Immune Mediated Thrombocytopenia

Rebecca Roles Gelé

Mississippi State University College of Veterinary Medicine

Class of 2018

Clinicopathologic Conference

Presented on January 12, 2018

CPC Advisor:

Dr. Alyssa M. Sullivant, DVM, MS, DACVIM

Introduction

Immune mediated thrombocytopenia (IMT) is a common cause of bleeding in small animal patients, especially canines.[2] There are many different causes of IMT, but it is characterized by decreased platelet lifespan, enhanced platelet destruction by the mononuclear phagocytic system and high levels of antibodies associated with platelets.[2] The normal platelet count in canines is between 200,000-500,000/uL, and spontaneous hemorrhage isn't seen until marked thrombocytopenia occurs below 50,000 platelets/uL.[6] One of the major obstacles in treating IMT is distinguishing secondary and primary disorders in order to guide your treatment plan.

History and Presentation

IMT most commonly affects middle-aged female dogs, but any breed or sex may be affected.[2] The average age of onset is about six years old, and the breeds that are more predisposed include: poodles, English sheepdogs, and cocker spaniels.[2] It is crucial to get a thorough history on these patients. Some important history questions include: travel history, recent vaccinations, current medications, and is the patient on monthly tick-preventative? These are all important questions that can be helpful in determining whether or not the patient has primary or secondary IMT. The physical exam findings that are the hallmark sign of thrombocytopenia are petechial hemorrhages. These pinpoint hemorrhages can be found throughout mucosal surfaces including the oral, nasal, conjunctival and urogenital mucosa.[2] Cutaneous and mucosal petechiae can often merge and become larger ecchymotic bruising that is commonly found throughout the ventrum.[2] You may also see evidence of mucosal bleeding in the form of epistaxis, hematemesis, melena, hematochezia and hematuria.[2] Clinical signs

associated with IMT are very nonspecific and are typically due to anemia from blood loss and include: weakness, lethargy, anorexia, pyrexia, pale mucous membranes, tachycardia, heart murmur, tachypnea and bounding pulses.[1] During physical exam it is important to look for any potential underlying causes of the IMT, for example an abdominal mass, organomegaly, lymphadenopathy, or crackles and wheezes on lung auscultation.

Pathophysiology

Platelet role in Hemostasis

There are two steps to hemostasis, known as primary and secondary hemostasis, and platelets are crucial to both of these processes. Primary hemostasis is defined as the formation of a platelet plug.[4] Typically, an intact endothelium acts as a physical barrier between circulating platelets and substances that induce thrombosis located in the extracellular space.[4] When the endothelium is injured, the subendothelial matrix is exposed and initiates primary hemostasis.[4] Primary hemostasis has three main events: platelet adhesion, platelet activation, and platelet plug formation.[4] Platelets bind to exposed matrix proteins to initiate platelet adhesion.[4] They adhere to exposed collagen and interact with a series of different receptors on the cell surface known as glycoproteins.[4] The adhesion of platelets to the matrix of the exposed endothelium activates platelets leading to a change in shape from discoid to a more elongated cell with cytoplasmic extensions, therefore increasing their surface area.[4] The activation of platelets is important because it leads to their degranulation of alpha dense granules.[4] Alpha granules contain Von Willebrand factor and coagulation factors including fibrinogen, factor X and factor XIII.[4] Dense granules are rich in platelet agonists that help recruit and activate additional platelets such as ADP and serotonin.[4] The final step of primary hemostasis is platelet

aggregation. This is mediated primarily by fibrinogen which binds to its activated receptor (GPIIb/IIIa) on the surface of the platelet.[4] This binding allows for platelets to link together and form a platelet plug that is sufficient enough to stop small blood vessel bleeding.[4] If more serious vessel damage occurs, the platelet plug has to be stabilized in order to stop bleeding, which is accomplished through secondary hemostasis.[6] Platelets are necessary to initiate amplification and propagation of thrombin as well as fibrin formation.[6] Activated platelets promote fibrin formation and are a physical network for fibrin formation.[4] A normal platelet count for canines is between 200-500,000/uL.[6] When platelet levels are between 30,000-50,000/uL or less, spontaneous bleeding can occur.[5] This leads to the hallmark petechial and ecchymotic hemorrhages seen with moderate to marked thrombocytopenias.[5] It is important to mention that there is constantly a balancing act occurring between pro- and anti-coagulants. The endothelium of blood vessels typically acts to inhibit coagulation when it is intact.[3] Normal blood flow reduces the interaction between clotting factors and acts as an anti-coagulant.[3] Scavenger proteins will bind and get rid of activated coagulation factors in circulation to prevent coagulation, and there are anti-coagulants such as anti-thrombin III.[3]

