Recurrent episodes of severe bleeding caused by congenital factor XIII deficiency in a dog

Lyndsay R. Kong, DVM; Elisabeth C. R. Sneed, DVM, MS; Hilary Burgess, DVM, DVS; Marc P. Dhumeaux, DEDV, MVS

**Case Description**—A 5-year-old castrated male Toy Poodle cross was evaluated at the Veterinary Medical Centre at the Western College of Veterinary Medicine on an emergency basis because of lethargy and decreased appetite of 4 days’ duration and suspected abdominal hemorrhage. The dog had been evaluated on 4 other occasions for episodes of excessive bleeding associated with trauma or surgical procedures.

**Clinical Findings**—At previous evaluations, results of repeated measurements of prothrombin time, partial thromboplastin time, and buccal mucosal bleeding time were unremarkable; activated clotting time, plasma von Willebrand factor concentration, results of platelet function testing, and plasma factor VII, VIII, IX, X, XI, and XII concentrations were considered normal. At this evaluation, clinicopathologic analyses revealed mild regenerative anemia that progressed over a 4-day period to moderate regenerative anemia and acute inflammation with panhypoproteinemia. Abdominal ultrasonography revealed a large mass (suspected to be a hematoma) near the urinary bladder. Rotational thromboelastometry revealed that clotting times were within reference limits, with abnormal clot formation times and clot firmness. The result of a factor XIII (FXIII) clot solubility assay confirmed FXIII deficiency.

**Treatment and Outcome**—The dog’s bleeding diathesis resolved with inpatient care and IV fluid therapy, although plasma transfusions had been required at previous evaluations. Seven months after discharge from the hospital, the dog continued to do well clinically, although it had several additional episodes of excessive bleeding.

**Clinical Relevance**—To the authors’ knowledge, this is the first reported case of congenital FXIII deficiency in a dog. In addition to more common inherited coagulopathies, FXIII deficiency should be a differential diagnosis for dogs with episodes of excessive bleeding and apparently normal results of standard coagulation tests. (J Am Vet Med Assoc 2014;245:1147–1152)

---

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPTT</td>
<td>Activated partial thromboplastin time</td>
</tr>
<tr>
<td>CFT</td>
<td>Clot formation time</td>
</tr>
<tr>
<td>FXIII</td>
<td>Factor XIII</td>
</tr>
<tr>
<td>MCF</td>
<td>Maximum clot firmness</td>
</tr>
<tr>
<td>PT</td>
<td>Prothrombin time</td>
</tr>
</tbody>
</table>

[reference range, 4.80 × 10^9 WBCs/L to 13.9 × 10^9 WBCs/L]; neutrophil count, 16.796 × 10^9 neutrophils/L [reference range, 3.0 × 10^9 neutrophils/L to 10 × 10^9 neutrophils/L]; and band neutrophil count, 2.431 × 10^9 band neutrophils/L [reference range, 0.0 × 10^9 band neutrophils/L to 0.1 × 10^9 band neutrophils/L]; slight toxic change detected). Panhypoproteinemia (total protein concentration, 44 g/L [reference range, 55 to 71 g/L]; albumin concentration, 29 g/L [reference range, 32 to 42 g/L]; and globulin concentration, 15 g/L [reference range, 20 to 34 g/L]) was identified via serum biochemical analysis. The dog subsequently developed hematuria and was referred to the Veterinary Medical Centre for a possible blood transfusion.

