ($P = .002$), shorter OSPT ($P = .0002$) and higher plasma fibrinogen ($P < .0001$), and AP ($P = .001$) concentration were detected at the time of bleeding compared with preoperative values in the dogs that bled excessively. The same findings were observed postoperatively for the control group, except for the decrease in hematocrit.

Conclusions and Clinical Importance: The results indicate that this excessive postoperative bleeding is not attributable to a primary or secondary hemostatic defect, but could result from altered fibrinolysis.

Key words: Antiplasmin; Fibrinolysis; Hemostasis; Surgery; von Willebrand factor.

Greyhounds have physiologic and hematologic peculiarities specific for the breed.[1–5] The mean packed cell volume (PCV), hemoglobin concentration, red blood cell (RBC) count, and whole blood viscosity are higher, whereas the white blood cell count, neutrophil count, and platelet count[1–3] are lower than in other breeds of dogs. The serum total protein, globulin, α-globulin, and β-globulin concentrations are also lower than in non-Greyhound dogs.[1,3–5] In addition, platelet aggregation under high shear as determined with PFA-100[a] is significantly shorter in retired racing Greyhounds (RRG) than in non-Greyhound dogs.[5]

We have observed a tendency for RRG to develop bleeding after minor trauma or a simple surgical procedure, including ovariohysterectomy, orchiectomy, dewclaw removal, or laparotomy. In the majority of the dogs, one-stage prothrombin time (OSPT), activated partial thromboplastin time (APTT), and platelet counts (PLT) were within the reference ranges. Although anecdotal references to excessive bleeding can be found in some Greyhound websites or Greyhound health manuals, there are no published controlled studies documenting or investigating this phenomenon in the breed.

Recently, a website-based survey documented the prevalence of diseases and major causes of death in 747 RRG in the United States in 2006.[6] The mortality rate during a 2-year period was 15%; and bleeding disorders were one of the 4 most prevalent causes of death reported, accounting for 8% of all deaths. Hematologic diseases had a prevalence of 3.3%, with thromboembolic disease and spontaneous and postoperative bleeding accounting for 46% of these.[6]

Intra-operative and immediate postoperative bleeding can be attributed to surgical technique (surgical bleeding) or systemic abnormalities (nonsurgical bleeding).[7] The latter includes primary hemostatic defects such as thrombocytopenia, platelet dysfunction, or von Willebrand disease (vWD), and secondary hemostatic defects such as hypofibrinogenemia, hypoprothrombinemia, hemophilia A or B, factor VII deficiency, or combined clotting factor deficiencies.[7] Delayed postoperative bleeding is more likely caused by abnormal fibrin stabilization, factor XI deficiency, or enhanced fibrinolysis.[8] Finally, systemic endothelial damage or dysfunction after postoperative septic complications or hypertensive crises can result in thrombocytopenia and generalized bleeding, as described in women with HELLP (elevated liver enzymes, low platelet count) syndrome associated with preeclampsia and in children with hemolytic-uremic syndrome.[9–10]

In our experience, limb amputation for osteosarcoma (OSA) also frequently results in severe postoperative bleeding in Greyhounds. In the past 3 years, 36% (10/38) of the RRG that underwent limb amputation for OSA at The Ohio State University Veterinary Teaching Hospital (OSU-VTH) had severe delayed postoperative bleeding that required intensive care management.[b] In six of these dogs, the severity of the bleeding required transfusion of packed RBCs and fresh frozen plasma, leading to a transient poor quality of life postamputation, lengthy hospitalization, and high medical bills.

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Our hypothesis was that Greyhound "bleeders" would have one or more preoperative abnormalities in primary or secondary hemostasis or in the fibrinolytic pathway that could be used to predict the risk and severity of postoperative bleeding. To test this hypothesis, primary and secondary hemostasis were prospectively evaluated preoperatively in RRG who underwent routine ovariohysterectomy or castration at OSU-VTH. The hemostasis assays were repeated in those RRG who developed bleeding complications at the time of the event, and in an age- and sex-matched control group of RRG that underwent ovariohysterectomy or castration at the same time and did not bleed.

Material and Methods

Animals and Blood Samples

This study was approved by the Hospital Executive Committee of The Ohio State University Veterinary Teaching Hospital. Eighty-eight RRG from Greyhound Adoption of Ohio (Chagrin Falls, OH—www.greyhoundadoptionofoh.org) were spayed or neutered at the OSU-VTH between November 2004 and February 2006 as part of a 3rd-year veterinary student teaching laboratory. A physical examination was performed on each dog, and blood was drawn preoperatively for a CBC and hemostasis assessment. The following tests were used to evaluate primary hemostasis: PLT, platelet function with PFA-100[a] closure time (CT), von Willebrand factor antigen concentration (vWF:Ag), and von Willebrand factor activity with collagen binding assay (vWF:CBA). Secondary hemostasis was evaluated with APTT, OSPT, fibrinogen concentration, antithrombin (AT) activity, and Factor XIII (FXIII). The fibrinolytic pathway was evaluated by measuring plasminogen activity (Plmg), antiplasmin activity (AP), and D-dimer concentration.