Platelet Pathophysiology

The process of platelet production is known as thrombopoiesis. At a stable platelet count the level of platelet production and destruction is constant.[3] The shorter the platelet survival, the higher the resulting platelet production and turnover rate.[3] There are four main pathways that cause thrombocytopenia. Platelets are either being destroyed, not enough are being produced, they are being consumed, or there is unequal distribution and sequestration of platelets.[1] Thrombopoietin (TPO) is the protein responsible for regulation of platelet

production. Thrombopoietin is mostly produced in the liver and binds to a receptor (c-Mpl) on the surface of megakaryocyte membranes.[3] This binding of TPO to its receptor stimulates platelet production and megakaryocyte proliferation and differentiation.[3] At high platelet counts TPO is taken up by platelets and internalized so that there is very little TPO remaining in circulation to bind free megakaryocytes, therefore inhibiting platelet production and maintaining homeostasis.[3] A drop in platelet counts results in increased TPO and increased megakaryocyte binding to its receptor.[3] The average lifespan of a platelet in canines is five to seven days.[6] In patients with IMT the average survival is drastically decreased to as little as less than one hour.[2] Platelet lifespan is inversely correlated to platelet-associated antibody levels, so the greater the antibody the shorter the lifespan of platelets due to immune-mediated destruction.[2] IMT patients are typically able to increase their production of platelets to turnover rates as high as ten times that of normal patients.[2] This is one target area of treatment of IMT, increasing platelet survival times in our patients with IMT. In rare cases, the immune system can create antibodies against megakaryocytes located in the bone marrow.[15] This leads to what is known as megakaryocytic aplasia.[15] Megakaryocytic aplasia is more severe and typically does not respond as well to immunosuppressive therapy, if at all, because antibodies are directed towards the stem cells that make platelets.[15]

With immune-mediated thrombocytopenia (IMT), platelets are being destroyed by the immune system. This occurs via antibody production directed towards glycoproteins on the platelet surface. Once the antibodies bind to the antigens on the platelet surface these platelets are cleared by the mononuclear phagocyte system.[1] The spleen is the major organ involved in removing the platelets, as well as a source of the anti-platelet antibodies.[2] IMT

patients are having their platelets destroyed faster than their own platelet production. Antibodies directed towards glycoproteins on the surface of platelets known as GP IIb/IIIa and GP Ib/IX are produced by the body and can also lead to overall platelet dysfunction in IMT patients.[2] GPIIb/IIIa is the fibrinogen receptor located on platelets.[4] This receptor is necessary for platelets to form platelet aggregates and plugs.[4] These glycoproteins are essential for normal platelet function, so even the few platelets that IMT patients have may not be functioning normally due to these disturbances.[2]

Just as with immune-mediated hemolytic anemia, there are two types of IMT, primary and secondary. Secondary means that there is an underlying cause of the IMT. These secondary causes can be infectious, drug-induced, neoplastic, or potentially vaccine-induced.[1] Some of the common infectious causes of IMT in dogs are ehrlichiosis, Rocky Mountain spotted fever, anaplasmosis, histoplasmosis, leishmaniasis and distemper.[1] Multiple malignant neoplasias are assoiciated with thrombocytopenia, the most common include hemangiosarcoma, lymphoma, and melanoma.[14] Primary IMT is a spontaneous autoimmune disease that is typically a diagnosis of exclusion of any of the other secondary causes.[2]

Differential Diagnoses

It is important to rule out disseminated intravascular coagulation and platelet consumption or sequestration when diagnosing immune-mediated thrombocytopenia.[13] Bone marrow disease, leukemia and paraneoplastic conditions are also on the differential diagnosis list for thrombocytopenia.[13] There are many causes of secondary IMT that need to be ruled out in order to confirm a diagnosis of primary IMT, which is a diagnosis of exclusion.

