Primary Immune Mediated Thrombocytopenia

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Introduction

Hematologic disorders are a common finding in veterinary medicine. Of the hematologic disorders observed, thrombocytopenia accounts for 5% of cases.[1] Thrombocytopenia can result from one or more of the following: decreased platelet production, excessive platelet destruction, platelet consumption, and/or sequestration.[2] Disease categories associated with thrombocytopenia include immune-mediated, neoplastic, infectious, drug and toxin exposure, disseminated intravascular coagulation (DIC), and inherited disorders.[3] From this list, immune-mediated disease is the most common reason for severe thrombocytopenia.[4] Immune-mediated thrombocytopenia (IMT) occurs when antibodies against platelets are produced, causing destruction of the circulating platelets. IMT can be further categorized into primary (idiopathic) or secondary based on the presence of an inciting cause[3]. Because primary immune-mediated thrombocytopenia is defined as an isolated thrombocytopenia in the absence of a known cause or disorder for thrombocytopenia, primary immune-mediated thrombocytopenia can only be diagnosed when all other reasons for thrombocytopenia have been ruled out.

Clinical signs consistent with thrombocytopenia and a primary hemostatic disorder can include petechiae, ecchymosis, mucosal bleeding, epistaxis, melena, hematochezia, hematemesis and scleral hemorrhage. Non-specific clinical signs may include lethargy, weakness and anorexia upon presentation.[3] Initial diagnostics to evaluate platelet number include a complete blood count and a manual blood smear analysis. Once a diagnosis of thrombocytopenia is confirmed on bloodwork, the cause should be further investigated to see if IMT is the cause. If IMT is the underlying cause, it should be further investigated to see if it is primary or secondary in nature. When all possible secondary causes of IMT have been ruled out, a final diagnosis of primary IMT is established.

Case History and Presentation

The case presented is a thirteen-year-old neutered male Maltese that presented on emergency to the Animal Medical Center of Charlotte, NC for ongoing hematochezia for a 12-hour duration on May 13th, 2016. The patient presented to his referring DVM earlier in the day with loose stool containing a small amount of blood. He also was anorexic at the time of presentation to the rDVM. The rDVM performed a complete blood count, chemistry profile and fecal examination. The owners stated the rDVM said the platelet count was "52" and the fecal was negative. The patient was given a dexamethasone SP injection subcutaneously and prescribed prednisone tablets with instructions to start the prednisone the next morning. The patient has a history of elevated liver enzymes and hypothyroidism. Current medications at presentation to the Animal Medical Center include levothyroxine 0.1mg (1/2 tab SID) and ursodiol 250 mg (1/4 tab SID). The bloodwork results and complete patient record from the rDVM were not available to us at the time of presentation to the Animal Medical Center.

Upon presentation to the Animal Medical Center, the patient was bright, alert and responsive in the examination room. Further history from the owners revealed that they had noticed small bruises appearing on his abdomen and legs throughout the day. Completion of the physical exam revealed a body condition score of 6/9 with 2-5% dehydration. Temperature, pulse and respiratory rate were within normal limits. Mucous membranes were pink but slightly tacky with no petechial hemorrhages noted. Abdominal palpation revealed a tense abdomen with cranial organomegaly. There were multiple petechial hemorrhages noted on the limbs and ventrum, and large areas of ecchymosis present on the ventrum and prepuce. Rectal exam revealed hematochezia.

At this time, a primary hemostatic disorder was suspected. A CBC was performed which revealed a platelet count of zero. This was confirmed by a manual blood smear analysis. A PCV/TP was also performed and was within normal limits (PCV of 48% and TP of 8.0g/dL). Further questioning of the owners determined that the patient had been feeling normal and the only ongoing health issues were a history of elevated liver enzymes and hypothyroid disease. They said he was not recently on any medications aside from levothyroxine and ursodiol, had no exposure to toxins, and did not receive any vaccinations within the last few months. He was also up to date on his routine heartworm prevention, Heartgard. Initial treatment options were discussed with the owners including hospitalization for IV fluid administration to prevent dehydration from the diarrhea and close monitoring of thrombocytopenia. We also discussed that the bruising was most likely not from improper handling as the clients had previously suspected, but part of their pet's disease process.

