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Enoxaparin Treatment of Spontaneous Deep Vein Thrombosis in a Chronically Catheterized Rhesus Macaque (*Macaca mulatta*)

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A chronically catheterized 14-y-old male rhesus macaque (*Macaca mulatta*) was reported for recurrent scrotal swelling. The scrotum was enlarged and warm to touch, and associated skin was noted to be lichenified on physical examination. The penis could not be extruded due to preputial swelling. Results from the following diagnostic tests were all unremarkable or within normal limits: scrotal aspirate, hematology, serum biochemistries, urinalysis, and radiography of the thorax, scrotum, and abdomen. Ultrasonography of lower extremities identified thrombi in bilateral iliac veins and left femoral vein. Collateral circulation surrounding the left femoral vein permitted some compensatory venous return. The left femoral vein of this animal had been catheterized approximately 2 mo before initial presentation. A coagulation panel revealed a positive D-dimer test, indicative of elevated levels of fibrin degradation products due to active thrombus breakdown. Enoxaparin sodium, a low-molecular-weight heparin for human use, was administered at 20 mg subcutaneously once daily for 10 d to treat occlusive venous thrombi. After enoxaparin treatment, the edema was greatly decreased. To achieve complete resolution, a second course of enoxaparin was administered 2 months after the first. Ultrasonography of the pelvic vasculature 6 mo after completion of therapy showed marked thrombus resolution, allowing for bilateral patency in the iliac and femoral veins. Follow-up evaluation revealed that D-dimer values were negative as well. This case demonstrates the novel application of the human medication enoxaparin to treat clinical signs of deep vein thrombosis in a chronically catheterized rhesus macaque.

Abbreviations: DVT, deep vein thrombosis; LMWH, low-molecular-weight heparin.

Chronic catheterization in nonhuman primates is often a biomedical necessity for emulation of clinical conditions of human disease. Nonhuman primates may be catheterized long-term for serial blood withdrawal, drug administration, and administration of parenteral nutrition.[8,13,19] To eliminate the need for daily sedation to restrain animals for catheter manipulations, nonhuman primates are commonly housed by using a catheter and tethering system.[2,21] These systems are designed to enable chronically catheterized nonhuman primates to move freely within their housing units while allowing either drug administration or blood sampling.[4,8,13] Complications of chronic catheterization include catheter failure due to leaks, catheter migration, catheter tract infection, catheter-associated thrombus formation, and vascular injury.[3,17,29-31,35] In addition, catheter occlusion (and subsequent catheter failure) can be the sequella of intraluminal thrombus, fibrin tail, mural thrombus, or fibrin sheath formation.[7]

The specific purpose of this case report is to describe a case of deep vein thrombosis (DVT) that was diagnosed by ultrasonography in a macaque that had been chronically catheterized over the course of 53 mo. To our knowledge, spontaneous DVT in nonhuman primates secondary to chronic catheterization has not been published previously. We were able to use treatment techniques typically applied in human medicine as a novel approach in this case because of institutional expertise on animal models of human DVT.[24]

Case Report

A 14-y-old, 13.3-kg captive-bred male rhesus macaque (*Macaca mulatta*) was reported for dependent swelling of the scrotum. This animal was housed individually in a stainless-steel nonhuman primate cage, fed a commercial primate diet (Lab Diet 5038, PMI Nutrition International, Brentwood, MO), and provided water ad libitum by means of an automated system.

This rhesus macaque was used in a chronic alcohol addiction and withdrawal study and was a part of a colony of 120 animals in approximately 6 housing rooms (approximately 20 macaques per room). Animals in this colony were on related experimental studies and were maintained on protocols approved by our institutional animal care and use committee. The affected macaque and the clinically healthy nonhuman primates that were included as normal animals in this study were chronically catheterized using polyethylene tubing (50-durometer translucent silicone rubber; inner diameter, 0.030 in.; wall thickness, 0.036 in.; Saint-Gobain, Akron, OH) with ligation of the catheterized vessel. Pre- and postoperative cefazolin (25 mg/kg IM; Sandoz, Princeton, NJ) was administered, with few postoperative complications noted. The surgical history of this animal included chronic vascular catheterization to facilitate self-administration of alcohol in the following chronologic order: right femoral vein (36 mo), left femoral vein (13 mo), right internal jugular vein (3 mo), and left external jugular vein (1 mo). Between each site change, the animal underwent 1 mo without catheterization, according to the laboratory standard for minimal washout periods between catheter replacements. No other catheterized

