Acquired factor V inhibitors after allogeneic hematopoietic stem cell transplantation in a dog

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Abstract
Objective: Describe the clinical course and management of a dog that underwent hematopoietic stem cell transplantation (HSCT) for treatment of B-cell lymphoma and developed acquired circulating factor V (FV) inhibitors.

Case Summary: An 8-year-old male castrated Briard dog diagnosed with lymphoma (IVb, B-cell) presented for allogeneic HSCT. Despite multiple platelet, fresh frozen plasma, and red blood cell transfusions prolonged recovery and clinical bleeding occurred. Circulating acquired FV inhibitors were identified and hemorrhage subsequently was managed by immunosuppression. The dog was discharged when clinical resolution of bleeding was achieved.

New or Unique Information Provided: This case report describes a dog undergoing curative intent treatment for lymphoma, and subsequently acquiring factor inhibition, and was successfully managed. Specific coagulation screening to assess for coagulation factor deficiencies or inhibitors is essential in the diagnosis and treatment of patients with refractory bleeding or only transient response to blood transfusion.

KEYWORDS
coagulopathy, hemostasis, lymphoma, platelet, transfusion

1 | INTRODUCTION

Factor V (FV) is a coagulation factor located in the common pathway with effects in both the intrinsic and extrinsic pathways of the coagulation cascade. Information on factor inhibition in both the human and veterinary medical literature is sparse.2 Acquired factor inhibitors have been identified in human patients in association with medications, malignancies, autoimmune disorders, pregnancy, infections, idiopathic causes, and less commonly in patients receiving bovine thrombin products for hemostasis.2-6 To our knowledge, a single case report described a human patient who developed FV inhibition after hematopoietic stem cell transplantation (HSCT).7 Factor inhibition can be difficult to diagnose because of a lack of clinical bleeding. We describe the clinical management of FV inhibitors in a dog after allogeneic HSCT.

2 | CASE SUMMARY

An 8-year-old castrated male Briard dog, weighing 35 kg, was diagnosed with Stage IVb high-grade B-cell lymphoma at an out-of-state referral hospital. Treatment was initiated on a modified CHOP chemotherapy protocol (cyclophosphamide, doxorubicin, vincristine, and prednisone). Dog leukocyte antigen (DLA) typing and matching was
initiated on related relatives, similar to previously published studies. After achievement of both molecular and complete clinical remission, the dog presented to our specialty hospital for transplantation.

