Factor X deficiency in a cat

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- Factor X is a vitamin K-dependent glycoprotein that participates in the common pathway of blood coagulation.
- Factor X deficiency should be considered in cats with clinical evidence of hemostatic dysfunction, prolonged coagulation times, and lack of response to administration of vitamin K_1.
- Spurious coagulation times may be recorded by photo-optical clot detection instruments; therefore, identification of abnormal coagulation times may require an alternate method of clot detection (eg, visual) or specific coagulation factor analysis.
- Definitive diagnosis of factor X deficiency requires analysis of individual coagulation factor activities.

A 3-year-old castrated male domestic shorthair cat was referred to the veterinary teaching hospital because of acute onset of generalized seizures and prolonged bleeding from venipuncture sites. Administration of diazepam by the referring veterinarian (cumulative dose, 3.5 mg/kg [1.6 mg/lb] of body weight, IV) had not altered seizure activity. The history did not include episodes of spontaneous hemorrhage. The cat was castrated and declawed at 5 months of age without perioperative complications but was reexamined 5 days after surgery because of continued bleeding from 1 of the digits.

Episodes of ptyalism and facial twitching followed by lateral recumbency and hind limb paddling were observed during physical examination at admission. Asymmetric neurologic deficits were not identified. Extensive forelimb bruising and prolonged bleeding after jugular venipuncture were also observed. Results of a CBC, serum biochemical analyses, and urinalysis were within reference limits. Serologic test results for FeLV, feline immunodeficiency virus, feline infectious peritonitis, and *Toxoplasma gondii* were negative. Markedly prolonged **prothrombin time** (**PT**; > 60 seconds; reference range, 8.7 to 10.7 seconds) and **activated partial thromboplastin time** (**aPTT**; > 100 seconds; reference range, 11.8 to 16.4 seconds) were identified. Platelet count (349 × 10^3 platelets/µl) and fibrinogen (200 mg/dl) and fibrin degradation product (< 10 µg/ml) concentrations were within the laboratory's reference ranges of 300 to 800 × 10^3 platelets/µl, 50 to 300 mg of fibrinogen/dl, and < 10 µg of fibrin degradation products/ml, respectively.

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Seizure activity resolved after treatment with phenobarbital (7 mg/kg [3.2 mg/lb], IV, loading dose and 2 mg/kg [0.9 mg/lb], IV, after each of 3 additional seizures), diazepam (1 mg/kg [0.45 mg/lb], IV, as needed), and methylprednisolone sodium succinate (30 mg/kg [13.6 mg/lb], IV, q 24 h). Because results of a coagulation profile were consistent with vitamin K deficiency or antagonism, treatment with vitamin K_1[a] (1 mg/kg, IM, q 8 h) was initiated. Results of a second coagulation profile performed 24 hours later were within reference ranges (PT, 8.6 seconds; aPTT, 13.5 seconds). Rodenticide toxicity was suspected; however, the cat's history did not include exposure and it was kept strictly indoors. Treatment with vitamin K_1[b] (5 mg/kg [2.3 mg/lb], PO, q 12 h) was continued for 2 months from the time of initial examination. Anticonvulsant medication was not prescribed.

On 4 occasions during a 9-month period after initial examination, slight prolongation of PT without concurrent bleeding from the venipuncture site was detected. At the end of this 9-month period, the cat was returned for examination because of recurrence of generalized seizures and prolonged bleeding after venipuncture. Results of a CBC, serum biochemical analyses (including pre- and postprandial bile acid concentrations), and coagulation profile were all within reference ranges. Treatment with phenobarbital (1.5 mg/kg [0.7 mg/lb], PO, q 12 h) was initiated.