The dog had no history of trauma prior to evaluation by the referring veterinarian on this occasion, but pertinent past medical history included several episodes of excessive bleeding associated with trauma or surgical procedures. At 6 months of age, scrotal hemorrhage occurred after the dog was neutered. The surgical...
incision was reopened multiple times over the following 5 days to remove blood clots, and a blood transfusion was required 6 days after neutering. At that time, the dog’s platelet count was estimated as normal (automated count, $111 \times 10^9$ platelets/L; reference range, $200 \times 10^9$ platelets/L to $900 \times 10^9$ platelets/L) with platelet clumps on blood smear examination. Plasma concentration of von Willebrand factor antigen was within reference range (76%; reference range, 49% to 180%). The PT was not prolonged (7.1 seconds; reference range, 7.5 to 9.9 seconds), and the aPTT, although mildly prolonged (13.9 seconds; reference range, 9.6 to 13.8 seconds), was not deemed sufficiently high to be responsible for the amount of hemorrhage seen.

At 9 months of age, the dog was evaluated by the Veterinary Medical Centre ophthalmology service; a presumptive diagnosis of congenital glaucoma that was nonresponsive to medical management necessitated left eye enucleation. Results of preoperative hematologic and serum biochemical analysis and buccal mucosal bleeding time (<4 minutes; reference range, 1.7 to 4.2 minutes) were apparently normal. The dog underwent blood typing and cross-matching prior to surgery. Minimal hemorrhage occurred during surgery, but moderate oozing of blood was noted from the enucleation incision after surgery. The dog’s PCV was 41% (reference range, 36.5% to 57.3%). Given that its condition appeared stable, the dog was discharged from the hospital.

However, the dog was reevaluated that evening because of excessive bleeding from the surgical site. The PT (7.7 seconds), aPTT (13.3 seconds), and activated clotting time (<120 seconds; reference range, 60 to 125 seconds) were all within reference limits. The dog’s PCV had decreased to 25%; hence, the dog was administered diphenhydramine (2 mg/kg [0.9 mg/lb], IM) and a transfusion of stored whole blood (110 mL). Histologic examination of the enucleated eye confirmed congenital glaucoma. Two weeks after the enucleation, the dog was evaluated at a private veterinary clinic because of signs of pain on opening its mouth and signs of depression; palpation of the left side of the face elicited signs of pain. The dog was sedated, and an examination of the oral cavity was performed. The examination revealed a swelling at the left caudal aspect of the mouth, which, on aspiration, was found to contain blood. The dog was again referred to the Veterinary Medical Centre, but no treatment was given because the mass was resolving.

The mass was attributed to hemorrhage secondary to a hitherto undiagnosed coagulopathy. A platelet function test was performed, and results were considered normal (96 seconds; reference range, 48 to 105 seconds).

At 2 years of age, the dog was evaluated at another private veterinary clinic because of a bite wound to its right ear that developed into a severe aural hematoma. During the 3 days preceding referral, the dog’s PCV had decreased from 45.3% to 29.2%, and its platelet count decreased from $275 \times 10^9$ platelets/L to $48 \times 10^9$ platelets/L (reference range, $200 \times 10^9$ platelets/L to $900 \times 10^9$ platelets/L). The PT and aPTT were again within reference ranges. The hematoma eventually resolved with repeated application of pressure bandages over a 4-day period of hospitalization. Although FXIII deficiency testing was recommended at this time, the owners failed to bring the dog back for the necessary follow-up.