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colony animals had been reported for a similar clinical presentation. A trained laboratory staff member performed the catheter placement surgeries. Each catheter remained in a particular site as long as the tubing remained patent. Catheter maintenance in this lab included monthly sodium chlorite flushes (Alcide disinfectant, Alcide, Westport, CT). Catheters that lost patency were flushed with a thrombolytic agent (Activase, Genentech, San Francisco, CA).[1]

The affected animal was acquired at the age of 2 y from a National Primate Research Center (Atlanta, GA). Health records from the primate center indicated that this animal had been generally healthy, with no ongoing clinical issues. Over the 12 y that this animal was housed at our facility, the animal had an unremarkable health history. A clinically normal semiannual physical examination was recorded 5 mo prior to the presentation of dependent scrotal swelling. On initial presentation, the nonhuman primate was anesthetized using ketamine (10 mg/kg IM; Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) for a physical examination, at which time auscultation revealed 3 heart sounds, with the point of maximal intensity over the 4th intercostal space on the left, in what appeared to be a gallop rhythm with a split S2 or S1. Diagnostics included blood sample collection to perform a complete blood count and serum chemistry as well as a urinalysis. The results for the complete blood count, serum chemistries, and urinalysis were unremarkable, and the scrotal swelling resolved in 10 d without any specific treatment beyond a single dose of dexamethasone sodium phosphate (3 mg IV; American Regent, Shirley, NY). Steroids were offered in lieu of extensive diagnostic testing in light of an otherwise healthy clinical assessment of the animal with expected levels of food and water consumption and normal behavior observed by both laboratory and veterinary staff.

To define a concrete reference point, all time points hereafter will follow the initial clinical presentation, which is designated as day 0 (Figure 1). On day 44, the second presentation of scrotal swelling occurred. The animal was sedated as described earlier, and physical examination once again identified the presence of 3 heart sounds during thoracic auscultation. Thoracic radiography was performed, including 2 orthogonal views, for evaluation of the heart. Dilated cardiomyopathy, hypertrophic cardiomyopathy, and right-sided congestive heart failure were ruled out, although endocarditis could not be ruled out at this time. Thoracic radiographs from a similarly-sized clinically normal male rhesus macaque were taken for comparison. No observable differences were identified between the nonhuman primate in this report and that of the clinically normal animal. Results from subsequent analyses of blood and urine were also unremarkable. The scrotal swelling resolved over time without specific treatment and was not reported again until day 104.

On the third presentation for scrotal swelling, which was the most severe, a firm swelling was also noted on the ventral abdomen and appeared to encompass the penis. At this time, marked edema enlarged the scrotum and prepuce, and the skin was lichenified (thickened and leathery) and warm to the touch (Figure 2 A). Upon sedated examination, the preputial swelling prevented the penis from being extruded manually. The heart abnormalities that were auscultated on the first and second presentations were not detected at the time of this evaluation. Diagnostics at this point included a follow-up complete blood count, serum chemistry, and urinalysis, which revealed no abnormalities. Palpation of the testicles revealed comparable consistency and size bilaterally. A fine-needle aspirate of the scrotum was performed to evaluate the possibility of orchitis. A small amount of fluid from the right side of the scrotum was obtained for aerobic–anaerobic culture, and a slide of this fluid was submitted for cytology. Cytology results revealed a virtually acellular fluid with a small amount of keratin, fat droplets, and other degenerated cellular debris. No infectious agents, evidence of inflammation, or neoplasia were observed or cultured, thereby ruling out the possibility of orchitis. Radiographs of the scrotum were performed in 2 orthogonal views and revealed a large amount of swelling but no herniation of abdominal organs into the scrotum. Full abdominal radiographs in 2 orthogonal views were all within normal limits. Follow-up thoracic radiographs remained within normal limits. After this evaluation, scrotal herniation and an inadequate inguinal lymphatic drainage were ruled out. Because of the severity of the swelling and possibility for discomfort, treatment at this time included 0.1 mg/kg buprenorphine hydrochloride (Buprenex, Reckitt Benckiser, Slough, United Kingdom) given intramuscularly twice daily for 4 d for pain management. In addition, silver sulfadiazine ointment (1% Silvadene, Monarch Pharmaceuticals, Bristol, TN) was applied to the prepuce to minimize the potential for urine scald. During these described incidences of scrotal edema, the animal maintained its ability to urinate spontaneously.

