Successful ovariectomy in a dog with Glanzmann thrombasthenia

David J. Brdecka, DVM; Christopher A. Adin, DVM, DACVS; Mary K. Boudreaux, DVM, PhD; P. Cynda Crawford, DVM, PhD; Stacy R. Randall, DVM, DACVIM

- Glanzmann thrombasthenia is a rare, congenital bleeding disorder associated with a functional platelet defect caused by a mutation in the gene encoding for platelet glycoprotein subunit α_{IIb}.
- A combination of preoperative transfusion with functional platelets and use of techniques to minimize tissue trauma may allow abdominal surgery to be performed successfully in dogs with functional platelet disorders.

A 7-month-old 28-kg (62-lb) sexually intact female Great Pyrenees was referred to the University of Florida Veterinary Medical Teaching Hospital for neutering. The primary veterinarian had referred the dog for surgery because of concerns about an underlying bleeding disorder. At 5 months of age, the dog had developed excessive bleeding during eruption of the permanent dentition. Abdominal petechiae were also seen at that time. The dog had not had an estrous cycle.

Results of a CBC, serum biochemistry profile, and von Willebrand factor antigen assay (88%; reference range, 70% to 180%) were unremarkable, and platelet count (350,000/µL; reference range, 175,000 to 500,000/µL), prothrombin time (6.4 seconds; reference range, 6 to 12 seconds), and activated partial thromboplastin time (12.1 seconds; reference range, 10 to 25 seconds) were within reference limits. The **buccal mucosal bleeding time (BMBT)** was prolonged (4.5 minutes; reference range, 2 to 3 minutes). On the basis of signalment, history, and results of coagulation function tests, a presumptive diagnosis of **Glanzmann thrombasthenia (GT)** was made. Glanzmann thrombasthenia is a severe disorder of primary hemostasis caused by platelet dysfunction.

Results of a physical examination performed at the time of admission to the veterinary teaching hospital were unremarkable, other than ecchymoses and petechiae in the nonhaired regions of the ventral aspect of the abdomen. Results of a CBC were within reference limits, the platelet count was 190,000/µL (reference range, 160,000 to 430,000/µL), results of a heartworm test were negative, and the BUN concentration was normal (5 to 15 mg/dL). The BMBT was > 10 minutes.

Because the dog's bleeding disorder was suspected to be a result of platelet dysfunction, a decision was made to supplement the dog with functional platelets before surgery. The recommended dose of **platelet-rich plasma (PRP)** is 1 U/10 kg (1 U/4.5 lb).[1] Therefore, 4 units (225 mL each) of PRP were prepared from cross-match-compatible, dog erythrocyte antigen-4 donors. Crossmatching was performed because of the large number of RBCs in PRP and to ensure that compatible RBCs would be available in the event of substantial hemorrhage during the perioperative period.

Platelet-rich plasma was prepared by centrifuging fresh whole blood at 1,000 × *g* for 4 minutes at 23°C, as described.[2,3] To maximize platelet recovery, plasma and WBCs in the buffy coat were expressed into a satellite bag until RBCs entered the bag.[2] Quality-control procedures performed by the Clinical Pathology Service at the University of Florida Veterinary Medical Teaching Hospital on 16 units of canine PRP prepared with this protocol indicated a mean yield of 5.4 ± 1.0 × 10^{10} platelets/U of PRP (mean ± SD). This represented a recovery rate of 65% to 75% of the platelets in the donor blood sample, which was consistent with values reported previously for dogs.[3,4] Platelet, WBC, and RBC counts of PRP units prepared for the dog were not determined. Instead, the number of platelets in each unit of PRP was estimated on the basis of platelet counts of the donor dogs, assuming platelet recovery of 70% with this protocol.

Transfusion with PRP was initiated at a rate of 10 mL/kg/h (4.5 mL/lb/h). Fifteen minutes after administration of the second unit of PRP had begun, the dog had signs of a transfusion reaction characterized by facial edema, wheals, and an increase in rectal temperature from 38.4° to 39.7°C (101.2° to 103.5°F). The transfusion was immediately terminated, and diphenhydramine (1 mg/kg [0.5 mg/lb], IV, once) and methylprednisolone sodium succinate (10 mg/kg [4.5 mg/lb], IV, once) were administered. The dog's rectal temperature decreased to 38.9°C (102.0°F) within 1.5 hours, and transfusion with the second unit of PRP was resumed at a rate of 4 mL/kg/h (1.8 mL/lb/h). The dog received a total of 1,000 mL of PRP containing an estimated 2.6 × 10^{11} platelets.