At the current evaluation, the dog was lethargic, and signs of pain were elicited during abdominal palpation. There were mild areas of ecchymotic hemorrhage on the right side of the abdominal wall. Abdominal ultrasonography revealed subcutaneous edema, moderate peritoneal effusion, and a hypoechoic mass near the urinary bladder. The dog was hospitalized. During a period of 4 days, the dog’s initial mild regenerative anemia progressed to moderate regenerative anemia (RBC count, $3.8 \times 10^12$ RBCs/L; Hct, 0.259 L/L; and proportion of reticulocytes, 6.1%). The platelet count, PT, aPTT, and buccal mucosal bleeding time were again within reference ranges. To verify the findings of the screening test at our laboratory and to rule out a mild factor deficiency not detected by the PT and aPTT testing, screening coagulation tests were repeated, and specific factor assay testing was performed through the Cornell University Animal Health Diagnostic Center. These analyses revealed no abnormalities, as follows: aPTT, 12.1 seconds (reference range, 10 to 17 seconds); PT, 14.6 seconds (reference range, 11 to 16 seconds); thrombin clotting time, 6.0 seconds (reference range, 5 to 9 seconds); plasma von Willebrand factor antigen concentration, 143% (reference range, 70% to 180%); plasma factor VII concentration, 118% (reference range, 50% to 150%); plasma factor VIII concentration, 87.5% (reference range, 50% to 200%); plasma factor IX concentration, 86% (reference range, 50% to 150%); plasma factor X concentration, 87% (reference range, 80% to 175%); plasma factor XI concentration, 97% (reference range, 60% to 150%); and plasma factor XII concentration, 92% (reference range, 60% to 150%). Given the undefined nature of the dog’s hemostatic defect, the owners were told to expect further bleeding episodes following any type of trauma and that administration of fresh frozen plasma would be necessary prior to any surgical procedure. Differential diagnoses for the dog’s bleeding disorder included a deficiency in antifibrinolytic proteins, a rare platelet function defect (eg, Scott syndrome), or a deficiency in FXIII; however, the owners declined further investigation.

At 3 years of age, the dog was again referred to the Veterinary Medical Centre because of a bite wound to its right ear that developed into a severe aural hematoma. During the 3 days preceding referral, the dog’s PCV had decreased from 45.3% to 29.2%, and its platelet count decreased from $275 \times 10^9$ platelets/L to $48 \times 10^9$ platelets/L (reference range, $200 \times 10^9$ platelets/L to $900 \times 10^9$ platelets/L). The PT and aPTT were again within reference ranges. The hematoma eventually resolved with repeated application of pressure bandages over a 4-day period of hospitalization. Although FXIII deficiency testing was recommended at this time, the owners failed to bring the dog back for the necessary follow-up.
the cause of the dog’s underlying coagulopathy. Thromboelastometry is an in vitro diagnostic technique that can continuously record changes in the viscoelastic properties of whole blood during clotting and fibrinolysis. It has clinical applications for diagnosis of hypercoagulable states, platelet function disorders, and defects in fibrin formation and fibrinolysis via a series of assays: an extrinsic pathway assay with tissue factor reagent (Figure 1), an intrinsic pathway assay with ellagic acid reagent (Figure 2), and a fibrinolysis assay with cytochalasin D in DMSO solution and 0.2M CaCl₂ in HEPES buffer (pH, 7.4; Figure 3). Although the dog had normal clotting times (measured in seconds from clot initiation to a clot amplitude of 2 mm) for thromboelastometry assays evaluating the extrinsic (43 seconds; reference range, 29 to 75 seconds) and intrinsic (139 seconds; reference range, 129 to 200 seconds) pathways, CFT (measured in seconds from a clot amplitude of 2 to 20 mm) could not be determined in the extrinsic pathway assay (reference range, 66 to 186 seconds) because the clot did not reach an amplitude of 20 mm and was delayed in the intrinsic pathway assay (744 seconds; reference range, 48 to 237 seconds). The MCF (maximum clot amplitude) was decreased for all assays (extrinsic pathway assay, 19 mm [reference range, 46 to 63 mm]; intrinsic pathway assay, 22 mm [reference range, 45 to 64 mm]; and fibrinolysis assay, 4 mm [reference range, 6 to 26 mm]). Assessment of the rotational thromboelastometry tracings revealed a small clot with a decreased slope of the line delimiting the \( \alpha \) angle, indicating slow clot formation.

A citrated blood sample from this dog along with a sample from an age- and breed-matched clinically normal dog was sent to Regina General Hospital to undergo an FXIII clot solubility assay. The clot solubility assay determines the interval to lysis of a stabilized clot after addition of either a 1% monochloroacetic acid or 5M urea solution; in blood samples obtained from individuals with an FXIII deficiency, clot lysis time is shortened (< 24 hours) owing to poor cross-linking of the fibrin clot. Results of this test were abnormal (clot dissolution occurred within 24 hours following incubation) for the sample from the dog of this report and were normal (the clot remained stable after 24 hours of incubation) for the sample from the control dog.

