Gracie's Gargantuan Gut

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Introduction

When defining abnormalities on blood work, a decrease in the number of platelets is referred to as a thrombocytopenia. There are four main mechanisms that can result in a thrombocytopenia. These include reduced production of platelets, platelet consumption, abnormal platelet sequestration, and platelet destruction.[1] A common cause of severe thrombocytopenia resulting from destruction is immune mediated thrombocytopenia (IMTP). IMTP is a disease process where the body's immune system targets and destroys platelets. Similar to immune mediated hemolytic anemia, IMTP can either not have an identifiable underlying cause (primary) or be the result of another systemic disease process such as neoplasia, infectious processes, medication reactions, or inflammatory (secondary).[1,2] The classic presentation for canines and felines with IMTP mimic the presentation of general thrombocytopenia which include petechial hemorrhages, mucosal bleeding, melena, epistaxis, hematochezia, hematuria, and hematemesis. As with all immune mediated disease, the mainstay of therapy includes immunosuppressive drugs. The most common of which are glucocorticoids (e.g., prednisone).[2,3,5] Depending on underlying cause, prognosis is usually good with appropriate treatment, though relapse is somewhat common.[3] The following case takes a look at an unusual presentation of immune mediated thrombocytopenia.

History, Presentation and Diagnostics

Gracie is an approximately 9-year-old, spayed female, mixed breed canine that presented to the Mississippi State University College of Veterinary Medicine on June 1st, 2020 on referral for an abdominal mass of unknown origin. The primary veterinarian first noted that her abdomen was firm on palpation in January of 2020. The owner and primary veterinarian noticed that the abdomen was enlarging over time and in April 2020 it was noted that the left side of the

abdomen was noticeably larger than the right. Finally, the owner noticed that the abdomen was enlarging at a faster rate between April and May. Abdominal radiographs were taken, which showed a mass effect in the abdomen of suspected splenic origin. No other diagnostics were performed. Prior to being seen for this, Gracie was seen at MSU-CVM's oncology service in May of 2019 for a nodule on her left mandible that was diagnosed as a liposarcoma on histopathology. Other than the enlarged abdomen, the only other complaints were a slightly decreased appetite and vomiting once the day prior to presentation.

On the initial physical exam Gracie was bright, alert and responsive. She weighed 17.4kg and had a body condition score of 3/9. Her mucus membranes were pink and moist with a capillary refill time of less than 2 seconds. Her vital perimeters were abnormal with a heart rate of 80 beats per minute, a respiratory rate of 72 breaths per minute and a rectal temperature of 102.6^{o}F. No ocular or nasal discharge was present. No to mild debris was present in both ear canals. Moderate to severe tartar was noted on most teeth. Cardiopulmonary auscultation was normal with no crackles, wheezes, murmurs or arrhythmias heard. Her abdomen was firm and distended, and individual organs could not be identified on palpation. No abnormalities were palpated on rectal exam. There were no enlarged peripheral lymph nodes. The remainder of the physical exam was unremarkable.

Initial diagnostics consisted of an abdominal ultrasound, thoracic radiographs, complete blood count and chemistry panel. There were no clinically significant findings on the thoracic radiographs, including no signs of metastatic neoplasia. On abdominal ultrasound, the spleen was severely enlarged with rounded margins. After confirming that the cause of her enlarged abdomen was the spleen, the initial plan was to perform a fine needle aspirate of the spleen. However, prior to the ultrasound Gracie's abdomen was shaved and while the ultrasound was

occurring, petechial to ecchymotic hemorrhages became apparent. This prompted a more thorough exam which led to finding a few mild petechial hemorrhages in her left pinna and the medial aspect of her left hindlimb. It is unknown whether these petechial hemorrhages were present at the time of the initial physical exam or occurred due to normal patient handling or the physical exam. Due to the risk of excessive hemorrhage, fine needle aspirates were not performed on the spleen at the time of ultrasound. Instead, it was elected to wait until the laboratory results were received.