Pathophysiology of IMT

Immune-mediated thrombocytopenia accounts for 5-15% of cases of canine thrombocytopenia reported in dogs but is rare in cats.[3, 5] In cases of immune-mediated thrombocytopenia, a platelet count will reveal a severely decreased platelet number, often less than 50,000 platelets/uL.[3] Normal platelet counts range from 200,000-500,000 platelets/uL and spontaneous bleeding generally occurs at a platelet count of 25,000-50,000 platelets/uL or lower.[1,3] Immune-mediated destruction of platelets is a type II hypersensitivity reaction and occurs when the immune system produces antibodies that bind to platelets which are destroyed by macrophages prematurely.[1,6] In cases of primary IMT, gamma Immunoglobulin G (IgG) is the primary antibody involved and is directed against glycoproteins IIa and IIIb on the surface of the platelet. With secondary IMT, the antibodies target non-self antigens on the platelet surface or

altered platelet antigens that may have resulted from an underlying disease process.[6] Once the antibody binds to the antigen on the platelet, Fc receptors on macrophages recognize the antibodies and trigger phagocytosis. Anti-megakaryoctye antibodies can also form, causing destruction of platelet precursors at the level of the bone marrow.[7] Features of IMT include high levels of platelet-associated antibody, enhanced platelet destruction by the mononuclear phagocytic system, and markedly decreased circulating platelet numbers. Thrombocytopenia develops when platelet destruction exceeds platelet production.

Primary IMT is a spontaneous autoimmune disease, with antibodies being produced against platelets for no known reason. Secondary IMT may be initiated by a diverse number of disease processes, including neoplasia, inflammatory diseases, and infectious diseases, as well as recent exposure to medications or vaccinations.[8] Common types of neoplasia associated with secondary IMT include lymphoma, hemangiosarcoma, adenocarcinoma, leukemia, multiple myeloma and many others. Infectious causes can include *Ehrlichia canis, Anaplasma phagocytophilum, Anaplasma platys, Rickettsia rickettsii, Dirofilaria immitis* as well as many others. Many medications have been associated with secondary IMT in dogs, and the most common include antibiotics (especially sulfonamides such as TMS). Recent vaccinations, often with modified live vaccinations, are also thought to be a trigger for secondary IMT.[3] Primary IMT is diagnosed only when no other disease known to trigger IMT can be found. The thoroughness of the investigation for secondary causes will dictate the confidence in the diagnosis of primary IMT.

Clinical Presentation

Clinical features of dogs with IMT include a median age of 6 years old (age range from 8 months to 15 years), with females two times more affected than males. Common breeds affected

include Cocker Spaniels, Poodles, German Shepherds and Old English Sheepdogs.[5,6] When there is a defect in primary hemostasis, clinical signs such as petechiae, ecchymosis, mucosal bleeding, epistaxis, melena, hematochezia, hematemesis and scleral hemorrhage can be noted. Non-specific clinical signs, including lethargy, weakness and anorexia, are commonly seen.[3, 6]

Diagnostic Approach

Primary IMT is a diagnosis of exclusion and can be challenging. Definitive confirmation of IMT requires demonstration of circulating anti-platelet antibodies. Unfortunately, these tests are not readily available[6]. Diagnostics performed are aimed to rule out a secondary cause of IMT. A thorough history should always be obtained, and information surrounding recent drug exposure and vaccinations should be specifically asked about. A complete physical exam should be done to search for any clues for a possible secondary cause of the IMT.

Routine hematology is the first diagnostic step in a patient suspected to have IMT. Platelet number is often determined by a platelet count on an automated counter as seen on a CBC. This will help document the presence and severity of the thrombocytopenia. Immune-mediated thrombocytopenia often demonstrates a severely decreased number of platelets in circulation. A low platelet count should be confirmed by a blood smear analysis. In a normal blood smear, there should be between 8-15 platelets per high power field. If there is a decreased number of platelets but evidence of macrothrombocytes, this likely indicates that this is a regenerative process and the bone marrow is responding to the thrombocytopenia.[1] Additional findings on a CBC besides thrombocytopenia may include anemia or alterations in the white blood cell count. Anemia may be present due to blood loss from the thrombocytopenia or because of immune-mediated destruction of red blood cells in addition to platelets, a disorder known as Evan's Syndrome. With IMT, the neutrophil count may be normal or increased.