Diagnostic Approach

The first step in diagnosing IMT is running routine bloodwork. Typically, patients with primary IMT will have drastically low platelet counts of less than 10,000/uL.[2] It is important to perform a direct blood smear to look for platelet clumping and megathrombocytes, which often lead to a misdiagnosis of IMT.[2] Typically a diagnosis of primary IMT is reached when the following criteria are met: moderate to severe thrombocytopenia, increased thombopoiesis and no evidence of DIC, platelet sequestration or destruction, and response to immunosuppressive therapy.[2] Ideally, to confirm a primary IMT diagnosis, you would be able to demonstrate that there are anti-platelet antibodies present at high levels.[2] Currently, there are not any methods that have a high enough sensitivity for primary IMT to rely on.[2] In order to diagnose primary IMT the following tests should be run to rule out a secondary problem: complete blood count with reticulocyte count if anemic, chemistry panel, urinalysis, coagulation profile, thoracic and abdominal radiography, retroviral testing in cats, bone marrow biopsy, rickettsial PCR and/or tick-borne disease titers, and a doxycycline treatment trial.[2] In older animals, it may be necessary to perform an abdominal ultrasound.[2] Often, client funds will be limited and an extensive workup to rule out other causes might not be possible. A response to immunosuppressive therapy and doxycycline trial may be done in these cases as long as the clients are fully informed that there might be an underlying cause leading to the thrombocytopenia that will not fully respond to, or may be worsened by, immunosuppressive therapy.

Treatment and Management Options

If a patient has secondary IMT, the most important treatment option is to treat the underlying cause. Often, rickettsial diseases can be difficult to diagnose so our canine patients

are typically put on doxycycline as well as immunosuppressive therapy until we get the results of a tick-borne panel.[7] For primary IMT, the treatment goals target three major mechanisms: decreasing antibody synthesis, decreasing the binding affinity between antibodies and platelets, and decreasing the destruction of antibody-coated platelets by the mononuclear phagocytic system.[7] The staple for treatment is glucocorticoid therapy starting at 2-4 mg/kg/day dose.[7] In the short-term, glucocorticoids act to decrease the destruction of platelets by the MPS, but can take 3-7 days to see an increase in platelets.[7] Steroids may also help decrease the overall production of antibodies as well as help support capillaries, decreasing bleeding tendencies in IMT patients.[9] Some patients take as long as one month to respond to glucocorticoids alone, so it is often necessary to start patients on a second immunosuppressive drug.[7] Side effects that are commonly seen with glucocorticoids include PU/PD, panting, polyphagia, increased liver enzymes, muscle atrophy, and GI ulceration.[9] Often, the choice of second line immunosuppressive agent is clinician dependent based on anecdotal information and/or comfort level with the medication. There are a number of options, most of which have not been investigated thoroughly in clinical studies.[12] Patients that do not respond to a second immunosuppressive may need a third drug with a different mechanism of action.

Vincristine

Vincristine is a chemotherapy medication that is used at lower doses (than chemotherapy doses) to stimulate megakaryocyte endomitosis allowing for maturation and release of platelets from the bone marrow.[9] Vincristine has been shown to stimulate thrombopoieisis in healthy dogs, but typically, IMT patients already have ramped up levels of thrombopoiesis. Vincristine may reduce phagocytosis of platelets by macrophages as well as

decrease antibody synthesis and binding.[8] Dogs that receive prednisone and vincristine in combination have significantly faster increases in platelet counts (5 days +/- 1 day SD, 6.8 days +/- 4.5 days SD) compared to those who just receive prednisone as well as shorter hospitalizations (5 days vs 7 days).[8] Vincristine is generally well tolerated but some of the side effects are anorexia, vomiting, diarrhea, and myelosuppression.[9] Vincristine is very cost effective, especially since it is given one time early on in treatment, and is about $8 for a 20kg dog.[8]