The complete blood count showed a macrocytic, hypochromic anemia with hematocrit of 27.7% and a packed cell volume of 28.0%, a severe thrombocytopenia of 10,000/µl platelets and a moderate lymphopenia of 348/µl (1,100 - 4,800). The only significant finding on the chemistry panel was a mild hyperchloremia of 126.5 mmol/L (106.0 - 122.0). A pathologist review of the CBC confirmed the thrombocytopenia with a manual platelet count that also showed only a few megakaryocytes and no platelet clumping. After confirming the thrombocytopenia, a coagulation profile 4Dx SNAP test, and reticulocyte count was performed. On the coagulation profile, PT and PTT times were within reference range. The reticulocyte count was 0.2% uncorrected and 0.12% corrected. On the 4Dx SNAP test, Gracie was positive for *E. canis/ewingii* antibodies but negative for *D. immitis*, *B. burgdorferi*, and *A. phagocytophilum/platys.*

With the current physical exam and diagnostic findings, a presumptive diagnosis of immune mediated thrombocytopenia was made. Other potential rule outs included megakaryocyte aplasia/hypoplasia and thrombocytopenia resulting from an *E. canis* infection. It was elected to hospitalize her and pursue empirical treatment to correct the severe thrombocytopenia before doing further invasive diagnostics to determine the exact cause of it.

Pathophysiology

Immune mediated thrombocytopenia is an autoimmune disease characterized by the host's immune system targeting platelets for destruction. In primary IMTP, this cycle occurs spontaneously with no external factors. Platelet destruction can occur in peripheral circulation and megakaryocytes can be targeted within the bone marrow.[4] There are several mechanisms occurring within the immune system that allow this destruction. The humoral component is responsible for targeting and binding. This is accomplished by autoantibodies targeting platelet membrane antigens, specifically glycoproteins IIb and IIIa.[1,2] IgG is the main antibody involved but IgM is also involved.[5,6] While not fully understood, it is assumed that complement plays a role in mediating attachment of immune complexes to platelets.[5,6] Many IMTP patients also have an increase in T helper cytokines that promote a cell mediated response leading to increases in macrophage function, autoreactive B-cell development, and T-cell cytotoxicity.[4] In secondary IMTP, the antigens are absorbed into the platelet membranes from an external factor such as neoplasia (most commonly lymphosarcoma or hemangiosarcoma), infectious processes (such as rickettsial diseases), medications, vaccines, or inflammatory processes.[1,2]

In both types of IMTP, clinical presentation is the same. The most common and apparent clinical signs stem directly from the reduced platelet count include petechial hemorrhages, mucosal bleeding, melena, epistaxis, hematochezia, hematuria, and hematemesis. Even though most signs involve some form of hemorrhage, intracavitary bleeding is uncommon unless it is due to a secondary disease process such as hemangiosarcoma. Non-specific signs that patients may present with include lethargy, inappetence, or splenomegaly. Diagnosis is usually based on exclusion of other potential causes. Specific diagnostic tests for the associated antibodies do exist, however, due to availability and cost these are usually not performed. Instead, most

diagnostics center around commonly performed tests such as complete blood counts and blood smears to observe platelet numbers. Manual platelet counts on blood smears should be performed to ensure the accuracy of laboratory machine results as platelet clumping may give an inaccurate count. Should you encounter a severe thrombocytopenia that is repeatable with a manual count, attempts should be made to collect the blood and perform the smear directly from the patient to rule out pseudothrombocytopenia. Coagulation profiles should be performed to rule out potential coagulopathies. In the IMTP patient, these results should be normal. Another diagnostic that may be performed is a bone marrow analysis, to rule out megakaryocyte aplasia/hypoplasia.

Immunosuppressive therapy is the most commonly used treatment approach in cases of primary IMTP.[2,3,5] Glucocorticoids such as prednisone are usually the first choice by most clinicians.[1,2,3] Steroids work by inhibiting macrophages, thus sparing the platelets that are targeted for destruction.[7] Additional therapeutics that can be used concurrently with glucocorticoids include administration of vincristine, platelet transfusions and human immunoglobulin administered intravenously. Second line therapies include but are not limited to cyclosporine, azathioprine, melatonin, and splenectomy.