Before surgery, all RRG were premedicated with buprenorphine[c] (0.05 mg/kg) and acepromazine[d] (0.05 mg/kg) intramuscularly, and a prophylactic dose of intravenous cefazolin sodium[e] (22 mg/kg IV) was administered. Anesthesia was induced with ketamine[f] (5 mg/kg) and diazepam[g] (0.25 mg/kg) IV, and maintained using isoflurane[h] in oxygen. Respiration was supported with intermittent positive-pressure ventilation, and intra-operative fluid therapy consisted of lactated Ringer's solution (10 mL/kg/hours IV). The dogs were monitored during surgery with electrocardiography, pulse oximetry, respirometry, capnography, measurement of peripheral arterial pressure, and body temperature. After surgery, analgesia was continued with one single intramuscular injection of carprofen[i] (4 mg/kg). The dogs were monitored postoperatively until recovery and then transferred to a boarding area.

After the surgical procedure (spay or neuter), dogs were kept at the OSU-VTH for a minimum of 4 days. All dogs underwent daily physical examination, their bleeding score was recorded once a day, and the areas of hemorrhage were photographed digitally. Although there is no standardized scale to evaluate the severity of bleeding in dogs, a system with scores ranging from 0 to 4 was adapted from the one proposed by Buchanan and Adix for children with idiopathic thrombocytopenic purpura (Table 1).[11] The final bleeding score assigned to each RRG corresponded with the highest score recorded during the postoperative period.

In dogs with clinical evidence of bleeding (ie, bleeding score 1–4), the assays of hemostatic function performed at baseline were repeated when the bleeding was detected. These assays were also performed in a group of age- and gender-matched Greyhounds who underwent surgery at the same time and did not have postoperative bleeding (ie, bleeding score 0). The results of the hemostasis assays in this "control" group were used to assess which hemostatic changes developed postoperatively as a result of the surgical procedure performed and for comparison with the postoperative results of the "bleeder" group.

Table 1. Scale for severity of bleeding.

Score 0	Definitely no new bleeding
Score 1	Questionable new petechiae or bruising
Score 2	Definite new cutaneous hemorrhagic lesions
Score 3	Moderate to severe cutaneous bleeding without measurable decline in hemoglobin concentration
Score 4	Severe external bleeding of sufficient magnitude to decrease hematocrit by $>6\%$

Adapted from the system for children with idiopathic thrombocytopenic purpura.[11]

Sampling

Twenty milliliters of blood were collected from the jugular vein by atraumatic direct venipuncture, and placed in a 3 mL siliconized tube containing sodium ethylenediaminetetraacetate and in 4.5 mL tubes containing 3.8% sodium citrate.[j]

One tube of blood collected in sodium citrate was placed in a rack at room temperature for at least 10 minutes (and for no longer than 3 hours); this sample was used to evaluate platelet function with the PFA-100.[a] The other tubes of citrated blood were immediately centrifuged at $1{,}380 \times g$ for 10 minutes, and the plasma was transferred to several polypropylene transfer tubes. One tube with plasma was used for immediate determination of OSPT, APTT, and fibrinogen concentration, and the rest of the tubes were stored in a freezer at -3°C for 1–2 weeks. Frozen plasma was shipped for the additional assays to the Comparative Coagulation Section laboratory (Animal Health Diagnostic Laboratory, Cornell University, Ithaca, NY).

Platelet Count and Platelet Function Assays

Platelet counts were performed using automated counters (CellDyn 3500[k] or LaserCyte[l]) or a manual hand count for those samples below the reference ranges. Whole blood (800 μL) platelet function assays were done by means of the PFA-100.[a] The PFA-100[a] simulates primary hemostasis after injury to a small vessel under high shear stress. Disposable cartridges with collagen and either epinephrine (10 μg) (COL/EPI) or adenosindiphosphate (ADP—50 μg) (COL/ADP) were used as platelet activating reagents. The test was performed twice with each type of reagent as described previously, and the closure times (CT) were recorded.[12,13] Greyhound-specific reference intervals were used for hematocrit, PLT, and CT with PFA-100.[a5]

Routine Hemostasis Assays

OSPT, APTT, and plasma fibrinogen concentration were determined by means of the automated analyzer ACL-200[m] and commercially available reagents,[n] as described previously.[14]

Antithrombin

Plasma AT activity was measured with a synthetic chromogenic substrate kit[o] following the manufacturer's recommendations for assay conditions and instrumentation.[p] The assay detects AT activity based on inhibition of a thrombin reagent (anti-IIa activity) and was modified by the substitution of pooled normal canine plasma, rather than human plasma, as the calibration standard. The AT activities of test plasma were reported as the percentage of the standard, which had an assigned value of 100%.[15]

Von Willebrand Factor Assays

Plasma vWF concentration [vWF antigen (vWF:Ag)] was measured by enzyme-linked immunosorbent assay (ELISA) configured

with monoclonal anticanine vWF antibodies.[16] The biologic activity of vWF was evaluated in a collagen-binding assay (vWF:CBA).[17] Deficiency of the high molecular-weight vWF multimers impairs the ability of vWF to effectively bind collagen. Determination of the ratio of vWF:Ag to vWF:CBA differentiates type 1 from type 2 vWD, based on the disproportionate lack of vWF:CBA in the latter. A ratio of vWF:Ag to vWF:CBA greater than 2:1 is considered to be diagnostic of type 2 vWD.[17–18]

Fibrinolysis Assays

The activities of Plmg and AP were measured using synthetic chromogenic substrate kits and the manufacturer's recommended instrument.[q,r,s] The Plmg assay was modified by substitution of urokinase, rather than streptokinase, to activate Plmg.[19] Standard curves for the Plmg and AP assays were derived from dilutions of a canine plasma standard, and the results were reported as a percentage of the standard. Plasma D-dimer concentration was measured using a semiquantitative latex agglutination assay,[t] as described previously.[20] The results of this assay were expressed semiquantitatively within 4 ranges: <250, 250–500, 500–1,000, and 1,000–2,000 ng/dL.

