A Siri-ous Situation

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Introduction

Canine immune-mediated thrombocytopenia (ITP) is a life-threatening condition in which antiplatelet antibodies destroy platelets.[1] This can either occur within circulation or at the level of the bone marrow.[1,2] Once platelet numbers have dropped below 30,000-50,000 /uL spontaneous bleeding can occur due to impaired primary hemostasis.[5] Immune-mediated platelet destruction can be categorized as either primary or secondary. Primary ITP is a diagnosis of exclusion; however, it is still the most common acquired form of thrombocytopenia.[1] Other secondary causes of thrombocytopenia are organized into broad categories of decreased production, increased destruction, sequestration, or increased consumption or loss.[1,2] Decreased production indicates a bone marrow disorder or overwhelming neoplasia.[1] Increased destruction describes infectious agents, drug administration, recent vaccination, or additional autoimmune etiologies.[1] Sequestration and or increased consumption can occur secondary to an existing neoplastic condition.[1]

ITP typically occurs in middle aged dogs with a median age of 6 years old.[2] Overly represented breeds include Poodles, Cocker Spaniels, Old English Sheepdogs, and German Shepherds; however, any breed can be affected.[1,2] Female dogs develop ITP twice as frequently as male dogs thought to be due to the influence of sex hormones.[2]

Patients with primary ITP often present with non-specific signs of lethargy, weakness, and inappetence.[1] Others may present with pale mucous membranes, petechiae and ecchymosis, melena, and or hematuria.[1] In either situation, the first step towards a diagnosis of primary ITP ruling out secondary causes of thrombocytopenia. Obtaining a detailed history of recent drug administration, tick exposure, and travel along with a thorough physical exam provides a vital

baseline prior to beginning diagnostic evaluation.[1,2] These steps should not be overlooked when working-up a case of thrombocytopenia.

Cornerstone diagnostic tests for ITP include a complete blood count with a direct blood smear and manual platelet count.[1] Additionally, a serum chemistry profile, coagulation profile, heartworm antigen test, and a tick panel is recommended.[1] It is imperative that blood samples be obtained prior to initiating immunosuppressive therapy and should be performed as atraumatically as possible to avoid bruising.[1]

Once secondary causes of thrombocytopenia have been excluded, immunosuppressive therapy can be considered. Corticosteroids are the mainstay of treatment due to their rapid onset of action and low cost. However, some cases require additional immunosuppression to gain appropriate disease control, and a second agent can be considered to allow for more rapid tapering of glucocorticoids following disease stabilization.[1,2,5] Such immunosuppressive agents include azathioprine, cyclosporine, mycophenolate mofetil, and leflumonide.[1,2,5] Vincristine may also be used in dogs with ITP to increase the platelet count.[6] Vincristine binds to tublin and inhibits cell division.[6] The exact mechanism by which vincristine increases the platelet count is not fully known; however, acceleration of platelet production and decreased phagocytosis of platelets via impaired phagocytic function have been suggested.[6] The end goal for ITP therapy is to maintain a normal platelet count with the lowest possible dose of immunosuppressive medication.[1,2] In most dogs, prognosis is good and they can return to their normal life.[1,2] If patients fail to respond to initial therapy, thorough re-evaluation may be necessary.[2] This paper will discuss a case of suspected primary immune-mediated thrombocytopenia in a Boykin Spaniel.

History and Presentation

Siri is a 5-year-old female spayed Boykin Spaniel that presented to MSU-CVM Emergency Service Department on August 22, 2019 for abdominal bruising, lethargy, and an episode of collapse. Siri lives an indoor-outdoor life with access to a large cattle farm in Mississippi. Prior to this event she had no major medical history and was up to date on vaccinations and preventative medications. The owners first noted that Siri was not acting herself on August 16, 2019, when she began vomiting. On Monday August 19, 2019 Siri was taken to her primary care veterinarian and abdominal radiographs were performed. She was diagnosed with colitis and was discharged on a penicillin-based antibiotic and maropitant citrate. Siri continued to vomit overnight and was re-evaluated on August 20, 2019. During this visit, she was prescribed lactulose and a sulfa-based antibiotic. On the morning of Wednesday August 21, 2019, the owners noted that Siri was having multiple bouts of bloody urine, so she was taken back to her primary care veterinarian. At this time, she was noted to have pale mucous membranes with multiple petechiae and ecchymoses on her ventral abdomen and inner thighs. She was presumptively diagnosed with rat bait toxicity and was prescribed vitamin K and iron supplementation tablets. She continued to decline throughout the day on Thursday, August 22, 2019 and became notably more lethargic. Siri then collapsed after going up the stairs in and was brought directly to MSU-CVM for further evaluation.