The cat was reexamined 3 weeks later because of bilateral supraorbital ecchymotic hemorrhages and petechiae of the oral mucous membranes. Seizure activity had not been observed. Diagnostic evaluation included a CBC, serum biochemical analyses, coagulation profile, buccal mucosal bleeding time test, thoracic radiography, and abdominal ultrasonography. Abnormalities identified were thrombocytopenia (47 × 10^3 platelets/µl; reference range, 300 to 800 × 10^3 platelets/µl), slightly prolonged aPTT (16.5 seconds), and a small amount of subcapsular fluid around the right kidney. Immune-mediated thrombocytopenia, possibly secondary to phenobarbital administration, was suspected, and treatment with prednisone (1 mg/kg [0.45 mg/lb], PO, q 12 h) was begun. The owner was instructed to taper phenobarbital (25% reduction, q 3 wk) and administer clonazepam[c] (0.05 mg/kg, [0.023 mg/lb], PO, q 12 h) for seizure control. During the next 4 months, generalized seizures occurred despite treatment with prednisone and clonazepam, using various dosage regimens. Thrombocytopenia (range, 40 to 120 × 10^3 platelets/µl) and prolonged coagulation times were detected on 4 occasions during this period.

Because of poor seizure control and a suspected congenital coagulation disorder, the cat was returned to the teaching hospital 14 months after initial examination. At reexamination, medication included clonazepam (0.05 mg/kg [0.23 mg/lb], PO, q 24 h) and

Table 1—Results of coagulation studies for a factor X deficient cat and its relatives

	Assays											
	Coagulation screening tests				Coagulation factor analyses							
	aPTT (s)	PT (s)	TCT (s)	RVVT (s)	vWF:Ag (%)	fII (%)	fVII (%)	fVIII (%)	fIX (%)	fX (%)	fXI (%)	fXII (%)
Control cat	16.0	18.0	8.0	16.0	NT	110	NT	77	82	100	87	NT
Patient	> 60	> 60	8.0	30.0	88	110	81	84	58	2	40	73
Dam of patient	32.0	NT	NT	23.0	NT	NT	NT	NT	NT	63	NT	NT
Sibling 1	15.0	NT	NT	16.0	NT	NT	NT	NT	NT	80	NT	NT
Sibling 2	18.0	NT	NT	15.0	NT	NT	NT	NT	NT	73	NT	NT
Sibling 3	18.0	NT	NT	16.0	NT	NT	NT	NT	NT	89	NT	NT
Pooled feline plasma	16.0	19.0	8.0	15.0	100	100	100	100	100	100	100	100
Reference range	14.0–18.0	14.0–20.0	5.0–8.0	11.0–19.0	75–180	> 50	> 50	> 50	> 50	> 80	> 60	> 60

aPTT = activated partial thromboplastin time; PT = prothrombin time; TCT = thrombin clotting time; RVVT = Russell's viper venom time; vWF:Ag = von Willebrand factor antigen concentration; fII = factor II coagulant activity; fVII = factor VII coagulant activity; fVIII = factor VIII coagulant activity; fIX = factor IX coagulant activity; fX = factor X coagulant activity; fXI = factor XI coagulant activity; fXII = factor XII coagulant activity; NT = not tested.

prednisone (3 mg/kg [1.4 mg/lb], PO, q 48 h). Phenobarbital (1.5 mg/kg [0.7 mg/lb], PO, q 12 h) had also been given during the previous 2 weeks. For coagulation screening tests, determination of plasma von Willebrand factor concentration, and specific coagulation factor analyses,[d] blood was collected by jugular venipuncture into a one-ninth volume of 3.8% sodium citrate and centrifuged at 1,286 × g for 10 minutes. The plasma was separated immediately and submitted frozen on dry ice. Plasma from a clinically normal cat, processed identically to plasma from the patient, was submitted as a control for sample handling.

Coagulation screening tests included aPTT,[1,e] PT,[1,f] thrombin clotting time,[1,g] and **Russell's viper venom time (RVVT).**[2] The RVVT assay was performed, using a commercial reagent[e] (diluted 1:8 in imidazole-buffered 0.9% NaCl solution) as a source of phospholipid and Russell's viper venom reagent[h] diluted 1:100,000 in imidazole-buffered 0.9% NaCl solution. A pooled plasma sample prepared from 22 healthy cats was assayed simultaneously with submitted samples.