Five and 4 days (days –5 and –4) before conditioning, gastrointestinal sterilization and immunosuppression were begun with, respectively, cyclosporine 175 mg PO q12h (Atopica, Elanco, Switzerland), enrofloxacin 408 mg PO q24h (Baytril, Bayer HealthCare LLC, Animal Health Division, Shawnee Mission, Kansas), neomycin sulfate 190.5 mg PO q8h (Neomycin Sulfate, Sigma-Aldrich, St Louis, Missouri), polymyxin B 265500 IU PO q8h (Polymyxin B sulfate, PCCA, Houston, Texas). On day 0, the dog received 24 Gy (8 cGy/min) fractions of total body irradiation (TBI) delivered by a Varian Trilogy Linear Accelerator (Varian Medical Systems, Inc. Palo Alto, California). Immediately after the second fraction, related DLA-matched previously-harvested allogeneic hematopoietic stem cells (CD34+) were administered at 5.98 × 10⁶ cells/kg IV over 30 minutes. The cells were obtained from the donor dog using a cell separator machine described in previous studies. The patient was pretreated with diphenhydramine (Benadryl, Pfizer, Karlsruhe, Germany) and moved into reverse isolation. During the dog’s hospital stay, plasma cyclosporine concentrations were monitored continuously and dosage was adjusted to maintain target trough plasma concentrations of 400-600 ng/mL. On days 8+ and 9+ post-allogeneic HSCT, leukoreduced platelets were administered (2 × 10¹¹ and 3 × 10¹¹, respectively) according to the established protocol and anticipated platelet nadir. The patient’s platelet count on this day was 80 000/μL. On day 9+ post-allogeneic HSCT, the patient received 3 units of leukoreduced platelets (300 × 10¹² platelets). On day 9+, increasing white cell counts and engraftment was documented (platelet count 79 000/μL, total white blood cell count 100/μL) and the patient was removed from isolation. On day 14+, delayed platelet recovery was noted because of recurrent severe thrombocytopenia (49 000/μL). Over the next 7 weeks, platelet transfusions (leukoreduced, fresh, frozen, and lyophilized) were administered (Table 1). During this time, serial chimera testing indicated increasing numbers of circulating donor white cells, ruling out failure of engraftment and relapse of lymphoma. In addition, peripheral blood clonality testing was negative for the presence of clonal lymphocytes. Cyclosporine was discontinued on day 28+. In an effort to speed platelet recovery on day 30+, a cryopreserved aliquot of the related DLA-matched donor CD34+ (1.84 × 10¹⁰) cells was administered. On day 32+, cyclosporine again was initiated to treat possible underlying graft versus host disease leading to severe consumptive thrombocytopenia, possible underlying autoimmune disease directed against megakaryocytes, and perceived improved platelet status before previous discontinuation of cyclosporine (Table S51). The patient required daily platelet transfusions because of persistent thrombocytopenia at this time. A bone marrow aspirate was performed on day 33+ and disclosed normocellular bone marrow and moderate to marked megakaryocytic hypoplasia with a slight left shift. Thromboelastography (TEG) was performed in-hospital (TEG 5000 Haemostasis Analyzer, Haemoscope Corporation, Niles, Illinois). Results were consistent with thrombocytopenia or thrombocytopenia (normal R time, no K time available, normal alpha angle, low MA; Table S51). On day 35+, the patient was not improved; cyclosporine was discontinued and replaced by prednisone (Cadista, Salisbury, Maryland; 30 mg PO q12h) to treat potential platelet-directed alloimmunization.

On day 37+, hyphema and uveitis were found OD, improvement was noted until day 39+ when OS developed new hyphema and bilateral iris bulging was observed. Petechiation and ecchymosis continued to progress in new areas and older areas slowly resolved. At this time, the patient was given 1 U pRBC transfusion for anemia (hematocrit, 26%). Antimegakaryocyte antibody testing was negative and T-cell PCR was negative. Doxycycline (Epic Pharma, Laurelton, New York) 200 mg PO q12h was started on day 42+ because the patient historically resided in areas endemic for tick-borne disease while a tick-borne disease PCR panel was pending. Repeat bone marrow cytology on day 46+ identified increasing but persistently low numbers of megakaryocytes compared to the previous sample. On day 53+, the patient was noted to have scleral hemorrhage, recurrent and

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**TABLE 1 Total estimated transfusion volumes**

| Product type                        | Average volume/unit | Amount of units | Total estimated volume infused |
|-------------------------------------|---------------------|-----------------|-------------------------------|
| Leukoreduced platelet concentrate (from donor apheresis) | 200 mL | 61 | 12 200 mL (406.7 mL/kg) |
| Leukoreduced frozen platelets⁴      | 100 mL              | 4               | 400 mL (13.3 mL/kg)          |
| Lyophilized platelets⁵              | 56 mL (1.05 × 10¹¹ particles per dose) | 3              | 168 mL; 3.15 × 10¹¹ particles (5.6 mL/kg) |
| Packed RBC²ᵃ                        | 190 mL              | 9               | 1710 mL (57 mL/kg)          |
| Fresh frozen plasma⁴                | 120 mL              | 8               | 960 mL (32 mL/kg)          |
| IVIG⁶                               | 200 mL              | 3 vials (6 g diluted with 200 mL diluent) | 600 mL (20 mL/kg) |