Human Intravenous Immunoglobulin

Human intravenous immunoglobulin (hIVIg) is sterile Ig preparation that contains 90% IgG from healthy human donors.[9] There are trace amounts of IgM, IgA, CD4, CD8 and human leukocyte antigen molecules.[10] The mechanism of action has been described as a blockage of the Fc receptors on mononuclear phagocytes, therefore decreasing B cell antibody production.[9] In one study with 18 dogs, there was a significant decrease in the days until platelet count increased above 40,000/uL in dogs that received hIVIg compared to the placebo group that received only glucocorticoids.[10] In the same study, all dogs in the hIVIg group responded by day 7 of therapy, whereas the placebo group of 9 dogs had four that that failed to respond to glucocorticoids alone during those 7 days.[10] There were no adverse effects due to hIVIg in this study that followed up with the patients for the first six months following discharge, but side effects can include allergic reactions secondary to foreign proteins.[10,9] The cost of hIVIg treatment for IMT patients is a limiting factor in veterinary medicine. The one-time treatment for a dog can be anywhere between $500-$1,000.[9,10] One study comparing the use of vincristine and hIVIg in IMT patients receiving glucocorticoid therapy did not find a significant difference

between the average platelet recovery times in the two groups, or time of hospitalization; however, there was a significant price difference in the two groups.[11] The group that received vincristine had an average cost of about $2,500 and the hIVIg group had an average cost of about $4,000.[11]

Second Line Immunosuppressive Therapies

Azathioprine

Azathioprine is a thiopurine analog which has a mechanism of action of competing with endogenous purines for incorporation into RNA and DNA creating, nonfunctional RNA and DNA.[9] This drug has a narrow therapeutic range and is typically started at a 2 mg/kg dose.[9] Side effects include gastrointestinal, myelosuppression including pancytopenia, pancreatitis and hepatitis.[9] Azathioprine should not be used in cats due to such a narrow therapeutic window and severe myelosuppression.[9] Azathioprine is not ideal for emergency situations because it can take at least a few weeks to reach peak efficacy, and up to as long as six weeks.[9]

Cyclosporine

Cyclosporine inhibits phosphatase activity and therefore inhibits the transcription of many cytokines that are needed for maturation and proliferation of T-cells, in particular IL-2.[12] The dose used in canines is 5-10 mg/kg daily day in order to maintain drug concentrations between 400-600 ng/mL.[12] Monitoring of IL-2 levels are likely a more reliable way to monitor cyclosporine and is available through the Mississippi State University College of Veterinary Medicine Pharmacodynamic Laboratory. Cyclosporine is generally well tolerated in canine patients, and can be a sole agent used as glucocorticoids are tapered. The side effects of

cyclosporine include vomiting, diarrhea, anorexia, weight loss, gingival hyperplasia, predisposition to secondary infections, and alopecia.[12]

Mycophenolate mofetil

Mycophenolate is a newer immunosuppressive that is more affordable than cyclosporine and gaining interest in veterinary medicine.[14] The mechanism of action is by inhibiting inosine monophosphate dehydroxygenase (IMPDH) production by B and T lymphocytes.[14] By blocking this compound, guanosine triphosphate is decreased leading to a reduction in DNA production.[14] The most common side effects are gastrointestinal in origin, but it seems to be very well tolerated at a dose of 10 mg/kg twice a day.— A study comparing the use of mycophenolate vs cyclosporine as a secondary immunosuppressive in conjunction with glucocorticoids showed that hospitalization and survival times were similar between the two groups.[14] The group receiving mycophenolate in combination with glucocorticoids experienced fewer side effects than those get cyclosporine, and the cost was much less expensive.[14]

Leflunomide

Leflunomide is metabolized to malononitriloamide, which is a selective pyrimidine synthesis inhibitor.[9] Malononitriloamide also inhibits tyrosine kinase activity.[9] Overall, its immunosuppressive properties inhibit B and T cell proliferation and inhibit Ig production.[9] The main side effects include GI as well as lethargy.[9] The dosing of leflunomide is 2-3 mg/kg once a day.[9]