Supplementary treatment with scrotal hydrotherapy and massage was implemented to accelerate the resolution of the confirmed scrotal edema by manually assisting the resorption of fluid from the scrotum. With the animal under ketamine sedation, tissue massage was performed while the entirety of the scrotum was submerged into a warm water bath once daily for 4 d. Following scrotal hydrotherapy, silver sulfadiazine ointment was applied to the preputial swelling. After intensive edema reduction measures, the scrotal edema showed moderate resolution, but the preputial edema remained largely unchanged. At this time, because experimental studies on the animal had ceased, the left external jugular catheter was removed.

To elucidate the underlying cause of the recurrent scrotal edema, ultrasonography with a multiHertz probe and Doppler flow was used to evaluate the heart, scrotum, and lower extremity vasculature. The heart valves appeared to be within normal limits, and no endocarditis was noted. After bilateral visualization of the femoral and iliac veins, manual compression was applied by the ultrasonographer to assess the compressibility of the vessels. Whereas the normal response is a collapse of the vessel upon compression with the ultrasound probe, the left femoral and bilateral iliac vessels of our macaque did not compress or 'flatten' as expected. The lack of normal compression was due to the presence of multiple adherent venous thrombi. The thrombi present in the left femoral vein of our macaque almost totally occluded the vessel (Figure 3 A). Adherent venous thrombi were detected in the left femoral, left iliac, and right iliac veins. The ultrasonographic findings confirmed the diagnosis of DVT. Our clinical team, which consisted of a vascular surgeon, vascular ultrasound technician, and laboratory animal veterinarians, hypothesized that blockage of the left femoral and iliac veins compromised normal venous return from the scrotum and prepuce, leading to edema formation. The previously observed alterations in normal heart rhythm were likely a secondary effect of the extensive DVT that our patient exhibited.

The hypogastric vein normally takes venous return from the pelvic region to the external iliac vein. Ultrasound evaluation confirmed that the nonhuman primate in this case had developed collateral circulation that enabled venous blood to bypass the left femoral vein, allowing normal venous return from the legs. This vascular abnormality most likely occurred due to

the patient's previous bilateral femoral vein catheterizations, residual partially occlusive venous thrombi, and vein wall fibrosis due to the presence of the catheter.

To further assess DVT, a blood sample was collected for a coagulation panel, which tested activated partial thromboplastin time, thrombin clotting time, fibrinogen concentration, and D-dimer level. The D-dimer test measures the amount of fibrin cleaved by plasmin. The test is an indication of secondary fibrinolysis (the breakdown of a formed clot) rather than primary fibrinolysis, such as seen with the administration of thrombolytic agents. This test is a method to rule out disseminated intravascular coagulation, in which both clot formation and secondary fibrinolysis occur.[28] In this clinical case, we used a latex-enhanced automated semiqualitative tubidometric assay, in which 20 µL of sodium citrated macaque blood was evaluated.[24] From the results of the coagulation panel, only the D-dimer test revealed an abnormal finding (1.20 ± 0.4 µg/mL), which is a highly positive result based on extrapolation from human values available: according to the manufacturer of the human assay (Dade-Behring, Deerfield, IL), concentrations of plasma D-dimer greater than or equal to 0.20 µg/mL are considered positive results in humans. The positive value in this test indicated active thrombolysis in the described animal. To evaluate the panel of a clinically normal animal, a coagulation panel was performed on another sex- and study-matched rhesus macaque, which was housed in the same study room and approved on the same protocol. The analysis of this control animal yielded results within the normal reference range.