Factor XIII Deficiency Screen

A screening test to detect the presence of cross-linked fibrin was performed by comparing the urea solubility of fibrin from control dogs and test plasma.[21] Briefly, 2 volumes of citrated plasma were combined in a glass tube with 1 volume of an imidazole buffer and 1/10th volume of 0.25 M $CaCl_2$. After incubation for 1 hour at 37 °C, the resultant fibrin clot was transferred to a 5 M urea solution, and the presence or absence of residual clot was recorded at 5-minute intervals. In the absence of Factor XIII, complete clot lysis should occur within 5 minutes. Cross-linked fibrin, formed by the action of Factor XIII, is insoluble in urea and remains intact. Results are reported as normal or abnormal.[22]

Statistical Analysis

Graph Pad Prism software[u] was used for statistical analysis. Descriptive statistics were performed for all the variables. The Kolmogorov-Smirnoff test was used to assess normal distribution of the variables before using parametric methods. The Student's *t*-test for independent variables was used to compare hemostatic variables between groups ("non-bleeders," "bleeders," or control group). Comparisons within groups ("bleeders" and control group) were done using the Student's *t*-test for paired samples. Contingency tables were used to compare categorical variables between groups. Differences were considered statistically significant when $P < .05$.

Results

Signalment and Prevalence of Postoperative Bleeding in RRG

Eighty-eight Greyhounds were evaluated. There were 36 intact males and 52 intact females, with ages that ranged from 1 to 11 years (mean 4 years). Although none of the dogs experienced intra-operative or immediate postoperative bleeding, 26% of the dogs (23/88) had delayed postoperative bleeding 36 to 48 hours after surgery. The bleeding was scored as grade 1 in 2 dogs, grade 2 in 14 dogs, grade 3 in 5 dogs, and grade 4 in 2 dogs. The observed signs of bleeding consisted of cutaneous bruising that extended from the area of the surgical incision toward the periphery (Fig 1). There was no bleeding from mucosal surfaces or in areas distant from the surgical site. None of the dogs required transfusion of blood components and the bleeding was self-limiting; bruising was still present at the time the dogs were discharged, 4 days after the surgery.

There was no significant difference for sex between "bleeders" and "non-bleeders." The age of the "bleeders" (range 1–11 years, mean 3 years) was significantly lower than in the "non-bleeders" (range 2–11 years, mean 4 years) ($P = .02$). On physical examination, we found that the prevalence of a left basilar systolic murmur in the "non-bleeder group" was 69%, and it was significantly higher than in the "bleeder" group, where it was 52% ($P = .013$). The murmurs were graded as I or II/VI in all dogs, except for 1 RRG from the "non-bleeder group" that had a III/VI murmur.

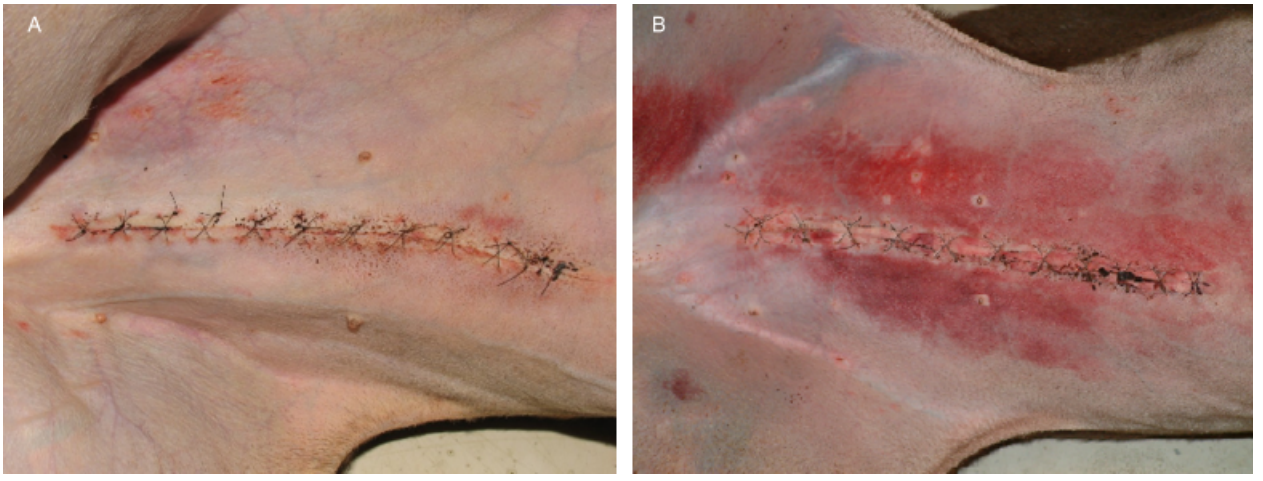

Fig 1. **(A)** Image of the surgical incision in one of the Greyhounds of the control group 36 hours post-surgery (bleeding score 0). **(B)** Image of the surgical incision in one of the Greyhounds of the "bleeder" group 36 hours postsurgery (bleeding score 3).

Comparison of Hemostatic Parameters between "Bleeders" and "Non-Bleeders" Preoperatively

Preoperatively, there were no significant differences between "bleeders" and "non-bleeders" for any of the following factors: PLT, hematocrit, OSPT, APTT, fibrinogen, vWF:Ag, platelet function using the PFA-100[a] with COL/EPI and COL/ADP, Plmg, D-dimer, and F-XIII (Table 2). The D-dimer concentration was <250 ng/dL in all but two of the "non-bleeders," which had values of 250–500 and 500–1,000 ng/dL, respectively; all "bleeders" had values of <250 ng/dL, except 1 dog that had 250–500 ng/dL before surgery. Factor XIII activity was normal for all the dogs in both groups.