Increased numbers of segmented neutrophils may be seen simply due to a marrow stimulation which is present because of the low platelet count. If all cell lines are down, a bone marrow disorder should be suspected. Beyond the CBC and blood smear analysis, further diagnostics to be considered for identifying a possible secondary cause of IMT include serum chemistry testing, urinalysis, coagulation testing (PT and PTT), thoracic radiographs, abdominal radiographs, abdominal ultrasound, infectious disease testing, and lymph node aspiration. A bone marrow analysis may be indicated in cases that do not respond to immunosuppressive therapy to rule out megakaryocytic hypoplasia or aplasia.[3] In cases with primary IMT, additional diagnostic testing is within normal limits.

To summarize, primary immune-mediated thrombocytopenia is diagnosed when all other causes of thrombocytopenia have been ruled out using appropriate diagnostic testing. This diagnosis can be challenging because there is no one test to definitively confirm primary IMT, as it is a diagnosis of exclusion. Also, response to treatment with immunosuppression therapy may therapeutic as well as diagnostic.

Treatment and Management

When primary IMT has been diagnosed, it is important to start treatment rapidly to suppress the immune system and stop the destruction of platelets. While the goal of therapy is to bring the platelet count back to normal, ideal initial therapy is to raise the platelet count enough to stop spontaneous bleeding. The mainstay of treatment is glucocorticoid therapy because of its immunosuppressive effects. Glucocorticoids are used initially because they are effective, fast acting and affordable for clients. This can include dexamethasone, prednisone and prednisolone. The most common glucocorticoid used in dogs is prednisone, an intermediate- acting

corticosteroid. The immunosuppressive effects of glucocorticoids are multifactorial, with actions including downregulation of Fc receptors on macrophages, inhibiting phagocytosis by macrophages, reduction of antigen presentation and processing, inhibition of the release of proinflammatory cytokines IL-1 and IL-6, directly inhibiting T-cell function, and stabilization of cell membranes.[1,3,9] The recommended immunosuppressive dose of prednisone is 2mg/kg/day. Common side effects include polyuria, polydipsia, polyphagia, and muscle wasting. Dogs treated with prednisone therapy alone show a variable response to treatment with an increase in platelet numbers in 1-15 days.[9] Prednisone can be used alone or it can be combined with a second immunosuppressive agent.[3] A second immunosuppressive medication can be considered to further suppress the immune system beyond that of glucocorticoids. Also, because dogs are so susceptible to the side effects of glucocorticoids, a secondary immunosuppressive medication is often used so that the dosage of glucocorticoids can be reduced sooner in the course of therapy. Secondary immunosuppressive medications which can be considered include mycophenolate mofetil, azathioprine, cyclosporine, and leflunomide. Other secondary treatments for IMT in dogs which can be considered include vincristine, human IVIG, and splenectomy.[3]

Secondary oral immunosuppressive drugs in addition to steroid therapy may be indicated if severe side effects of steroid therapy are present or if the IMT is refractory to sole steroid therapy. Common immunosuppressive agents used include mycophenolate mofetil, azathioprine, cyclosporine, and leflunomide. All of these drugs have pros and cons with their use, and the specifics of each drug should be considered for each individual case. Mycophenolate mofetil was the drug of choice used to treat the present case in discussion, and will be expanded upon here. Mycophenolate mofetil is the prodrug form of mycophenolic acid. This drug inhibits synthesis of purine nucleotides by inhibiting a key enzyme, inosine monophosphate

dehydrogenase (IMPDH). This leads to suppression of B and T lymphocytes and the subsequent decrease in antibody production.[3,10] The immunosuppressive effects appear to be as fast in onset as other secondary immunosuppressive options such as cyclosporine, and this drug is generally well tolerated, with the exception of the common side effect of gastrointestinal upset. The dose often used to treat primary IMT is 10mg/kg PO q12h.[3] In one study, mycophenolate was used as the only agent to treat 5 dogs with IMT and showed adequate results for treatment. In this study, all 5 dogs obtained remission of disease with a median dosage of mycophenolate of 8.5 mg/kg PO BID, and the median time for platelet counts to reach above 50,000/uL was 3 days.[10]