In light of the diagnosis of DVT, the macaque was dosed with 20 mg of the low-molecular-weight heparin (LMWH) enoxaparin sodium injection (Lovenox, Sanofi-Aventis, Bridgewater, NJ), which was given subcutaneously once daily for 10 d. This dose was based on the typical treatment dose administered to human patients with DVT and to baboons with experimentally-induced DVT used as animal models for human disease processes.[24]

After treatment with enoxaparin, the patient's scrotal and preputial edema dramatically abated, although the preputial edema was not resolved completely at the end of the 10 d treatment period and persisted with mild severity without further resolution. In an attempt to achieve complete resolution, a second round of enoxaparin treatment according to the same dosing regimen was initiated 2 mo after the first. On reevaluation 2 wk after the second round of enoxaparin treatment, the preputial edema had resolved completely, and the penis could be extruded manually (Figure 2 B). Lichenification of the scrotal skin and scrotal edema were resolved at this time also. At follow-up 6 mo after completion of all treatment, ultrasound analysis of the lower extremity vasculature, including the bilateral femoral and iliac veins, revealed resolution of the DVT as evidenced by the ability of the veins to flatten in response to manual compression with the ultrasound probe (Figure 3 B). The coagulation panel taken at 6 mo after treatment yielded a negative D-dimer test.

After complete resolution of the index animal, the veterinary staff elected to assess D-dimer values in a sampling of chronically catheterized macaques from the same colony to understand whether subclinical DVT could be correlated with chronic catheterization in other animals. To this end, 6 macaques from the same colony, chronically catheterized (duration, 48 to 56 mo), and maintained under the same approved protocol were selected randomly and tested for D-dimer levels. Five of the macaques had negative test results, whereas the remaining 1 had a positive D-dimer level (0.6 µg/mL), indicative of active thrombolysis. This level was lower than that of the index case with confirmed DVT (1.2 µg/mL).

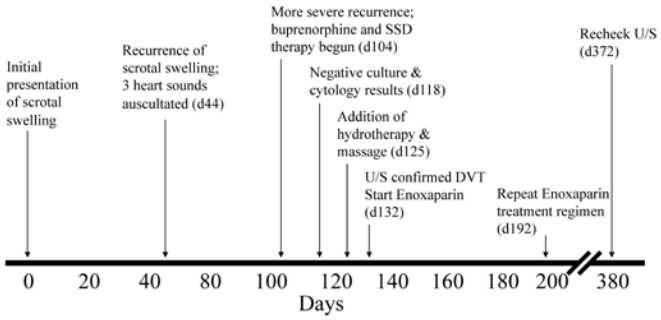

Figure 1. Timeline of the medical management of deep venous thrombosis in a rhesus macaque.

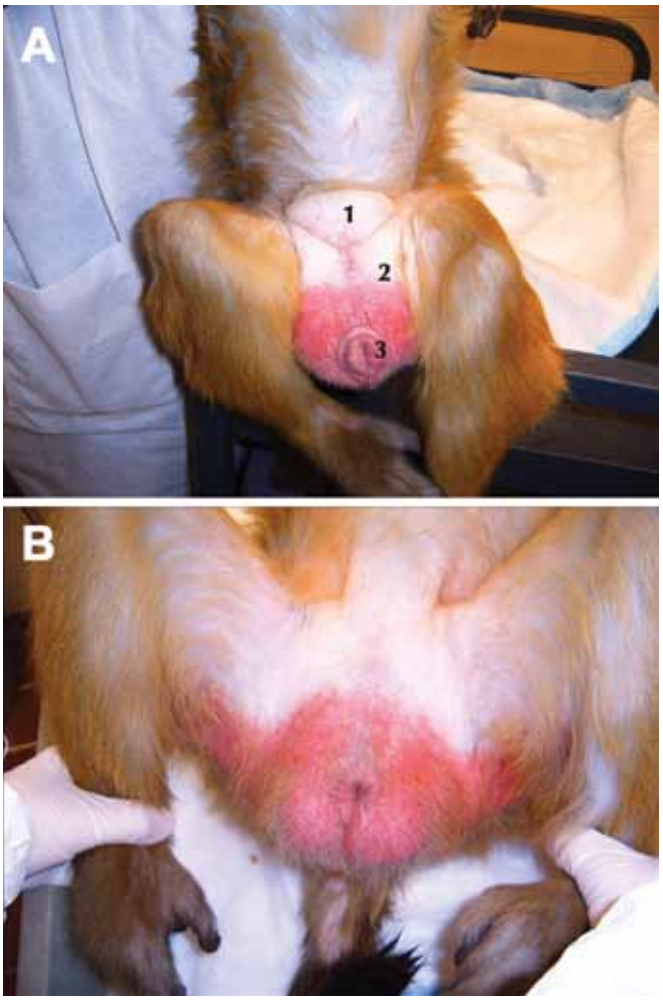

Figure 2. (A) This photograph of our patient was taken at the third recurrence of scrotal swelling. In addition, a firm swelling on the ventral abdomen appeared to encompass the penis (1). The macaque had an enlarged scrotum (2) and prepuce (3) due to edema formation. (B**)** This photograph was taken after the second regimen of enoxaparin treatment: the scrotal and preputial edema was completely resolved, and the penis could be extruded manually.