The dog was treated IV with isotonic electrolyte solution supplemented with...
20 mEq of KCl/L (26 mL/h) from the day of admission until discharge. The dog was discharged from the hospital 5 days after evaluation. The owners were instructed to return the dog for follow-up ultrasonography in 1 month.

Two months later, the dog was evaluated by its regular veterinarian for routine vaccination. Findings of follow-up ultrasonography of the abdomen performed by the referring veterinarian at that time were unremarkable. Following vaccination, the dog developed a large subcutaneous hematoma leading to non-weight-bearing lameness, which subsequently resolved. Five months after vaccination, the dog jumped out of a car, resulting in severe abdominal hemorrhage that limited its ability to walk; ambulation was assisted with sling support, and the dog did not require hospitalization. The injury resolved. At the time of the injury, hematologic analysis revealed moderate anemia (RBC, 3.47 × 10¹² RBCs/L; reference range, 5.50 × 10¹² RBCs/L to 8.50 × 10¹² RBCs/L; Hct, 0.223 L/L; reference range, 0.370 to 0.550 L/L). An ultrasonographic examination performed a week later confirmed resolution of the bleeding.

Discussion

Factor XIII deficiency is a coagulopathy that is rare in people and, to the authors' knowledge, has not been previously described for domestic animals. Factor XIII is involved in stabilizing the initial fibrin clot formed during secondary hemostasis. Clinical signs of congenital FXIII deficiency in people include umbilical stump bleedings, hemarthrosis, poor wound healing; bleeding after surgery, minor trauma, or strenuous exercise; rebleeding; hemarthrosis; poor wound healing; bleeding resulting from a congenital FXIII deficiency in humans has been reported, and knockout mice have been bred specifically for an FXIII deficiency. In people, wound healing occurs extremely slowly; thus, wounds continue to bleed for a prolonged period (as long as several weeks) despite surgical intervention or compressive bandages, similar to the clinical features of the case described in this report. Diagnosis of FXIII deficiency is challenging because the most commonly used results of screening tests for primary and secondary hemostatic diatheses are expected to be normal. Tests for secondary hemostasis (PT and aPTT) detect only the appearance of polymerized fibrin but not its stabilization by FXIII. In the dog of this report, the repeatedly unremarkable PT, aPTT, and thrombin clotting time made abnormalities in factors I, II, V, VII, VIII, IX, X, XI, XII, prekallikrein, and high-molecular-weight kininogen unlikely, although a mild factor deficiency could not be conclusively ruled out well-known coagulopathies early, leaving only a few remaining possible differential diagnoses (rare platelet function defect; a deficiency in plasminogen activator inhibitor-1, α₂-antiplasmin, or thrombin-activated fibrinolysis inhibitor [the latter leading to a hyperfibrinolytic state]; and an FXIII deficiency). A thrombopathy was less likely on the basis of the dog's apparently normal result on platelet function testing, which has a high reported sensitivity (95.7%) and specificity (100%) with use of the ADP-collagen cartridge for detection of a platelet function defect in dogs; however, because the platelet function test may not consistently detect milder forms of platelet dysfunction and certain platelet disorders (storage pool disease or Scott syndrome), a platelet function abnormality was not definitively ruled out on the basis of this result. The platelet function test can also be used to detect abnormalities in von Willebrand factor function; normal test results for this dog made a von Willebrand factor dysfunction unlikely. Rotational thromboelastometry findings also conclusively ruled out both a thrombocytothria and a fibrinolytic system abnormality, and the clot solubility test confirmed the dog's FXIII deficiency.