Other Treatments

Melatonin

Melatonin has been shown to stimulate platelet production in human immune-mediated thrombocytopenia purpura patients.[12] There currently is not any data in veterinary medicine in regard to efficacy, but some clinicians feel that melatonin is a very affordable treatment with few side effects. The dose is 3-6mg PO q12-24 hours.[12] The proposed mechanism of action of melatonin in people is increasing megakaryocyte fragmentation and alteration of cytokines that increase platelet production.[12]

Splenectomy

The spleen is the primary organ responsible for the removal of antibody sensitized platelets.[12] Splenic platelet destruction is greatly increased up to ten times that of normal in IMT patients, so removing this source is the overall rationale behind a splenectomy.[2] In human medicine, splenectomy is considered a second line treatment for patients whose platelet levels have failed to respond to first line glucocorticoid therapy.[12] In veterinary medicine, dogs with IMT have been reported to have extremely variable results in response to splenectomies.[12] Splenectomy is typically reserved for patients where multiple immunosuppressive medications have been applied and the patient has failed to respond, or keeps relapsing.[12] Prior to performing a splenectomy it is important to rule out any potential infectious cause of IMT. The spleen is the major organ responsible for the clearance of antigenic red blood cells and platelets. Animals that are infected with Babesia and undergo a splenectomy will not be able to clear the infection and will have an increase in parasitemia.— There are no recent studies evaluating splenectomy as a treatment for dogs with IMT, only retrospective studies.[12]

Transfusion

Platelet transfusions typically are not commonly used because the platelets are destroyed or consumed within minutes to hours of transfusion.[10] Life threatening hemorrhage is rare in dogs with IMT.[12] Frequently, IMT patients require whole blood transfusions due to anemia from blood loss and depending on how long the patient takes to respond to therapy, more than one transfusion might be necessary.[12]

Monitoring

Platelet counts should be monitored every day or every other day initially during hospitalization, but at discharge patients should have at least 40,000 platelets in order to be at a safe level to avoid spontaneous bleeding.[12] It is important to perform a manual platelet count while your patient is in hospital, as well as after discharge during rechecks before altering immunosuppressive doses. Not only is there potential for spurious errors with automated counters, but there could be sample or collection errors as well. When you perform a manual platelet count you can look for the presence of megaplatelets, platelet clumping, or immature platelets. Once the patient's platelets have stabilized and is discharged, strict exercise restriction should be enforced until platelet counts normalize.[12] Medications should start to be tapered very slowly after the platelets have been normal for multiple weeks. Tapering by about 25% every two-four weeks with a platelet recount before and one week after adjusting medications is necessary for IMT patients.[12] It is important to culture the urine of patients that are on long-term immunosuppressive medications due to their increase risk of infections.[12]

Prognosis

Historically, IMT was thought to have about a 30% mortality rate, but more recent studies have shown much lower rates between 10-20%.[12] IMT overall has a much better

prognosis than immune-mediated hemolytic anemia. More than 70% of dogs with IMT will have greater than 50,000/uL platelets within one week of initiating therapy.[12] However, recurrence is common with IMT, but rates are decreasing to about 26% recurrence.[12] One study reported as low as 9% recurrence rate in 54 dogs with IMT.[12] Poor prognostic indicators are elevated BUN and melena on presentation, which have been correlated with decreased probability of survival.[13] In cases where a secondary cause of the IMT is suspected, such as recent vaccination or medications, these causes should potentially be avoided. There have been reports of IMT relapses after being revaccinated, so avoiding vaccinations in these patients may be warranted and patient risk-benefit analysis should be considered.[16]

Conclusion

Immune-mediated thrombocytopenia is a common disease process in canines and felines. Primary IMT is more common in canines and is a diagnosis that is typically made after excluding many of the common causes of secondary IMT including rickettsial diseases, neoplasia, vaccine, and drug-induced. Each patient's response to therapy is unique, and cost of diagnosis and treatment can really add up. IMT patients have a good prognosis, but relapse is common and clients need to be informed that their dog could have another episode. Diagnosis of primary IMT is difficult without pursing extensive diagnostics. There are many different treatment options, but glucocorticoids are the cornerstone to treatment of IMT. Glucocorticoids have many side effects that are less desirable for owners, so weaning of steroids slowly and potential maintenance on another immunosuppressive drug may be ideal for many patients. Secondary immunosuppressive agents have little data with regards to which is best to use, and selection is often by clinician preference, cost, comfort, and/or patient

response to therapy. Patients platelet count needs to be monitored closely during the tapering process. Some patients will be able to be completely weaned off of immunosuppressive medications, while others will have to stay on these drugs for life in order to keep their IMT in remission.