Discussion

Deep venous thrombosis is of great public health importance in the United States, with an estimated 900,000 cases of DVT reported annually.[5,9,10] In light of the prevalence of DVT in the human population, this disease has been studied extensively in

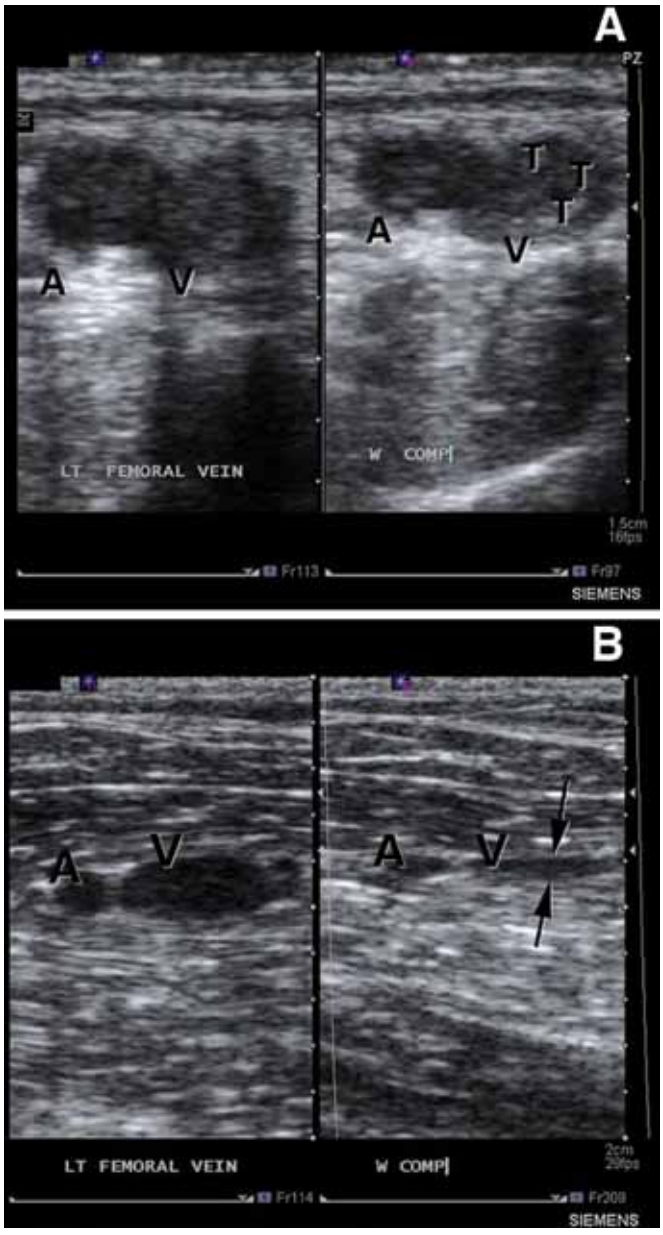

Figure 3. (A**)** Representative ultrasound targeting the left femoral vein, showing no additional compression (left) and with manual compression (right). Ultrasound evaluation showed that the left femoral vein could not be compressed manually, because of the presence of partially occlusive venous thrombi. The same technique was applied also to the right femoral vein, which was within normal limits, and to bilateral iliac veins, which (like the left femoral vein) failed to flatten. (B**)** At the time of follow-up, ultrasound analysis of the entire lower extremity vasculature was performed. This representative ultrasound focuses on the left femoral vein 240 d after diagnosis of DVT. The flattening of the left femoral vein after compression with the ultrasound probe (arrows) confirmed thrombi resolution. This finding also was representative of both the left and right iliac veins. A, femoral artery; T, thrombus; V, femoral vein; W COMP, with manual compression.