On the basis of a search of the veterinary medical literature, this is the first reported case of congenital FXIII deficiency in a dog, to the authors' knowledge. Congenital FXIII deficiency in humans has been reported, and knockout mice have been bred specifically for an FXIII deficiency. In people, it is considered a rare coagulation disorder that has a much lower incidence than either hemophilia A or B and most commonly results from a deficiency in the A subunits (type 2 defect), although it can also result from a lack of B subunits (type 1 defect). Patients with congenital FXIII deficiency have a severe bleeding diathesis, typically apparent after trauma. Bleeding after a traumatic event will often be delayed because the blood clots formed are loose and therefore contribute to rebleeding when not stabilized. This diathesis and delay in onset of bleeding were evident in the dog described in the present report, wherein all but 1 incidence of abnormal bleeding were subsequent to surgical or accidental trauma and bleeding was often delayed following surgical procedures. In addition to its hemostatic role, FXIII is also important for maintenance of pregnancy, for wound healing and tissue repair, and for angiogenesis. Recurrent miscarriages are common in affected women. In affected people, wound healing occurs extremely slowly; thus, wounds continue to bleed for a prolonged period (as long as several weeks) despite surgical intervention or compressive bandages, similar to the clinical features of the case described in this report.

Diagnosis of FXIII deficiency is challenging because the most commonly used results of screening tests for primary and secondary hemostatic diatheses are expected to be normal. Tests for secondary hemostasis (PT and aPTT) detect only the appearance of polymerized fibrin but not its stabilization by FXIII. In the dog of this report, the repeatedly unremarkable PT, aPTT, and thrombin clotting time made abnormalities in factors I, II, V, VII, VIII, IX, X, XI, XII, prekallikrein, and high-molecular-weight kininogen unlikely, although a mild factor deficiency could not be conclusively ruled out.
out prior to specific factor testing. Although PT and aPTT are reliably used to detect severe deficiencies in clotting factors associated with secondary hemostasis, both tests have relatively low sensitivity (30%) for detection of mild factor deficiencies, and such sensitivities can vary depending on the deficient factor and the reagent used in the tests.19,20 Possible deficiencies in factors VII through XII were conclusively ruled out through factor testing for the dog of this report. In addition, patients with an FXIII deficiency will have a platelet count, platelet function test results, and plasma von Willebrand factor antigen concentration that are all within reference limits, as seen in the dog of this report, thereby ruling out common primary hemostatic disorders. Although the dog was thrombocytopenic during 2 episodes of bleeding, this was likely a result of platelet consumption during excessive hemorrhage. Platelet count was within reference range at most evaluations, ruling out thrombocytopenia as a cause of the dog’s excessive bleeding.

A clot solubility test has been traditionally used to diagnose an FXIII deficiency in people.2 For this test, a plasma sample from an affected patient is incubated with a buffer of calcium and thrombin to promote clot stabilization. The clot is then suspended in a freshly prepared solution of either 1% monochloroacetic acid or a 5M urea solution and left undisturbed at 37°C for 24 hours. In a patient with normal FXIII concentration, the clot remains stable after 24 hours. Clot lysis occurs rapidly (typically within hours) when an FXIII deficiency exists because the fibrin clot formed is poorly cross-linked and dissolves more quickly.6 Controls (blood samples from apparently normal individuals) must also be run when the test is performed, and considering that the test is manual, it must be performed in duplicate. Owing to the semiquantitative nature of this assay, only severe FXIII deficiencies (activity of FXIII, < 1% of activity in a clinically normal individual) may be detected, which can delay diagnoses in some humans with less severe deficiencies.8 Even the addition of a small amount of plasma from a healthy individual to the test system will elevate FXIII activity to 1% to 3% of that expected in clinically normal individuals and render the clot insoluble. Hence, this test should not be run if plasma has been administered to the patient within the preceding few weeks. Despite this shortcoming, the clot solubility test is still used by many laboratories for the diagnosis of an FXIII deficiency in humans because of its simplicity and the lack of readily available and more quantitative diagnostic tests. Confirmation of the diagnosis, especially for mild or moderate deficiencies of FXIII, is ideally provided by use of a more quantitative test that measures FXIII activity or antigen concentration.8 As part of the diagnosis of FXIII deficiency, PCR testing has been used in to identify the specific type of mutation (A or B subunit) responsible. Because there are no dog-specific assays for determining plasma FXIII activity or antigen concentration, the clot solubility test was used to establish the diagnosis for the dog of this report. Testing for a specific subunit deficiency in this dog was not done for financial and practical reasons.