After the PRP transfusion was completed, the dog was premedicated with hydromorphone (0.1 mg/kg [0.045 mg/lb], IV) and glycopyrrolate (0.01 mg/kg [0.005 mg/lb], SC) and anesthetized with diazepam (0.3 mg/kg [0.14 mg/lb], IV) and propofol (3 mg/kg [1.4 mg/lb], IV). An endotracheal tube was placed, and anesthesia was maintained with isoflurane.

High-frequency electrocoagulation was used to incise the skin, subcutaneous tissues, and linea alba. Each ovary was identified and isolated, and electrocoagulation was used to transect the suspensory ligament. Each ovarian pedicle was double ligated with

From the Department of Small Animal Clinical Sciences, Veterinary Medical Teaching Hospital, University of Florida, Gainesville, FL 32610-0126 (Brdecka, Adin, Crawford); the Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL 36849 (Boudreaux); and Affiliated Veterinary Specialists, 9905 S Highway 1792, Maitland, FL 32751 (Randall).
Address correspondence to Dr. Adin.

2-0 polydioxanone. The uterine horn and uterine artery and vein were double ligated with 2-0 polydioxanone and transected just caudal to the ovary, allowing complete ovariectomy. The abdomen was inspected for hemorrhage and closed routinely. Total blood loss was estimated to be 5 to 10 mL. Hydromorphone (0.05 mg/kg [0.023 mg/lb], IV, once at the time of extubation) and buprenorphine (0.01 mg/kg, IV, q 6 to 8 h) were given for analgesia. The dog was monitored in the intensive care unit for any signs of hemorrhage. Petechiae near the incision site and mild incisional bleeding were noted during recovery. The dog was released from the hospital the next day.

The diagnosis of GT was confirmed by isolation of genomic DNA from blood and amplification with a polymerase chain reaction assay of exon 13 and intron 13 of the gene encoding for platelet glycoprotein subunit α_{IIb}. The amplification product was visualized on an agarose gel and purified for sequencing, which confirmed the presence of a 14-base pair insertion near the end of exon 13. This insertion has previously been identified as the mutation responsible for GT in Great Pyrenees.[5]

Platelet adhesion and aggregation are the result of a complex and intricate system. For platelets to plug an endothelial defect, they must first adhere to the vascular subendothelium matrix proteins.[6] The mechanism by which this occurs, however, depends on whether the defect is in an area of low or high shear. In areas of low shear, platelet adherence involves direct interaction between the platelet and collagen, fibronectin, or laminin. In contrast, in areas of high shear, platelet adherence relies on the presence of von Willebrand factor.[6] Once platelets have adhered to the subendothelial matrix proteins, aggregation can begin. Aggregation is an active process that is initiated when an agonist, such as thrombin, binds to a platelet surface receptor associated with guanine nucleotide-binding, or G, protein.[6] Activation of G protein leads to a cascade of events in the platelet, ultimately resulting in exposure of the fibrinogen binding site on the platelet surface glycoprotein receptor GPIIb/IIIa ($\alpha_{IIb}\beta_3$).[6] In the presence of Ca^{2+} and fibrinogen, platelet aggregation occurs, resulting in the development of a platelet plug that stops hemorrhage.[6]

Glanzmann thrombasthenia is a rare bleeding disorder characterized by a defect in platelet aggregation.[7] It is an inherited, intrinsic platelet defect that involves the platelet surface glycoprotein receptor GPIIb/IIIa ($\alpha_{IIb}\beta_3$).[8] Affected individuals have normal platelet numbers and normal concentrations of von Willebrand factor; however, platelets from these individuals do not aggregate, even when exposed to specific agonists such as thrombin, collagen, and adenosine diphosphate.[9] In humans, 3 types of GT have been identified. Types I and II are quantitative defects in $\alpha_{IIb}\beta_3$ expression.[10] In individuals with type I GT, < 5% of the normal amount of $\alpha_{IIb}\beta_3$ is expressed on the platelet surface, severely inhibiting the ability of platelets to form platelet plugs. In individuals with type II GT, 10% to 20% of the normal amount of $\alpha_{IIb}\beta_3$ is expressed on the platelet surface, resulting in moderate to severe prolongation of platelet plug formation. Type III GT, in contrast, is characterized by a qualitative disorder in $\alpha_{IIb}\beta_3$.[10] Although normal amounts of $\alpha_{IIb}\beta_3$ are expressed on the platelet surface, its ability to bind with fibrinogen is impaired, resulting in severe impairment of platelet function.[10] Because both the α_{IIb} and β_3 protein subunits must be expressed on the platelet surface to form a functional and stable receptor, mutations in either of the genes encoding for these proteins can result in GT. More than 50 genetic mutations in the genes encoding for α_{IIb} and β_3 have been found in humans with GT.[11]