In the "non-bleeder" group, AP and AT activities were above the reference range in 20% and 23% of the dogs, respectively. The AP and AT activities, although within the normal range, were significantly lower in the "bleeder" group than in the "non-bleeders" ($P < .001$ and $P = .007$, respectively). The vWF:Ag was below the reference interval in 21% of the "bleeders" and in 50% of the "non-bleeders" without significant differences between groups ($P = .9614$), and the vWF:CBA was below the reference range in 8% of the "bleeders" and in 30% of the "non-bleeders" and was significantly higher in the "bleeders" ($P = .0284$). The ratio between the vWF:Ag concentration and the vWF:CBA was significantly lower in the "bleeder" group ($P < .0001$) (Table 2, Fig 2).

Control Group

The control group consisted of 8 Greyhounds who underwent spaying or neutering and had no signs of bleeding (ie, Score 0). Before surgery, there were no significant differences for any of the parameters evaluated between the control dogs and the rest of the Greyhounds of the "non-bleeder" group.

Comparison of Preoperative and Postoperative Values within "Bleeders" and within Control Group

When the hemostatic variables were compared before surgery and after surgery within the "bleeders," and before and after surgery within the control group, the PLT was significantly lower in both groups ($P = .001$, $P = .03$), the OSPT was significantly shorter ($P = .0002$, $P = .048$), and the fibrinogen ($P < .0001$, $P = .002$) concentration and AP ($P = .01$, $P = .04$) activity were significantly higher postoperatively in both groups (Table 2; Figs 3 and 4). Concentration of the D-dimer was significantly increased after surgery in both groups. In 2 dogs within the control group (25%), the D-dimer concentration increased to between 250 and 2,000 ng/dL ($P < .001$), whereas in 3 dogs within the "bleeder" group (13%), it increased to between 250 and 1,000 ng/dL ($P = .0238$). The hematocrit was the only variable that was significantly lower at the time of bleeding in the "bleeder" group ($P = .002$), but not in the control group ($P = .4533$); the hematocrit decreased more than 6% (ie, grade 4 bleeding) in 2/23 "bleeders" (10%) (Table 2, Figs 3 and 4).

Comparison of Postoperative Values between "Bleeders" and Control Group

There were no significant postoperative differences between "bleeders" and the control group for any of the hemostatic parameters evaluated, except for the ratio vWF:Ag/vWF:CBA, which was lower in the "bleeder" group ($P = .0389$).

Discussion

In this prospective study, we documented the development of delayed postoperative bleeding after spaying or neutering in RRG, with a prevalence of 26%. In 10% of the bleeders, there was a decrease in the hematocrit of

Table 2. Results of hemostatic parameters in "non-bleeder," "bleeder," and control group.

	Laboratory Reference Ranges	Non-bleeder Pre-Sx Mean (SD) n=65	Bleeder Pre-Sx Mean (SD) n=23	Bleeder Post-Sx Mean (SD) n=23	Control Post-Sx Mean (SD) n=8
Hematocrit (%)	46–64	52.3 (4.3)	53.7 (3.7)	50.6 (4.4)[a]	53.7 (7.8)
PLT ($\times 10^9$/L)	125–397	218 (51)	198 (46)	169 (49)[a]	181 (43)[b]
COL–ADP (seconds)	63–92	88.7 (22)	82 (13.6)	80 (11.4)	84.5 (21.9)
COL–EPI (seconds)	87–238	207 (71.3)	180 (59.1)	206.5 (82.5)	174 (64.8)
APTT (seconds)	9–21	14.4 (2.6)	13.7 (1.6)	13.7 (2.3)	15.6 (4.2)
OSPT (seconds)	6–7.5	6.8 (0.4)	6.8 (0.47)	6.3 (0.23)[a]	6.37 (0.48)[b]
Fibrinogen (g/L)	100–384	118 (29.7)	130 (40.8)	215 (37.5)[a]	200 (54.7)[b]
vWF:Ag (%)	70–180	73.7 (23)	74 (14)	81.4 (22.7)	71 (27.8)
vWF:CBA (%)	>50	62 (20)	72.7 (16.5)[c]	80 (26.8)	62.2 (24.1)
Ratio vWF:Ag/vWF:CBA	<2	1.2 (0.14)	1.03 (0.12)[c]	1.03 (0.13)	1.15 (0.11)
Antiplasmin (%)	65–120	107 (35.8)	82 (20)[c]	94 (26.4)[a]	108 (36.5)[b]
Antithrombin (%)	65–145	100.8 (36.4)	78.3 (17.2)[c]	74.8 (24.3)	95.6 (32.4)
Plasminogen (%)	60–170	71.6 (16.9)	65 (19.4)	62.3 (15.2)	71.1 (20.8)

PLT, platelet count; COL-ADP, closure time with collagen-adenosindiphosphate; COL-EPI, closure time with collagen-epinephrine; APTT, activated partial thromboplastin time; OSPT, one stage phrothrombine time; vWF:Ag, von Willebrand Factor antigen concentration; vWF:CBA, von Willebrand Factor collagen binding assay activity; Pre-Sx, preoperative; Post-Sx, postoperative.

[a]Hemostatic parameters significantly differ within the "bleeder" group.

[b]Hemostatic parameters significantly differ within the "control" group.