Plasma von Willebrand factor concentration was measured in an ELISA[3] and reported as percent von Willebrand factor:antigen of the pooled feline plasma (assigned value, 100% von Willebrand factor:antigen). Functional coagulation factor assays were performed to specifically evaluate activities of factors II, VII, VIII, IX, X, XI, and XII. Coagulant activities for the intrinsic system factors (VIII, IX, XI, XII) were assayed, using a modified one-stage aPTT technique[4,i] with a series of congenitally deficient animal plasmas as follows: canine substrate (factors VIII, IX), bovine substrate (factor XI), and feline substrate (factor XII). Activities of factors II, VII, and X were assayed, using a modified one-stage PT technique[1,f] with human or canine congenitally deficient plasma (factors II and VII, respectively) and an artificially deficient bovine plasma[5,6] (factor X). Clotting times were determined for submitted patient and control plasmas and were reported, after log-log transformation, as a percentage of factor activity, compared with pooled feline plasma, which had been assigned a value of 100% factor activity.

To detect coagulation inhibitors, aPTT was performed on a 1:1 mixture of patient plasma and pooled normal feline plasma. Specific factor X coagulant inhibitor assays were performed on a series of dilutions of patient plasma and pooled feline plasma.[5,7]

Results of coagulation screening tests and specific factor analyses of patient plasma were consistent with a severe deficiency of factor X activity (Table 1). **Specific factor X coagulant activity (fX:C)** was markedly reduced in assays performed on samples drawn at 3 time points, and a demonstrable change in activity in response to oral administration of vitamin K_1 was not detected. Lipemia was not observed in any sample. Results of an assay of serum trypsin-like immunoreactivity performed to determine whether fat maldigestion could be responsible for lack of response to orally administered vitamin K_1 were within the reference range. Results of inhibitor studies indicated correction of prolonged aPTT when plasma from a clinically normal cat was added to plasma from the patient, and specific factor X inhibition was not evident.

To investigate the possibility that factor X deficiency in this cat was inherited, the dam and 3 siblings were located. All cats were clinically normal and had been neutered without evidence of excessive bleeding. Their owners did not report spontaneous bleeding tendencies. Plasma from each cat was submitted for determination of aPTT, RVVT, and fX:C. Abnormalities included prolongation of RVVT in the dam and mild deficiency of fX:C in the dam and 1 sibling (Table 1).

Factor X is a vitamin K-dependent glycoprotein that participates in the common pathway of blood coagulation.[8] Deficiency of factor X is a rare disorder in human beings (identified in approx 50 families[9]) and has been detected in a Jack Russell Terrier[10] and a family of American Cocker Spaniels.[11] To our knowledge, an isolated deficiency of factor X has not been identified in cats.

In human beings, familial factor X deficiency is inherited as an autosomal recessive trait. Characteristic laboratory findings include prolongation of aPTT, PT, and RVVT, values of thrombin clotting time and fibrinogen concentration within reference ranges, and specific deficiencies of fX:C and factor X antigen.[9,12] Dysfunctional, rather than deficient, factor X glycoprotein has been discovered by means of western blot analysis and positive results on a factor X antigen assay to be the cause of reduced fX:C in a small number of people.[13,14] Variable or lack of prolongation of ≥ 1

screening test (eg, aPTT, PT, RVVT) has been reported for a subset of cases. Differential affinities of factor VIIa, factor IXa, and Russell's viper venom for the altered factor X substrate have been proposed to account for these observations.[13,14]

A congenital, rather than acquired, deficiency of factor X was considered most likely in the cat of this report. Systemic amyloidosis, infection, neoplasia, and administration of certain drugs have been associated with isolated factor X deficiency in human beings[9] but were not detected in this cat. Acquired coagulopathies often are associated with deficiencies of > 1 clotting factor, and evidence of acquired factor X inhibition causing deficiency of fX:C could not be detected in this cat.[7,15] Multiple factor deficiency can result from liver failure,[16] phenobarbital administration,[17] or vitamin K deficiency caused by exocrine pancreatic insufficiency, intestinal malabsorption, complete bile duct obstruction, or rodenticide toxicity.[18-20] Results of serum bile acid measurements and a trypsin-like immunoreactivity assay, lack of drug or rodenticide exposure, and lack of increase in factor X activity after administration of vitamin K make these causes extremely unlikely. Initial correction of prolonged clotting times after administration of parenteral vitamin K remains unexplained. Findings of prolonged RVVT in the dam and variable reduction in fX:C for the dam and 1 sibling indicate heritability of factor X deficiency in this cat and its relatives.