On presentation Siri was dull, but responsive. She weighed 15.9 kg with a body condition score of 5/9. Her temperature and respiratory rate were within normal limits at 101.8 degrees F and 28 brpm respectively. She was tachycardiac at 180 bpm. Her mucous membranes were pale with multiple petechiae noted. Her capillary refill time was ~2 seconds, and she was estimated to be 5% dehydrated. Cardiothoracic auscultation and gentle abdominal palpation revealed no

abnormalities. The skin on her ventral thorax and abdomen had multiple ecchymoses, the largest measuring 2.5 x 2cm. Triage examination revealed adequate oxygenation and varied blood pressures. Thoracic and abdominal FAST scans revealed no free fluid.

Blood was drawn for a CBC, serum chemistry, clotting times, a tick PCR panel and a slide agglutination test. The CBC revealed a moderate anemia with a packed cell volume (PCV) of 23% and a manual platelet count of zero. The serum chemistry revealed a mildly reduced total protein (TP), mild hypocalcemia, and mild hypomagnesemia. Clotting times were within normal limits. A slide agglutination test was negative for both microscopic and macroscopic agglutination. Siri was started on a course of doxycycline while the tick panel was pending. Thoracic and abdominal imaging were performed to rule out secondary causes of thrombocytopenia. The thoracic radiographs revealed no abnormalities. Abdominal imaging revealed a mildly rounded spleen, thickening of the urinary bladder wall and the presence of a suspected hematoma within the urinary bladder.

Siri's problem list at this time consisted of lethargy, vomiting, hematuria, hyporexia, melena, dehydration, ecchymosis, petechiae, mild anemia, severe thrombocytopenia, mild splenomegaly, and a suspected urinary bladder hematoma. The potential of an immune-mediated thrombocytopenia or thrombocytopenia secondary to tick-borne disease or drug administration were discussed with the owners. It was elected to pursue vincristine therapy in addition to immunosuppression with dexamethasone and cyclosporine. Whilst awaiting the results of the tick panel it was elected to pre-emptively treat for an underlying tick-borne disease with doxycycline. Additional supportive care was administered including anti-emetics and IV fluid therapy. Her PCV/TP and abdominal FAST scans were monitored periodically.

Pathophysiology

The goal of primary hemostasis is the formation of a platelet plug to temporarily prevent hemorrhage.[3] Then, the original platelet plug is stabilized with fibrin while clotting factors are activated during secondary hemostasis.[1,3] Thrombopoeisis occurs within the bone marrow where megakaryocytes fragment and release platelets into circulation.[3] Platelet lifespan is approximately 8-12 days.[3] After this time they are consumed by tissue macrophages within circulation or within the spleen.[3] In cases of immune-mediated thrombocytopenia, primary hemostasis is often affected due to increased platelet destruction.[1] Other causes include decreased platelet production, sequestration, or increased consumption or loss.[1] Two main categories of immune-mediated thrombocytopenia are primary ITP, also referred to as idiopathic thrombocytopenia purpura, and secondary ITP.[1]

In cases of primary ITP, antiplatelet autoantibodies are targeted against normal host platelet-surface antigens.[1,2,3] Glycoprotein IIb/IIIa is highly immunogenic and is targeted most often by immunoglobulin G (IgG).[1,2] Circulating macrophages then destroy any platelets that have been flagged with an antibody-antigen complex.[1] In addition to flagging platelets for destruction, some studies suggest that the antibody-antigen complexes formed on the outside of platelets may alter their function to further compromise primary hemostasis.[1]