[c]Hemostatic parameters significantly differ preoperatively between "bleeders" and "non-bleeders."

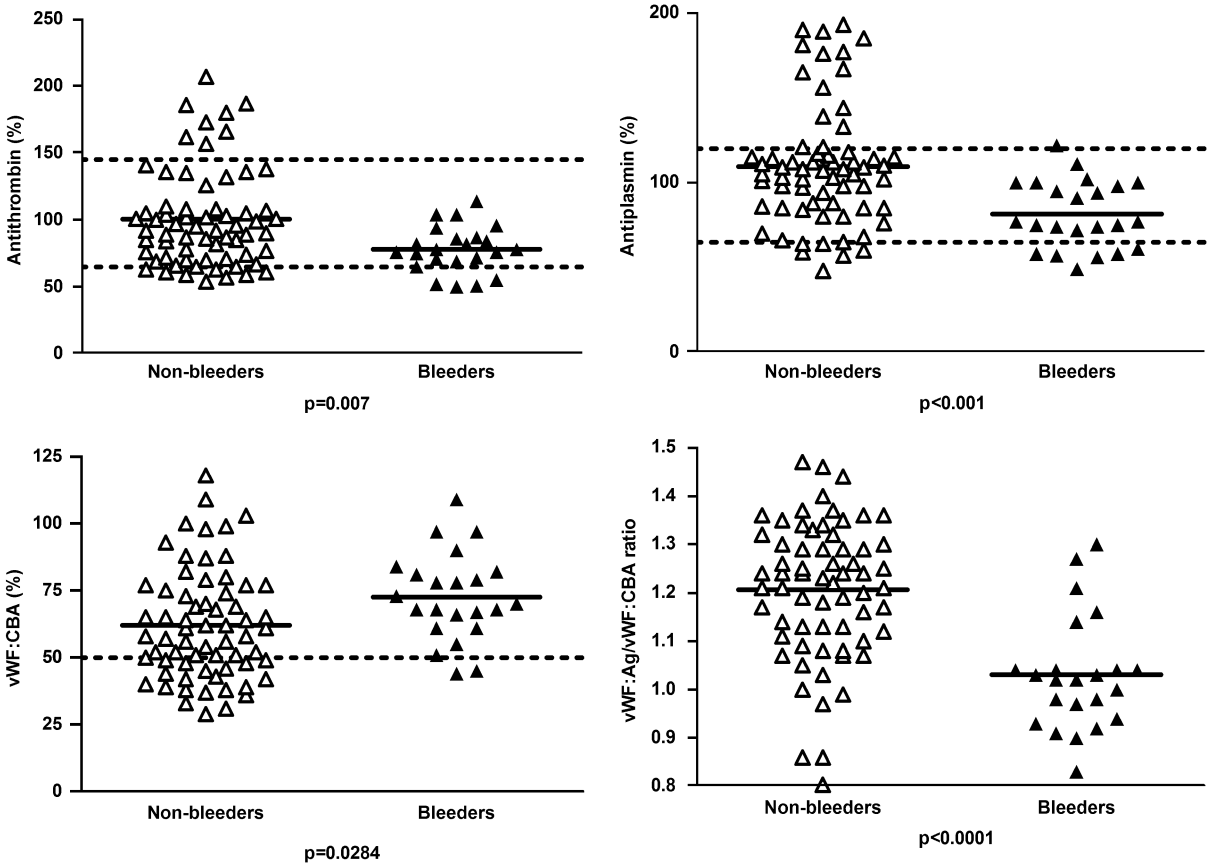

Fig 2. Scatter dot plots of the hemostatic parameters that differ significantly between the "bleeder" group (n = 23) and "non-bleeder" group (n = 65) preoperatively ($P < .05$). The dotted lines represent the reference range limits for a given parameter, and the solid line represents the mean of the values obtained for that parameter in each group of RRG.

more than 6% (ie, grade 4 bleeding) but blood component therapy was not required by these dogs. In both groups, platelet counts and platelet function were within the reference ranges for the breed[5] and were not significantly different between "bleeders" and "non-bleeders." Selective or combined clotting factor deficiencies were ruled out on the basis of normal fibrinogen concentration, OSPT, and APTT in the "bleeders." Factor XIII deficiency was also ruled out on the basis of normal Factor XIII assay.

All the RRG received the same analgesic and anesthetic protocol pre- and postoperatively; therefore, it is unlikely that differences between the groups are caused by the drugs administered perioperatively. The delayed bleeding makes drug-associated thrombocytopenia or platelet dysfunction unlikely as a cause of bleeding. Furthermore, in a previous study, acepromazine caused thrombocytopenia and platelet dysfunction that lasted <200 minutes.[23] Although all the Greyhounds in this study received carprofen perioperatively, the platelet function with PFA-100[a] was within normal limits in both groups at the time of bleeding. Previous studies reported decreased platelet aggregation by aggregometry in dogs that underwent surgery and received multiple doses of carprofen, but there was no clinical evidence of bleeding attributable to this drug in the postoperative period.[24] Another study by Gaal et al[25] demonstrated no changes in platelet aggregation at high shear using PFA-100[a] after treatment with carprofen for 5 days.

vWF is a large molecule composed of low-, intermediate-, and high-molecular weight (MW) multimers. The high-MW multimers are vital for platelet adhesion to the subendothelium in areas of high shear.[26] In this study, the vWF:Ag concentration was below the reference ranges in 21% of the "bleeders" (range 43–63%; mean 54.8%) and 50% of the dogs from the "non-bleeder" group (range 36–68%, mean 56%), without significant differences between the groups. Paradoxically, the "non-bleeder" group had significantly lower levels of high-MW vWF than the "bleeder" group measured by vWF:CBA ($P = .0284$); this unexplained phenomenon should have made the "non-bleeders" more predisposed to develop perioperative bleeding.