Vincristine and human intravenous immunoglobulin (hIVIG) are two treatment options that are administered in hospital and are associated with faster platelet recovery and shorter hospitalization time. Vincristine is a vinca alkaloid that is used as an antineoplastic and immunosuppressive agent. While the exact mechanism of action is not fully understood for vincristine, it is thought to inhibit phagocytosis of platelets, interfere with antibody formation, prevent antibody binding to platelets, and accelerate fragmentation of megakaryocytes to release more platelets into circulation.[11] Vincristine is administered intravenously as a single dose at 0.02mg/kg.[3, 11] Side effects include vomiting, diarrhea, and perivascular sloughing with extravasation.[11] At high doses, bone marrow suppression has been noted but is not common with the dose used to treat IMT. The median time for increased platelet count after vincristine administration is 3 days.[3] Human IVIG is another treatment that has also been shown to increase platelet numbers rapidly but is expensive. hIVIG is made from plasma from over 1,000 healthy human donors and contains IgG along with IgM, IgA, CD4 and CD8.[12] The mechanism for stopping platelet destruction with hIVIG is that the immunoglobulins bind to the Fc receptors on canine macrophages to prevent phagocytosis. Adverse effects include anaphylaxis, fever, and

thromboembolic events but are not commonly reported. The dose range is 0.25-1.5g/kg administered IV over 6-12 hours. Limitations of use include availability, expense and time for drug administration. Response time of platelet number is similar to vincristine.[9,11] Splenectomy can also be considered in refractory cases of IMT when nothing else seems to be working. The rationale for a splenectomy is that the spleen is the primary site of destruction of platelets with IMT.[3,9]

Another drug that was recommend due to the refractory nature of this case, was melatonin. Melatonin has been shown to stimulate platelet generation most likely by megakaryocyte fragmentation.[9] The dose suggested is 3 mg PO q12h for dogs weighing less than 20 kg, and 6mg PO q12h for dogs weighing more than 20 kg.[13]

In cases where there is continued bleeding and secondary anemia, blood transfusion products may be indicated. Platelet transfusions are not usually indicated because of the rapid platelet destruction occurring with IMT. Commonly, fresh whole blood transfusions can be used to replace red blood cell volume, and this product also contains platelets. Other platelet rich products include platelet rich plasma, platelet concentrate and frozen plasma concentrate, but these products are not as commonly used due to processing, availability and expense.[1,3]

Supportive care is also indicated with primary IMT. Patients should be carefully handled to prevent further bleeding. Only necessary diagnostics should be performed to limit the amount of venipuncture and hemorrhage. Cage rest and exercise restriction are recommended to prevent bleeding. Because steroid usage can be associated with a concern for the development of gastric ulcers, other medications such as H2 blockers, proton pump inhibitors and GI mucosal protectants may be considered.[3]

Patients are generally discharged from the hospital when platelet counts begin to increase and clinical signs are resolving. Cases of primary IMT are usually treated with high dose immunosuppressive medications for a minimum of 2-4 weeks, with the tapering of medications occurring over time as indicated by the response to therapy. Patients are generally treated over a 4 to 6 month time frame, with some patients needing to be managed medically for life. Once the platelet counts have normalized, tapering of immunosuppressive agents can be initiated. Only one drug should be tapered at a time and the choice of which to taper is often determined by cost and drug side effects. To taper medications, one medication should be decreased by about 25% every 3-4 weeks. A platelet count assessment is performed before any tapering of immunosuppressive medications to ensure a disease relapse has not occurred. Response is monitored by CBC results and clinical signs.[1,3] Discontinuation of all medications is indicated when the sole immunosuppressive medication is being administered at a very low dosage every other day and blood work results have remained within the normal range.[3] Prognosis for primary IMT is good to guarded depending on response to treatment, with survival rates ranging between 74-93%. Initially, poor prognostic indicators are melena and elevated BUN.[7] Relapse has been reported in 9-50% of patients.[1,3]

Case Outcome

The patient was hospitalized in the ICU for close monitoring. IV fluids were administered for ongoing fluid loss due to his diarrhea, which consisted of Plasmalyte with the addition of 2mL of B vits/L, started at an initial fluid rate of 15ml/hr. Additional medications started on Day 1 of hospitalization included hydromorphone (0.1mg/kg IV as needed for pain) and metronidazole (10mg/kg IV q12h). On the morning of Day 2, the patient was stable with a

normal TPR, but the petechia and ecchymosis were noticed to be spreading across his entire body. He continued to have hematochezia and show no interest in food. Initial blood work performed on Day 2 consisted of a PCV/TP (PCV of 45%, TP of 7 g/dL) and hyperglycemia (211 mg/dL). A CBC performed at 5 pm on Day 2, about twenty hours after hospitalization, showed a mild anemia (HCT 29.7%), and a platelet count of zero. That evening he started to eat Hill's i/d and was transitioned to oral prednisone at 1mg/kg SID. He was also started on famotidine 5mg PO SID.