Glanzmann thrombasthenia in a Great Pyrenees was first described in 1996.[12] In that dog, platelet number and morphology were normal, but platelets did not aggregate in vitro when an agonist was introduced. Clot retraction was impaired, and electrophoretic analysis revealed that platelets had decreased amounts of the α_{IIb} and β_3 subunit proteins.[12] A subsequent study[5] found that the genetic basis for GT in Great Pyrenees is a 14-base pair insertion near the end of exon 13 in the gene encoding for α_{IIb}. This insertion results in a frameshift as well as impairment of splicing of intron 13.[5] As of yet, no mutations in the gene encoding for β_3 have been identified in dogs with GT. The mutation causing type I GT in Otterhounds has been localized to the gene encoding for α_{IIb}.[13] In this instance, the mutation is a single nucleotide change in exon 12 that results in substitution of histidine for aspartic acid in a calcium-binding domain.[13]

Numerous blood products are available for the prophylactic treatment of dogs with platelet disorders undergoing elective surgical procedures, including fresh whole blood, PRP, and platelet concentrates.[1] Fresh whole blood for transfusion is readily available to most veterinary practitioners and has the advantage of providing both RBCs and platelets to dogs that have had substantial blood loss. However, dogs with thrombocytopathies that are undergoing elective surgical procedures generally do not require RBC replacement prior to surgery unless there is ongoing hemorrhage.[14] In addition, administration of unnecessary blood components can result in transfusion reactions and volume overload. Some large veterinary referral centers have access to the equipment required for preparation of PRP from whole blood, and transfusion with PRP provides platelets with optimal viability and function while reducing the potential for volume overload.[1,11] However, RBCs, WBCs, and plasma in PRP contribute to the risk of adverse reactions.[1,15] Platelet concentrates prepared from PRP contain large numbers of fresh platelets in small volumes.[4] The paucity of RBCs and plasma in platelet concentrates means that there is minimal risk of adverse reactions.[15] Frozen platelet concentrates prepared by use of apheresis[a] contain the highest numbers of platelets and the least amount of other components, but the function of platelets that have been frozen is controversial.[16] In the dog described in the present report, PRP was selected for platelet supplementation because of its availability and the financial and functional concerns associated with the use of frozen platelet concentrates. Even though crossmatching was done, the dog developed an acute hypersensitivity transfusion reaction characterized by fever, facial edema, and wheals. This reaction most

likely resulted from exposure to donor platelet and WBC alloantigens or to IgE in the donor plasma.[15,17]

Indications for performing elective ovariectomy in the dog described in the present report included the desire to avoid transfer of a genetic defect, the decreased risk of mammary neoplasia and pyometra, and the desire to avoid excessive vaginal bleeding during estrus.[18] Ovariectomy was performed rather than ovariohysterectomy because ovariectomy results in sterilization and decreases the incidence of hormone-related conditions, such as pyometra and mammary neoplasia, but requires minimal surgical exposure and manipulation of the urogenital tract.[19] The authors believe that minimizing surgical trauma through performing ovariectomy, rather than ovariohysterectomy, combined with the use of high-frequency electrocoagulation and the preoperative PRP transfusion resulted in the successful outcome in this dog.

To our knowledge, this is the first report of successful abdominal surgery in a dog with GT, although removal of a cutaneous mass from the antebrachium of an Otterhound with presumed GT has been described.[20] As was the case for the dog described in the present report, the Otterhound received a transfusion of PRP prior to surgery. In addition, a pressure bandage was applied after surgery, which may have assisted with perioperative hemostasis and decreased the risk of complications.

[a]Midwest Animal Blood Services Inc, Stockbridge, Mich.

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