Greyhounds are not one of the breeds with a reported high prevalence of vWD.[26] In a recent 2-year period (July 2002 to July 2004), approximately 10% (22 of 216) of the Greyhounds screened at the Comparative Coagulation Section had plasma vWF concentration of $\leq$30%.[v] In a previous study of platelet function with PFA-100[a] in Greyhounds, we also found that 13% (3/23) of the dogs had vWF:Ag concentrations below reference range, but normal CT with the PFA-100.[a5]

Greyhounds commonly have a high velocity aortic murmur because of relative aortic stenosis.[27] Type 2 von

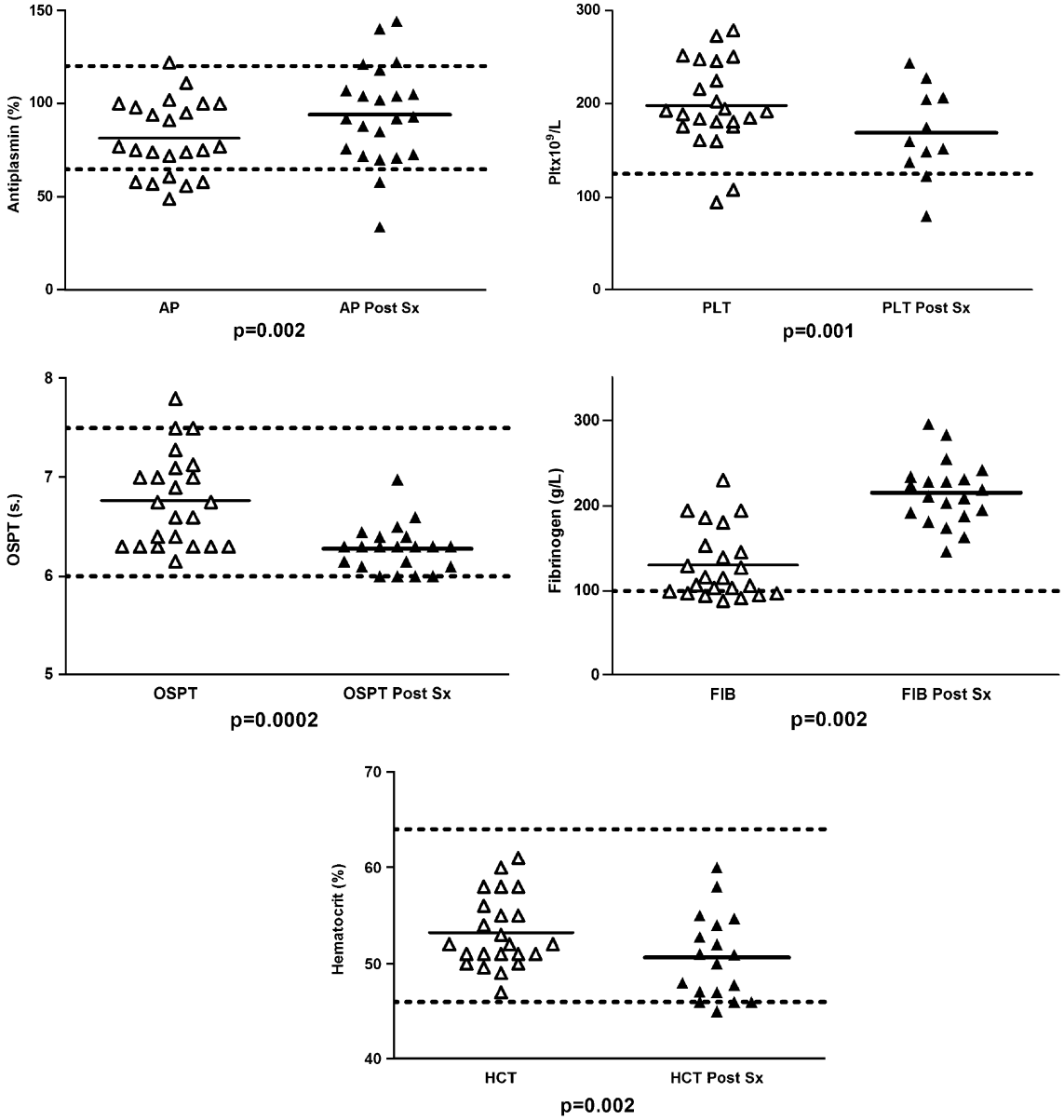

Fig 3. Scatter dot plots for the preoperative and postoperative values of the hemostatic parameters that differ significantly within the "bleeder" group (n = 23; $P < .05$). The dotted lines represent the reference range limits for a given parameter, and the solid line represents the mean of the group of values obtained for that parameter in the "bleeder" group before or after surgery.

Willebrand's syndrome (vWS), a depletion of high-MW vWF multimers secondary to high shear, has been described in humans with aortic valve mineralization, and recently in dogs with aortic and subaortic stenosis.[28] Interestingly, the prevalence of a left basilar systolic murmur in the "non-bleeder" group was significantly higher than in the "bleeder" group ($P = .013$). Type 2 vWS was ruled out because the vWF:Ag/vWF:CBA ratio was below 2. Because the decreases in vWF:Ag were moderate and the platelet function assays were normal in the "non-bleeder" group, it is possible that the "non-bleeder" group had lower vWF:CBA activity than the "bleeder" group owing to depletion of high-MW vWF multimers associated with the higher prevalence of a high-velocity aortic murmur.