³Animal Blood Resources International.
⁴Bode Vet Stable Plate RX.
⁵Hemopet.
⁶Privigen.
progressive ecchymosis diffusely, serosanguinous fluid around the anus, and bilateral epistaxis after sneezing. An in-house coagulation panel using a point-of-care coagulation monitor (VetScan VSPRO, Abaxis, Mountain View, California) was performed and identified prolonged prothrombin time (PT; 23.1 seconds; reference range [RR], 14-19 seconds) and prolonged partial thromboplastin time (PTT; 162.4 seconds; RR, 75-105 seconds) that was confirmed at a reference laboratory (Antech Diagnostic Laboratory; Table SS1). Vitamin K (VetOne, Sparhawk Laboratories, Lenexa, Kansas; 1 mg/kg SC) was initiated despite relatively normal liver enzyme activity on a serum biochemistry profile (AST, 15 IU/L; RR, 15-66 IU/L; ALT, 119 IU/L; RR, 12-118 IU/L; ALP, 198 IU/L; RR, 5-131 IU/L; GGT, 10 IU/L; RR, 1-12 IU/L), and serum total bilirubin concentration was 0.3 mg/dL (RR, 0.1-0.3 mg/dL). The next day (day 54+), the patient had an episode of dyspnea and multiple episodes of suspected syncope. Thoracic radiographs were performed and showed a severe mixed bronchial pattern in all lung lobes (accentuated in the caudal field) and subtle alveolar edema in the right middle lung lobe consistent with pneumonia or intrapulmonary hemorrhage. Ecchymosis and hyphema were persistent with new pitting edema in the limbs along with progressive anemia. An echocardiogram was unremarkable. Because of concerns for pulmonary hemorrhage, the patient received additional platelet transfusions, 2 U of pRBC, and 4 U of fresh frozen plasma (FFP). Immunosuppressive doses of prednisone (30 mg PO q24h) and cyclosporine (80 mg PO q8h) were initiated on day 55+, the patient and coagulation values were substantially improved (PT, 12.8 seconds; RR, 6-12.0 seconds; PTT, 17.0 seconds; RR, 12-25 seconds). Plasma fibrinogen and D-dimer concentrations also were within reference range whereas thrombocytopenia and anemia were persistent (platelet count 8000/μL; hematocrit, 20%). The patient received additional transfusions of 2 U of pRBC, 3 U of fresh platelets, and 10 mL/kg of FFP; a single transfusion of intravenous immunoglobulin (IVIG; Privigen, CSL Behring LLC, KOP, Pennsylvania) also was administered (0.5 mg/kg). Bleeding complications continued sporadically for the next 6 days with the patient continuing to receive pRBC, FFP, and platelet products as needed. Because of the unexpectedly large number of transfusions administered (platelets, pRBC, FFP) and transient clinical improvement with plasma transfusions despite only moderately prolonged clotting times (Table SS1), coagulation factor inhibition was suspected and on day 62+ a TEG again was performed and identified hypocoagulability, likely secondary to soluble factor deficiency based on prolonged R time (16.2 minutes; RR, 2-8 minutes), increased MA (70.9 mm; RR, 46-66 mm), with a normal α angle (51.4°; RR, 44-71°), and K time (3.3 minutes; RR, 1-4 minutes); vitamin K was continued at 25 mg PO q24h while full factor assay was submitted (Comparative Coagulation Section, Animal Health Diagnostic Center, Cornell University, Ithaca, New York). Because of no further ecchymosis or petechiation, good appetite, and good clinical appearance, the patient was discharged from the hospital on day 63+. Reevaluation on day 67+ showed no ecchymosis; a CBC, serum biochemistry panel, heartworm antigen, PT and PTT, and serum cyclosporine concentrations were performed, and the patient was returned to its home state.