Secondary immune-mediated thrombocytopenia occurs due to an underlying condition or disorder.[1,8] Many such disorders have been reported, however a causative agent has not been fully discovered in each scenario.[1] The most common autoimmune disorders reported with ITP include systemic lupus erythematosus (SLE), rheumatoid arthritis, and immune-mediated hemolytic anemia (IMHA), also known as Evan's syndrome.[1,2] Drugs including sulfa-based antibiotics, certain penicillins, cephalosporins, estrogens, and auranofin (gold salts) have been

known to induce ITP.[1,8] The drug binds to platelets creating antigenic drug-platelet complexes.[1] ITP usually develops within weeks to months of administration and may spontaneously resolve within two weeks of discontinuing the drug.[1] Lymphoma, mammary adenocarcinoma, mast cell tumor, hemangiosarcoma, nasal adenocarcinoma, and fibrosarcoma are among the reported neoplasms associated with ITP.[1,2] If neoplastic remission can be achieved, thrombocytopenia will resolve in most cases.[1] The most well researched causes of secondary ITP are associated with infectious agents, most notably tick-borne pathogens such as erlichia, babesia, and leishmania.[1,2] Viral, bacterial, and protozoal causes cannot be disregarded and should be included as possible differentials for secondary ITP.[1] Hematogenous infections result in antibody-platelet complexes and are later destroyed by circulating macrophages.[1]

Diagnostic Approach/Considerations

Mainstay diagnostic tests for ITP include a complete blood count with a direct blood smear and manual platelet count.[1,8] A serum chemistry profile, coagulation profile, heartworm antigen test, and a tick PCR panel may be performed.[1,8] It is imperative that blood samples be obtained prior to initiating immunosuppressive therapy.[1] Collection should be as atraumatic as possible to avoid activating tissue factor within the needle and avoid iatrogenic hemorrhage in the patient.[1] Thoracic and abdominal imaging with abdominal ultrasound are important to rule-out underlying neoplastic conditions.[1,2] Fine-needle aspirates of any abnormal lesions or enlarged organs for cytology may be helpful if the platelet count is adequate (often cited as >50,000/uL).[1]

A diagnosis of immune-mediated thrombocytopenia can be supported with six main criteria, but an ultimate diagnosis of primary ITP is one of exclusion.[2] The severity of thrombocytopenia (<50,000/uL versus 0/uL) should be considered, although this cannot reliably serve as a diagnostic indicator alone.[2] Microthrombocytosis and platelet fragmentation is

reported as a specific, but insensitive indicator if ITP with a specifity of 95% and sensitivity of 45%.[2] Bone marrow aspiration is not always necessary, but it is recommended if leukopenia is present or if inadequate platelet production is suspected.[2] Detection of antiplatelet autoantibodies can be accomplished with platelet factor 3 (PF3) test and the megakaryocyte direct immunofluorescence (MK-DIF) test.[2] Other indirect and direct assays for detection of serum antibodies to platelets have been developed as well.[2] Studies comparing these methods demonstrated that direct assays are 50-60% more sensitive than indirect assays, but not specific enough to directly diagnosis ITP.[2]

In Siri's case, a minimum database was established with a complete blood count, serum chemistry, coagulation profile, and a slide agglutination test. A tick PCR panel was submitted to NCSU for further evaluation for tick-borne diseases while pre-emptive doxycycline was started. Initial laboratory findings revealed normal coagulation times with a moderate anemia, severe thrombocytopenia, and a negative slide agglutination. These findings in addition to the clinical signs of bruising suggest a primary hemostatic disorder. Imaging revealed no evidence of a secondary thrombocytopenia and Von Willebrands disease was considered unlikely due to the prior history of an uncomplicated ovariohysterectomy when she was younger.

Diagnostic imaging was pursued to rule-out secondary causes of ITP and further evaluate complications of primary ITP. Her thoracic radiographs revealed no concerning abnormalities and abdominal radiographs were unremarkable. Mild splenomegaly and a large bladder hematoma were found on abdominal ultrasound. No evidence of abdominal masses or enlarged lymph nodes were noted which ruled-out an underlying neoplastic condition and increased our suspicion for a primary ITP disorder.