Although vWF has always been seen as a platelet adhesion molecule that is involved in only the primary hemostatic process, recent evidence in humans has documented that plasma vWF:Ag is higher in hypertensive than in normotensive human patients.[29] Greyhounds have high arterial blood pressure compared with mixed breed dogs[30,31]; therefore, the vWF:Ag in this breed may be artificially increased because of endothelial cell damage secondary to hypertension. It is possible that the "bleeders" had higher vWF:Ag concentrations than the "non-bleeders" because they were hypertensive, and that the high blood pressure contributed to the delayed bleeding (ie, increased hydrostatic pressure dislodging the hemostatic plug). Although this study did not examine arterial blood pressure as a potential contributing factor

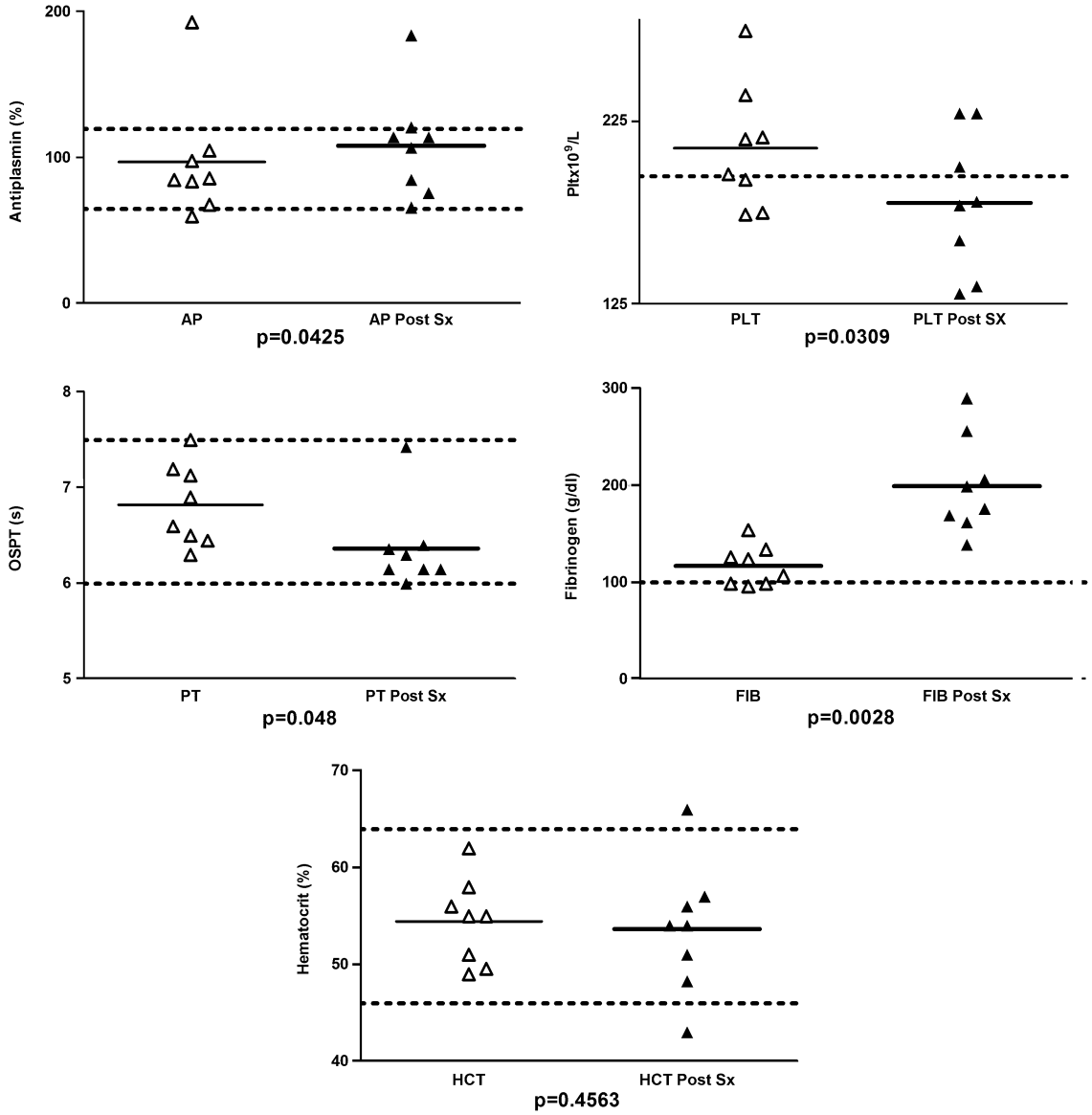

Fig 4. Scatter dot plots for the preoperative and postoperative values of the hemostatic parameters that differ significantly within the control group (n = 8; $P < .05$). The dotted lines represent the reference range limits for a given parameter, and the solid line represents the mean of the group of values obtained for that parameter in the "control" group before or after surgery.

to the bleeding, a prospective study evaluating this possibility is under way.