Treatment and Management

Gracie was hospitalized at her initial referral appointment to start receiving therapy for IMTP. Prednisone was started at 2mg/kg by mouth every 24 hours and doxycycline 5mg/kg by mouth every 12 hours. Serial PCV/TP and blood smears were performed daily to monitor for a platelet response and ongoing blood loss. On 6/3/2020 she was given a 0.02mg/kg dose of vincristine to stimulate an increase in platelet reduction. The following day, the hindlimb that the vincristine injection was administered in was edematous and diffusely covered in ecchymotic hemorrhages. While hospitalized, her lowest PCV was 23% and the highest platelet count

observed was approximately 14,000/µl. On 6/6/2020 cyclosporine was started, in addition to the prednisone and doxycycline, at 6mg/kg by mouth every 12 hours. Gracie was discharged on 6/10/2020 after not having an improvement in her platelet count. Her owner's were instructed to continue the medication regime, have her platelet count checked by her primary veterinarian and then return to MSU-CVM when her platelet count was greater than 50,000. At this time, a fine needle aspirate of the spleen would be performed to continue diagnostics.

On 6/18/2020 Gracie returned to MSU-CVM following a platelet count of 65,000 at her primary veterinarian. Unfortunately, the CBC done at this appointment showed a similar platelet count as the initial visit (approximately 10,000/µl) and the anemia (PCV of 21.5%) was still present. Melatonin was started at 3mg by mouth every 12 hours and she was discharged with the same instructions as before. This situation repeated itself on 7/15/2020, when Gracie returned for another appointment following an adequate platelet count at her primary veterinarian. She was again discharged with the same instructions and medication regime.

On 8/14/2020 Gracie presented to a specialty clinic where they found that her anemia was worsening (18%) and that her platelet count was still low. They performed a splenic FNA that showed a reactive spleen and was consistent with extramedullary hematopoiesis. At this point, the owner elected to have a splenectomy. On 8/31/2020 she presented to be hospitalized for her splenectomy that would occur the following day. Her CBC results were consistent with her previous results and her anemia was still severe with a PCV of 22%. On the morning of surgery (9/1/2020) a blood transfusion of 450ml of DEA 1.1+ blood was started. Once she received approximately half of the transfusion, Gracie was anesthetized for her splenectomy. After removing the spleen during surgery, she became hypotension and had an assumed vagal event

resulting in a brief period of cardiac arrest followed by spontaneous return to normal cardiac function. The remainder of the surgery and recovery from anesthesia was uneventful.

Case Outcome

The spleen was submitted for biopsy where the findings included: severe diffuse splenic vascular congestion with histiocytosis, plasmacytosis, extramedullary hematopoiesis, severe lymphoid depletion of the white pulp, rare intravascular fibrinous thrombi, multifocal capsular fibrosis and siderofibrotic plaques. These findings were not consistent with any one diagnosis but the top differentials were immune mediated thrombocytopenia and rickettsial diseases. No neoplastic features were observed.

A follow-up CBC was completed the day after Gracie's splenectomy, which had a platelet count of 116,000/µl. She remained hospitalized for 3 days in the ICU to ensure she was not having clinical signs from her anemia and give her time to transition to oral analgesics from intravenous analgesics. After discharge a plan was established with her primary veterinarian to begin weaning her off of the prednisone.

Gracie's owner was instructed to have routine CBC's completed to ensure her platelet count remains adequate and that her anemia is resolving. Since discharge, the CBC's received by MSU-CVM have shown a variance in platelet counts ranging from approximately 20,000/µl to 64,000/µl and a HCT between 26.2% to 35.8%. When asked for an update on 12/8/2020, her owner reported that the most recent platelet count was approximately 110,000/µl. He also stated that Gracie has been doing great, is regaining her appetite and is about 4lbs heavier than she was prior to surgery.

Conclusion

Immune mediated thrombocytopenia (IMTP) is a common autoimmune disease resulting in a dysfunction of primary hemostasis by destroying platelets and megakaryocytes. Patients typically present with hemorrhages on mucosal surfaces but may show other signs of bleeding. However, cavity bleeding is rare, and coagulopathies do not occur as coagulation factors are intact. Primary IMTP is usually diagnosed by exclusion, in a process that rules out any of the common causes of secondary IMTP. Once secondary causes are ruled out, treatment usually consists of glucocorticoids with concurrent therapy such as vincristine or adjunct immunosuppressants such as cyclosporine. Treatment is usually lifelong as relapse is somewhat common. Even with ongoing treatment, monitoring of platelet and red blood cell counts should be performed to ensure a thrombocytopenia or anemia is not present.

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