Results of rotational thromboelastometry proved useful in helping to support the diagnosis of an FXIII deficiency in the case described in this report because the data can reflect abnormalities with fibrin formation and cross-linking. Thromboelastography incorporates different assays that evaluate the intrinsic or contact pathway (analogous to aPTT assessment), the extrinsic or tissue factor pathway (analogous to PT assessment), and fibrinolysis (by irreversibly inhibiting platelets with cytochalasin D to eliminate the platelet contribution to clot formation).21 Thromboelastometry, a modification of thromboelastography, was used to test the patient in this report. Clotting time is primarily a function of coagulation factor concentrations, whereas CFT and MCF rely more on platelet function and fibrin formation and cross-linking.22 Normal clotting time, with delayed CFT and decreased MCF, as detected in the dog of this report, was consistent with either an FXIII deficiency or platelet abnormality. Thrombocytopenia at the time of testing could have influenced these results by falsely increasing CFT and decreasing MCF as determined by the intrinsic and extrinsic pathway assays; however, the fibrinolysis assay removes platelet contribution to clotting, and concurrent abnormal MCF results determined by the fibrinolysis assay ruled out thrombocytopenia (along with other platelet abnormalities) at the time of testing as a sole cause for abnormal CFT and MCF revealed by the intrinsic and extrinsic pathway assays. Thromboelastography has been validated as a specific and sensitive test for detection of FXIII deficiency in people.23 Thromboelastometry, as a modification of thromboelastography, is assumed to have similar sensitivity and specificity, and it was the only test other than the clot solubility test that yielded abnormal findings in this dog; therefore, thromboelastometry should be considered a useful diagnostic tool for investigating a coagulopathy where the underlying cause is not evident on the basis of standard screening test results.

Treatment of humans with FXIII deficiency traditionally consists of cryoprecipitate or fresh frozen plasma transfusions administered every 4 to 6 weeks. More recently, a new treatment that uses plasma-derived pasteurized FXIII concentrate has been available.9 The only treatment option currently available for veterinary patients would be frequent administration of either cryoprecipitate or fresh frozen plasma given the lack of species-specific FXIII concentrate. Prevention of bleeding by means of intermittent administration of cryoprecipitate has been attempted in humans,8 but this may not be financially viable for many veterinary clients. Gene therapy has been explored as a possible treatment option in dogs with hemophilia B (factor IX deficiency)24 or hemophilia A (factor VIII deficiency) and may represent an area for future research. In the case described in this report, treatment included advising the owners to minimize or prevent any traumatic events and providing transfusions to stabilize the dog after episodes of bleeding.

Factor XIII deficiency may be considered as a differential diagnosis in dogs with a coagulopathy that is not detected by standard coagulation screening tests. A thorough evaluation for more common inherited coagulopathies (von Willebrand disease, hemophilia A, and hemophilia B) should be performed before considering FXIII testing. Platelet function testing and rotational thromboelastometry may be helpful diagnostic...
tools, but specific testing for an FXIII deficiency in dogs currently relies on an abnormal clot solubility test. No highly practical or affordable treatment options are currently available for veterinary patients with FXIII deficiency, but prophylactic treatment with fresh frozen plasma transfusions should be provided before any elective or emergency surgical procedure, and owners should be warned that bleeding after trauma, even minor trauma associated with vaccination, is possible.

References