It is possible that the delayed onset of bleeding (ie, 36–48 hours) in the affected Greyhounds would be caused by enhanced fibrinolysis, because "bleeders" had lower AP than "non-bleeders" before surgery, suggesting activated fibrinolysis and, hence, a hypocoagulable state. D-dimer concentrations in the "bleeder" group were increased in only 3 dogs postoperatively, although theoretically D-dimer concentrations are expected to be high in patients with hyperfibrinolysis. Several studies in humans have shown that bleeding patients with increased fibrinolysis diagnosed by low plasminogen activator inhibitor type 1 (PAI-1) or short euglobulin lysis time (ELT) can have normal D-dimer concentrations, and that there was no correlation between D-dimer concentration and PAI-1 or ELT.[32,33] In this study, we did not measure tissue-plasminogen activator (tPA) or PAI-1, which are more specific fibrinolysis parameters whose results could have supported or ruled out this possible explanation for the postoperative bleeding in RRG. To our knowledge, assays for tPA in canine plasma have not been validated; we are currently evaluating an ELISA for PAI-1 in dogs (Marjory B Brooks, unpublished observations, Animal Health Diagnostic Laboratory, Cornell University, Ithaca, NY). At this moment, we are evaluating this assay in RRG plasma at our institution as a next step of the present study.

Aminocaproic acid is a prohemostatic agent that is beneficial not only in patients with hyperfibrinolysis but also in those with a variety of primary and secondary hemostatic defects.[34] Although the data in this study do

not confirm hyperfibrinolysis as the primary cause of postoperative bleeding in RRG, further clinical investigation of the role of perioperative administration of aminocaproic acid to decrease the prevalence or severity of this complication is warranted.

We propose that values for hemostatic assays in Greyhounds may differ from those of the general dog population, as it occurs with other clinicopathologic analytes in the breed,[1–5] and reference ranges for vWF:Ag, vWF:CBA, AT, AP in RRG should be generated.

In a previous study in dogs undergoing elective ovariohysterectomy, there were no differences in the hemostatic profiles before and after the procedure.[35] In our study, the hemostatic changes observed in both ''bleeder'' and control groups after surgery were identical, with the exception of the expected decrease in the hematocrit for the ''bleeders.'' The shortening in OSPT and the increase in fibrinogen and D-dimer concentrations observed postoperatively within the ''bleeder'' group and within the control group support the hypercoagulable state that has been widely described in humans after surgery attributable to release of TF intraoperatively.[36,37] The postoperative hypercoagulable state is most likely responsible for the increase in AP in the ''bleeders'' compared with their baseline values, supporting the fact that these dogs, regardless of the initial hemostatic profile, are capable of generating a compensatory response after surgical tissue trauma. This was clinically evident by the fact that the bleeding was self-limiting and the blood components were not required in any of the ''bleeders.'' This will also most likely explain the lack of differences in AP after surgery between the ''bleeders'' and the control group.

The low vWF:Ag, high AT, and high AP in RRG could be an adaptational mechanism to racing or an evolutionary trait designed to prevent clotting of blood with high viscosity (ie, Greyhounds have high hematocrit and whole blood viscosity) that circulates through large muscle masses, as it has been reported in human athletes.[38] A recent study in transgenic polycythemic mice with HCT of $\geq$85% reported thrombocytopenia, with platelet counts that did not differ from those in wild type mice when corrected for plasma volume, increased nitric oxide concentration, and hypocoagulability based on computerized thromboelastography and plasmatic coagulation activity.[39] It is very tempting to think that a similar phenomenon may occur in sight hounds and that they have evolved to be ''hypocoagulable'' in order to prevent intravascular thrombosis during strenuous exercise.

We did not evaluate the pedigrees of the ''bleeders'' and the ''non-bleeders''; it is possible that because racing Greyhounds are derived from a relatively small genetic pool, genetic disorders of clotting or fibrinolysis could be partially responsible for this disorder.

In conclusion, approximately 25% of RRG develop delayed postoperative bleeding 36–48 hours after a simple surgical procedure; this bleeding does not seem to be attributable to a primary or secondary hemostatic disorder. Our data suggest that enhanced fibrinolysis could play a role in the development of this complication. Further investigation is needed in order to determine its cause.

Footnotes

[a] PFA-100, Dade Behring, West Sacramento, CA
[b] Marin L, Couto CG, Iazbik MC, et al. Hemostatic complications after limb amputation in retired racing Greyhounds. J Vet Intern Med 2007;21:573 (abstract)
[c] Buprenorphine HCL, Bedford Laboratories, Bedford, OH
[d] Aceproject, Butler Animal Health Supply, Dublin, OH
[e] Cephazolin sodium, Sandoz Inc, Princetown, NJ
[f] Ketaset, Fort Dodge Animal Health, Fort Dodge, IA
[g] Diazepam, Hospira Inc, Lake Forest, IL
[h] Isosol, Vedco Inc, St Joseph, MO
[i] Rymadil, Pfizer Inc, New York City, NY
[j] Blue stopper tubes, Monoject, Sherwood, St Louis, MO
[k] Cell-Dyn 3500 R, Abbott Laboratories, Abbott Park, IL
[l] LaserCyte, IDEXX Laboratories, Westbrook, MD
[m] ACL-200, Instrumentation Laboratory, Lexington, MA
[n] IL Test APTT-C Activated Partial Thromboplastin Time and IL Test PT-Fibrinogen, Instrumentation Laboratory
[o] Stachrom AT III, Diagnostica Stago, Parsippany, NJ
[p] StaCompact, Diagnostica Stago
[q] StaCompact, Diagnostica Stago
[r] Stachrom Plasminogen, Diagnostica Stago
[s] Stachrom Antiplasmin, Diagnostica Stago
[t] Minutex D-dimer latex, Biopool, Wicklow, Ireland
[u] Prism version 4.0, GraphPad Software Inc, San Diego, CA
[v] Spectrolyse/pL PAI-1, DiaPharma, West Chester, OH

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