To confirm the presence of factor inhibition a citrated plasma sample was submitted to the Comparative Coagulation Section, Animal Health Diagnostic Center (Cornell University, Ithaca, New York). Measured factor concentrations were compared to a control of pooled canine plasma from healthy dogs in mixture (Bethesda Assay). The results indicated a marked decrease in FV activity at baseline and in mixture (FV : C = 8%; RR, 50-150%), prolonged PTT (26.5 seconds; RR, 8.5-15.5 seconds), prolonged PT (21.0 seconds; RR, 11-15.5 seconds) and normal thrombin clotting time (TCT; 6 seconds; RR, 5.0-9.0 seconds). The patient’s FV inhibitor titer was assessed and found to be increased (3.5 Bethesda Unit [BU]/mL). Over the next 6 months, cyclosporine was discontinued and prednisone was tapered (5 mg PO q12h) by a local veterinarian. The patient was monitored with coagulation tests sent to the same laboratory that performed the factor assay (Table SS1). Three months after discharge, the FV : C was improved (28%). Eight months after discharge, the FV : C was 157%. At time of writing, the prednisone has been discontinued, normal coagulation testing has been achieved, and the patient has remained cancer-free for 30 months.

3 | DISCUSSION

Risk factors for development of factor inhibitor are not fully understood, but the condition has been linked to surgical procedures when bovine thrombin is used, antibiotic administration (lactams, aminoglycosides, cephalosporins, tetracyclines, and cholinics), blood transfusions, cancer, and autoimmune diseases.2-5,9-12 In human patients, most published reports of acquired inhibitors tend to describe older adults, with males being more prevalent.2,4,7,13-16

A potential factor inhibitor or factor-specific coagulopathy should be considered in patients with transient response to multiple transfusions, continued coagulopathy despite transfusions, or continued coagulopathy of unknown origin. Fresh frozen plasma, FP, and platelet transfusions supply factors for coagulation, platelets, and building blocks for stable clots. The goal of these treatments is to stop clinical bleeding. When bleeding continues or standard coagulation tests remain abnormal despite multiple transfusions, testing for inhibitors should be considered.

Identification of FV inhibition is considered a coagulopathy when prolonged PT, prolonged PTT, and a normal TCT are present (except in cases in which bovine thrombin-induced antibodies are present). This consideration is based on FV’s critical role in the common pathway and its lack of effect on the actual formation of a clot when excess thrombin is added. Factor inhibition is confirmed using the Bethesda method as previously noted. The antibody titer sufficient to inhibit 50% of the FV activity in normal canine plasma is defined as 1 BU/mL of inhibitory activity. Factor V inhibitors tend to neutralize FV almost immediately.5,9 The coagulation factor percentage below the reference interval supports the presence of FV inhibitor activity in our patient plasma sample.

In our patient, the delayed recognition of a factor inhibitor prolonged the hospital stay, incurred the cost of multiple transfusions...
that transiently supported the patient, and increased the risk associated with multiple transfusions. One of the risk factors for developing a coagulation factor inhibitor is transfusion-based treatment and therefore we cannot say definitively at which point factor inhibitors developed before factor assay testing. The speculation of factor inhibition was made after transfusions (plasma and platelets) had begun to resolve the clinical signs of hemorrhage, but in-house coagulation tests (PT and PTT) were beginning to become abnormal again. The delay in recognizing potential factor inhibition delayed more targeted immunosuppressive treatment for the patient.

Consideration of factor inhibition should be made when a patient is only transiently improved by transfusions, clinical hemorrhage occurs despite transfusions, or coagulopathy testing remains abnormal. A common pattern for FV inhibition, even noted as pathognomonic, is prolonged PT, prolonged PTT, but normal TCT. During the TCT, thrombin is added to the samples. Thrombin converts inactive FV to the active Fva, which has not been found to be sensitive to FV inhibitors. Therefore, prolongation of the TCT would indicate fibrin pathology. Unfortunately, TCT is not commonly evaluated by in-house testing and mostly is available at commercial laboratories such as used in our patient. This likely further delayed establishing the diagnosis.

When coagulation factor testing was performed in this patient, FV was not the only abnormal factor identified. As noted in Table SS1, FVII, FVIII, FIX, FX also were mildly to moderately decreased, but, factor activities >25-30% generally are sufficient to support in vivo fibrin clot formation and prevent spontaneous hemorrhage. With the detectable FV inhibition on the Bethesda assay results, normal protein C concentration, normal fibrinogen concentration, and normal TCT, these other decreased factor activities were considered within reference limits for in vivo fibrin clot formation and FVIII consumption.