multiple animal models of venous thrombosis.[6,22,23] Expertise at our institution in these animal models was invaluable in diagnosing and then translating treatment from human research to our nonhuman primate. Development of DVT is believed to be dependent on a triad of events including blood stasis, alterations to the vessel wall, and blood hypercoagubility.[18,32] Although clinical symptoms are absent in some cases of DVT in the human population, clinical signs typically include 1 or more of the following: pain, swelling, warmth, and redness of the affected limb(s), or chest pain associated with pulmonary embolism. Sequellae of DVT include chronic venous insufficiency, pulmonary embolism, and subsequent chronic pulmonary hypertension.[25,26] These additional conditions can result in extensive costs for treatment and longterm management in human patients, estimated at billions of dollars annually.[11,12]

To our knowledge, the clinical management of spontaneously occurring DVT in nonhuman primates has not previously been addressed in the literature. Therefore, our clinical team initiated consultations with human and veterinary surgeons to help determine the best treatment plan for this unique case of DVT in a nonhuman primate. Low-molecular-weight heparins are currently the standard of care for the treatment and prophylaxis of DVT in hospitalized humans. Low-molecular-weight heparin (LMWH) can be dosed once daily effectively, and, unlike heparin, does not require continuous intravenous infusion to be effective. These properties of LMWH made it an ideal choice for the clinical management of DVT in this nonhuman primate. In addition, in human patients with DVT the use of LMWH is associated with a decreased risk of heparin-induced thrombocytopenia (HIT) syndrome.[16,27,33,34]

The LMWH enoxaparin primarily inactivates factor Xa, with less specificity for factor IIa (thrombin), which enables it to prevent or lessen thrombus formation. Contraindications of enoxaparin use in humans are active major hemorrhaging, thrombocytopenia with a positive in vitro test for antiplatelet antibody, and hypersensitivity to heparin or pork products. The adverse reactions most common in humans receiving enoxaparin include bleeding, anemia, thrombocytopenia, increased serum aminotransferase, diarrhea, and nausea. Low-molecular-weight heparins have the advantage over heparin due to their longer duration of action and predictable effects after dosing. The therapeutic effects of LMWH are monitored by evaluating antifactor Xa activity in the blood. In the current case, treatment with enoxaparin therapy was not monitored over the 10-d administrative course; the treatment regimen (1.5 mg/kg SC daily) was chosen based on human treatment regimens and efficacious DVT lysis studies in baboons with experimentally induced venous thrombosis.[20,24] Studies of these LMW formulations of heparin have been performed; most recently an oral formulation was proven to be nontoxic at multiple doses in cynomolgus macaques.[14,15] In our case, after the first round of enoxaparin, the animal's clinical signs were decreased but not eliminated entirely. The veterinary staff therefore determined that an additional therapeutic regimen of enoxaparin potentially would be beneficial in achieving complete resolution of the patient's clinical signs. The marked resolution of venous thrombi in our patient was verified by ultrasonography after the second dosing regimen (Figure 3 B). Further treatment with enoxaparin was not continued prophylactically because the nonhuman primate was removed from further catheterization studies.

Enoxaparin treatment regimens in human patients with DVT can include its use continuously as a preventative. Our clinical team discussed whether prophylactic enoxaparin treatment might be useful to minimize potential DVT formation in chronically catheterized nonhuman primate subjects within the colony. The financial burden of implementing prophylactic therapy with LMWH was determined to be cost-prohibitive and was not indicated, given the lack of reports of spontaneous DVT in chronically catheterized nonhuman primates. The additional testing of D-dimer levels in 6 other colony animals revealed that only 1 had increased levels. In agreement with the nature of the test itself, the animal with the low-positive D-dimer levels

was presumed to be undergoing thrombolysis without clinical signs of DVT. At our institution, the described animal was the only confirmed case of DVT in a colony of chronically catheterized nonhuman primates that consistently maintained a daily census of more than 100 animals. There had been 2 clinical cases in this colony in which localized edema (facial and upper limb) possibly could have been linked to thrombosis, although these cases resolved spontaneously.

The inciting cause of DVT in this patient likely was related to the animal's history of chronic catheterization; however catheterization may not have been the sole etiology due to the complex pathophysiology of venous thrombosis. This condition in nonhuman primate colonies likely is underdiagnosed, especially considering factors such as the intermittent nature of DVT and possible absence of clinical signs. The clinical presentation in this nonhuman primate included dependent edema in the scrotum and prepuce due to the location of the venous thrombi. After treatment with the LMWH enoxaparin and resolution of DVT, the nonhuman primate was maintained in the colony and enrolled in laboratory studies not requiring venous catheterization.

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