On the morning of Day 3, his stool was formed but dark, consistent with melena. His PCV remained stable at 26.9%, and his petechia and ecchymosis were stable from the previous day. A CBC continued to show zero platelets, which was confirmed with a blood smear. An Idexx 4Dx Snap test was performed at this time and was negative for all agents tested. On Day 3, vincristine (0.02mg/kg IV) was administered, and the prednisone dose was also increased to 2mg/kg orally once daily. He was also started on Carafate (½ gram PO TID) and transitioned to oral metronidazole. The IV fluid rate was decreased to 6ml/hr.

On Day 4, a CBC was performed at 8 am, which showed a platelet count of zero. The HCT had now dropped from 27% down to 18%. These findings were confirmed with manual PCV and a blood smear analysis. His heart rate, respiratory rate and attitude were normal at this time. The owners were made aware of the drop in HCT and the potential need for a blood transfusion, however, a transfusion was not clinically indicated at this time. In attempt to provide additional immunosuppression due to his persistently low platelet count, mycophenolate mofetil therapy (100mg/ml oral suspension dosed at 10mg/kg BID) was started. Overnight he vomited a few times, most likely secondary to the mycophenolate. He was started on Cerenia

(1mg/kg IV q24h) and transitioned back to IV dexamethasone SP, IV metronidazole and IV famotidine. Overnight thoracic and abdominal radiographs were performed. The thorax radiographs showed no abnormalities, and the abdominal radiographs showed mild hepatomegaly.

On the morning of Day 5, the patient was clinically stable and bright. His stool was formed and brown. The petechia and ecchymosis were beginning to resolve. A CBC showed a stable HCT at 18% and a platelet count of zero. His appetite was normal and he was transitioned back to oral medications. He did great throughout the day, but started to appear lethargic overnight. Recheck bloodwork the morning of Day 6 showed a PCV of 14% and TP of 5.2g/dL. His CBC continued to show a HCT of 18% and zero platelets. A slide agglutination test was performed to rule out a concurrent immune mediated hemolytic anemia, consistent with Evan's Syndrome. Evan's syndrome is characterized as immune mediated thrombocytopenia and concurrent immune mediated hemolytic anemia. This test was macroscopically and microscopically negative. At this time, a consult call was placed to Critical Care Consults for a second opinion. A whole blood transfusion at 20ml/kg was recommended in addition to administering human IVIG. The consultation also emphasized that it could take 5-7 days to see a response but to continue with the current therapy.

That afternoon a whole blood transfusion of 100 mL (20ml/kg) was administered over 8 hours. No reactions or complications were noted during the blood transfusion. Post-transfusion the patient was bright and alert, and the post-transfusion PCV was 19.6% and TP was 6.2g/dL. Overnight he rested comfortably and was monitored closely for any signs of transfusion reaction. On the morning of Day 7, a CBC was performed and revealed a HCT of 25.7% and a platelet

count of 3,000/uL. The platelet count was confirmed with a blood smear analysis. The patient was scheduled to be discharged that afternoon. Medications to go home included prednisone (2mg/kg SID), mycophenolate (10mg/kg BID), metronidazole (13mg/kg BID), famotidine (1mg/kg BID) and melatonin (3 mg SID). A recheck was scheduled for 24 hours after discharge. On Day 8, the CBC showed a HCT of 35.1% and a platelet count of 69,000/uL. The anemia at this time was considered regenerative, as indicated by an elevated reticulocyte count. The patient was doing great at home on all medications. No signs of melena or hematochezia were noted.

Recheck on Day 11 revealed that the patient had improved and was doing great! A CBC was performed, showing a HCT of 36.5% with a high reticulocyte count, consistent with a regenerative anemia. Platelets were well into the normal range, measuring 258,000/uL. All medications were continued as previously instructed at discharge. A recheck was scheduled in one week. In this patient, it took six days to start to see an increase in platelet numbers. The approach used to treat this case of primary IMT was multimodal therapy including steroids, mycophenolate mofetil as a secondary immunosuppressive medication, vincristine, a whole blood transfusion, as well as supportive care. The continued management included the monitoring CBCs with a controlled tapering of medications. So far, this patient has had a positive outcome, but relapse is a possibility. Hopefully this will be avoided with proper monitoring and drug tapering.

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