Diagnosis of factor X deficiency and management of this cat was complicated by transient thrombocytopenia. A buccal mucosal bleeding time and von Willebrand factor concentration within reference ranges and a moderate reduction in platelet count ($> 40 \times 10^3$ platelets/μl) indicate that failure of primary hemostasis alone was unlikely to account for all signs of hemorrhage. The cause for thrombocytopenia is uncertain but may have been related to phenobarbital administration.[21,22]

Intracranial hemorrhage in several infants with factor X deficiency has been reported.[23-25] Because factor X deficiency is rare and intracranial hemorrhage is uncommon in people with congenital coagulopathies, these reports may reflect an increased risk of this complication with factor X deficiency. The association of acute onset of generalized seizures and prolonged bleeding from venipuncture at the time of initial examination suggest that intracranial hemorrhage was the cause of seizures in the cat of this report. Results of hematologic, serum biochemical, urine, bile acid, and serologic tests that were within reference ranges, and the nonprogressive character of the seizures make other causes unlikely. Computerized tomography and CSF analysis were recommended for definitive diagnosis but were declined by the owner. Telephone contact was made with the owner 27 months after initial referral. The cat was receiving phenobarbital (2 mg/kg [0.9 mg/lb], PO, in the morning and 1 mg/kg [0.45 mg/lb], PO, in the evening), and seizures had not been observed in > 1 year.

A coagulation disorder was first suspected in this cat because of persistent, albeit variable, prolongation of clotting times in screening tests and recurrent signs of bleeding. Identification of a specific reduction in fX:C was determined on the basis of results of a series of functional clotting assays, although differentiation of a quantitative from qualitative factor X deficiency requires determination of plasma factor X concentration.[13,14]

We found marked discrepancies in results of coagulation screening tests (ie, PT, aPTT) between our laboratories, using the same sample of plasma. The initial laboratory[j] used a photo-optical instrument[k] for clot detection and commercial reagents.[i,l] The second laboratory[d] used a manual tilt tube method[l] with visual clot detection and different reagents.[e,f] To better define the effect of these differences on assay performance, a series of aPTT were performed, using various dilutions of pooled normal cat and factor X deficient plasmas (bovine depleted[6] and plasma from the cat of this report). Marked prolongation of aPTT was found using the first laboratory's reagent[i] and visual inspection or a different instrument[m] for photo-optical clot detection, indicating that instrumentation, rather than the reagent, was responsible for the discrepancy in results. False detection of clot formation by the photo-optical instrument[k] used by the first laboratory[j] was confirmed by observation that the plasma removed from the instrument had not completely clotted. Photo-optical clot detection techniques are designed on the basis of the principle that light passing through plasma is scattered as fibrinogen is converted to fibrin. As a clot forms, light scatter increases and light transmission reaching the photodetector is concomitantly decreased. This rate of change in light transmission is converted by a predetermined algorithm, set for each instrument, to report a clotting time. The parameters for threshold level and duration of light transmission are developed, calibrated, and optimized for detecting fibrin clot formation in human plasma.[26,27] For factor deficient plasmas from animals other than human beings,[28] photo-optical instruments may report spurious or irreproducible results, because the actual kinetics of clot formation differ from preset values. Although this is an unusual circumstance, definitive diagnosis for animals with clinical signs of coagulopathy may require repetition of coagulation screening tests, using different assay methods or specific coagulation factor analyses.

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[a] K_1 veterinary (phytonadione), Wendt Professional Laboratories, Belle Plaine, Minn.

[b] Mephyton (phytonadione), Merck & Co Inc, West Point, Pa.

[c] Klonopin, Roche Pharma Inc, Manati, Puerto Rico.

[d] Comparative Coagulation Section, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY.

[e] Dade actin FS reagent, Baxter Diagnostics, Edison, NJ.

[f] Thromboscreen, Pacific Hemostasis, Huntersville, NC.

[g] Fibriquick, Organon Teknika, Durham, NC.

[h] Russell's viper venom reagent, Wellcome Diagnostics, Dartford, England.

[i] Dade actin, Baxter Diagnostics, Edison, NJ.

[j] Clinical Pathology Laboratory, College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

[k] MLA electra 750, Medical Laboratory Automation Inc, Mount Vernon, NY.

[l] Dade thromboplastin-C plus, Baxter Diagnostics, Edison, NJ.

[m] Coag-a-mate XM, Organon Teknika, Durham, NC.

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