References

1. Chartier, Marie. Canine (Primary) Idiopathic Immune-Mediated Thrombocytopenia. Clinicians Brief. September 2015. https://www.cliniciansbrief.com/sites/default/files/attachments/CoC_Canine%20Primary%20(Idiopathic)%20Immune-Mediate%20Thrombocytopenia.pdf. Accessed December 23, 2017.
2. Mackin, Andrew. Immune-Mediated Thrombocytopenia: Pathophysiology and Diagnosis. Western Veterinary Conference 2012.
3. Gernsheimer, Terry. The Pathophysiology of ITP Revisited: Ineffective Thrombopoiesis and the Emerging Role of Thrombopoietin Receptor Agonists in the Management of Chronic Immune Thrombocytopenia Purpura. American Society of Hematology 2008. Internet source?
4. Primary Hemostasis. Eclinpath. http://www.eclinpath.com/hemostasis/physiology/primary-hemostasis/. Accessed December 23, 2017.
5. Secondary Hemostasis. Eclinpath. http://www.eclinpath.com/hemostasis/physiology/secondary-hemostasis/. Accessed December 23, 2017.
6. Platelet number. Eclinpath. http://www.eclinpath.com/hemostasis/disorders/platelet-numbers/. Accessed December 26, 2017.
7. Mackin, Andrew. Immune-Mediated Blood Disorders: Chronic Management. Wester Veterinary Conference 2012.
8. Rozanki et al. Comparison of platelet count recovery with use of vincristine and prednisone or prednisone alone for treatment for severe immune-mediated thrombocytopenia in dogs. JAVMA. Vol 220 No. 4, February 15, 2002.
9. Viviano, Katrina. A Practical Approach to Immunosuppressive Therapies. Clinical Pharmacology. AAHA. Accessed December 27, 2017. https://ams.aaha.org/eweb/images/AAHAnet/phoenix2009proceedings/pdfs/01_scientific/024_A%20PRACTICAL%20APPROACH%20TO.pdf.
10. Bianco, D et al. A Prospective, Randomized, Double-Blinded, Placebo-Controlled Study of Human Intravenous Immunoglobulin for the Acute Management of Presumptive Primary Immune-Mediated Thrombocytopenia in Dogs. Journal of Veterinary Internal Medicine: 2009; 23:1071-1078.
11. Balog, K et al. A Prospective Randomized Clinical Trial of Vincristine vs. Human Intravenous Immunoglobulin for Acute Adjunctive Management of Presumptive Primary

Immune-Mediated Thrombocytopenia in Dogs. Journal of Veterinary Internal Medicine: 2013; 27:536-541.
12. Nakamura, Reid et al. Therapeutic options for immune-mediated thrombocytopenia. Journal of Veterinary Emergency and Critical Care. 2012; 22:59-72.
13. O'Marra, Shana et al. Treatment and predictors of outcome in dogs with immune-mediated thrombocytopenia. JAVMA. 2011; 238:346-352.
14. Cummings, F. Rizzo, S. Treatment of Presumptice Primary Immune-Mediated Thrombocytopenia with Mycophenolate Mofetil versus Cyclosporine in dogs. Journal of Small Animal Practice. 2017. 58:96-102
15. Nelson, Richard W., and C. Guillermo. Couto. Small Animal Internal Medicine. 5th ed., Mosby, 2014.
16. Huang, A. Moore, G. Scott-Moncrieff, J.C. Idiopathic Immune-Mediated Thrombocytopenia and Recent Vaccination in Dogs. Journal of Veterinary Internal Medicine. 2012. 26:142-148.