Treatment and Management

Immunosuppressive medications are the mainstay of treatment for primary ITP.[1,2,5] Vincristine can also be administered for short-term increases in platelet count. Potential mechanisms include acceleration of platelet production and decreased phagocytosis of platelets by impaired phagocytic function.[2,6] In cases of severe anemia, blood transfusions may need to be administered until the immunosuppressive therapy begins working.[2] The end goal for ITP treatment is to maintain a normal platelet count with the lowest possible dose of steroids and or without the need for additional medications.[1] Despite appropriate therapy, recurrence rates range from 26-58% in certain cases.[6]

When beginning therapy for primary ITP patients, immunosuppressive doses of corticosteroids should be the first-line of treatment due to their rapid onset of action.[1,2,6] Steroids impair the destructive effects of macrophages on antibody-sensitized platelets[2]. Dexamethasone can be administered intravenously at a dose of 0.28 mg/kg every 24 hours[1,2]. Prednisone or prednisolone should be administered orally at a dose of 2 mg/kg/day[1,2]. Patients will typically remain on corticosteroids at this dose until adequate platelet numbers are reached (>50,000/uL).[1] This usually occurs within seven days. The initial immunosuppressive dose should be continued for two to four weeks before tapering the dose[1]. After this time, the platelet count should be rechecked and if the numbers are still adequate (>50,000/uL), then the corticosteroid dose can be tapered slowly.[1] Potential adverse effects of steroids include polyuria, polydipsia, panting, and other signs of iatrogenic Cushing's disease.[1,2]

The most common immunosuppressive agents used as adjunctive therapy for ITP include cyclosporine, azathioprine, and myphenolate mofetil.[1,2,5,7] These drugs are typically reserved for use in patients that have failed to respond to corticosteroids, dogs that have relapsed, or dogs that

have severe corticosteroid adverse side effects.[2] In one study, mycophenolate use with corticosteroids was just as effective as cyclosporine use with corticosteroids and showed less adverse side effects.[4] Cyclosporine was chosen as the second immunosuppressive therapy of choice in Siri's case in addition to the corticosteroid therapy because it has a more rapid onset of action, and a favorable adverse effects profile.[2,7]

Case Outcome

On Saturday, August 24, 2019, Siri collapsed on her morning walk outside and her PCV had fallen to 11% from 23% only 24 hours prior. This was suspected to be secondary to a bladder or gastrointestinal bleed due to her severe thrombocytopenia. A DEA 1.1 positive whole blood transfusion was given (22.6 mL/kg whole blood, cross match compatible) over a 5.5 hour time period. Siri was much brighter after her transfusion and her post-transfusion PCV had increased to 24%. On the morning of August 25, 2019, a CBC revealed that her platelet count had increased to 16,000/uL and her PCV remained static at 25%. On the morning of August 26, 2019 her platelet count had increased to 96,000/uL. Siri's clot in her urinary bladder had decreased in size drastically and she was no longer hematuric. She was transitioned to oral medications in preparation for discharge although she did not have a ravenous appetite. While in hospital she began urinating frequently and having accidents in her kennel. A urine culture was submitted as a precaution due to her immunosuppression to rule out a potential secondary urinary tract infection. The urine culture returned negative after 24 and 48 hours.

Siri was successfully discharged on the evening of August 27, 2019 with a platelet count of 188,000/uL and a PCV of 27%. She was sent home on immunosuppressive doses of prednisone and cyclosporine. Doxycycline was continued while the tick PCR panel was still pending. Due to her reduced appetite and melena, she was prescribed omeprazole as a

gastroprotectant and maropitant citrate as an antiemetic. Her owners were given strict instructions to return if they noticed any signs of worsening anemia that may indicate a relapse of her thrombocytopenia, such as pale mucous membranes or abdominal bruising.

A recheck was scheduled on September 5, 2019 for a manual platelet count. At this recheck her owners reported that Siri still had a decreased appetite and was urinating more frequently but was tolerating her medications well otherwise. Her platelet count had jumped to 880,000/uL and she had a PCV of 34%. On September 13, 2019 Siri's tick PCR panel returned from NCSU which revealed a weak positive IFA for *Babesia canis*. These results were interpreted as a past-exposure or a latent infection. An additional recheck was scheduled for September 19, 2019 for another manual platelet count, a repeat tick PCR panel, and cyclosporine level monitoring. Her platelet count was within normal limits at 512,000/uL and she had a PCV of 40%. The cyclosporine levels and tick PCR panel are still pending. If this second tick panel still exhibits a weak positive IFA, then Siri's treatment regimen will need to be adjusted to address the potential *Babesia canis* infection. Otherwise, Siri's owners report that she is back to her happy self at home; running, playing, and eating as usual. Siri's case serves as a great reminder that although an initial diagnosis of primary ITP can be complicated, appropriate treatment can be successful and allow affected animals to return to a normal life.

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