Treatment for acquired FV inhibition is similar to treatment for other coagulation factor inhibitors. First, symptomatic hemorrhage is controlled. Management of hemorrhage is based on the patient’s needs (red blood cells, platelets, plasma, or other component treatments). If the patient is not symptomatic, component administration usually is not recommended in human patients. Second, eradication of the inhibitor (autoantibody) is needed to prevent further hemorrhage and coagulopathy. Several treatments have been described including FFP, platelet transfusions, prothrombin complex concentrates, high dose IVIG, plasmapheresis, and immunoadsorption with variable success.

Immunosuppression is a staple of treatment to eradicate the inhibitor while still identifying and treating the underlying trigger. Treatment with corticosteroids (with and without additional chemotherapeutic agents) has been reported to be successful in 76-83% of cases. Cytotoxic agents such as cyclophosphamide have been used, and recently the monoclonal antibody rituximab has been used to successfully treat 3 patients with severe symptomatic FV inhibition and pulmonary hemorrhage. Treatment of the underlying cause that triggered factor inhibitor production often results in disappearance of the inhibitor. Occasionally, spontaneous remission occurs and appears related to initiation and then withdrawal of a new medication (usually antibiotics).

The prognosis for human patients who develop FV inhibition largely depends on presence, location, and extent of bleeding. Overall, the mortality rate is reported to be 12-30%. Patients who develop central nervous system hemorrhage or hemolytic uremic syndrome appear to have the worst prognosis. Two patients who developed FV inhibition after treatment with FFP have been reported, but, both patients had congenital FV deficiencies before receiving transfusions. To our knowledge, the prevalence of FV inhibitors occurring solely secondary to blood (or component) transfusions has yet to be described.

During our patient’s hospital stay, several component transfusions were administered (64 U of platelets, 168 mL of lyophilized platelets, 8 U of FFP, 9 U of pRBC). Unfortunately, all amounts were not available for review in the medical records, and the number of units was; therefore only an estimated total volume, based on average volume per unit. No current recommendations are available on the number of platelet transfusions to administer to patients after HSCT because treatment is dependent on the extent, location, and nature of the patient’s bleeding. Some studies in the human medical literature cite 1 U of platelets per day whereas others have used 2 U per day during diffuse bleeding episodes. These differences highlight the difficulty of diagnosing this condition after a procedure that can confound coagulation results as a result of myeloinactivation.

Our goal was to treat the patient’s clinical bleeding during immunosuppression, neutralize the existing inhibitors, and prevent further inhibitor creation. The patient originally was started on prednisone 1 mg/kg PO q12h and cyclosporine 5 mg/kg PO q12h. At the time of hospital discharge, the patient was receiving 1.65 mg/kg prednisone PO q12h and cyclosporine 1.8 mg/kg PO q8h with directions to taper to 1.5 mg/kg PO q8h after a week. Six months after discharge, the patient was receiving 0.07 mg/kg prednisone PO q24h and, at the time of writing, was not receiving any additional immunomodulatory medications, and has had no additional episodes of bleeding.

Although factor inhibition is uncommonly reported in veterinary patients, circulating factor inhibitors are a risk when patients have neoplastic or immune-mediated conditions. Patients that continue to have persistent clinical and hematologic signs of hemorrhage should be screened for factor inhibition at specialized laboratories. Persistent thrombocytopenia despite multiple whole blood and platelet transfusions can be a sign of a consumptive coagulopathy secondary to hemorrhage and not solely immune-mediated destruction. It is possible that hospitalization could have been minimized if advanced factor activity testing had been performed after the first abnormal TEG results. If platelet counts and clotting times do not return to normal, or do not remain normal after transfusion treatment, further evaluation for underlying coagulopathy should be performed. During this investigation, appropriate immunosuppressive treatment should be started and all elective procedures with risk of hemorrhage should be delayed.
CONFLICT OF INTEREST DECLARATION
Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION
Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION
Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION
Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

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