“Gracie: My ‘Pred’cious Blood”

Jennifer Hu

Mississippi State University

College of Veterinary Medicine

Class of 2022

Clinicopathologic Conference
March 25, 2022

Advisor: Doug Margarucci, DVM

Introduction

Immune mediated hemolytic anemia can be classified as either primary or secondary with primary accounting for most cases. Primary immune mediated hemolytic anemia (IMHA) is an idiopathic autoimmune disease where the immune system attacks red blood cells. Primary IHMA is thought to have a genetic component with predisposed breeds including cocker spaniels, springer spaniels, and Old English sheepdogs although any breed can be affected. It is more commonly seen in middle aged to older spayed females.[4] It can be a frustrating disease to manage with mortality rates ranging between 29% and 70%.[4] Primary IMHA can be classified as extravascular or intravascular with extravascular hemolysis being more common.[4] Intravascular hemolysis occurs with destruction of erythrocytes within the vasculature through the complement system. Extravascular hemolysis occurs when there are not enough antibodies for complement fixation and these red blood cells are removed through the liver and spleen. In both extravascular and intravascular IMHA, clinical signs related to the resulting anemia vary depending on the chronicity of the disease. In chronic cases, the most commonly seen clinical signs include lethargy, weakness, inappetence, vomiting, diarrhea, and pigmenturia whereas in acute cases, collapse might be first noticed.[4] Primary IMHA is a diagnosis of exclusion, when other causes of IMHA cannot be found, although supportive care for the treatment of both primary and secondary IMHA are similar.

History and Presentation

Gracie, an approximately 7-year-old female spayed Bichon Frise presented to MSU-CVM Emergency Services on May 24, 2021 from her referring veterinarian for suspected IMHA. Her owner first noticed she was trembling on May 22nd and was lethargic on May 23rd. She had not urinated, defecated or eaten since May 19th. On the morning of May 24th she had

white mucus membranes. At her referring veterinarian, a complete blood count was performed revealing a hematocrit of 19% with a positive slide agglutination.

On presentation, she was panting, quiet, alert and responsive. Her vital parameters included a mild tachycardia characterized by a heart rate of 136 beats per minute, panting, and a normal rectal temperature of 102.4F. On cardiothoracic auscultation, no crackles or wheezes were appreciated but a 2/6 systolic heart murmur was heard. Her mucus membranes were slightly icteric and moist with a capillary refill time of less than 2 seconds. She had an ideal body condition score of 5/9. The remainder of her physical examination was unremarkable and within normal limits.

Differential Diagnoses

Causes of anemia can include hemorrhage, non-immune mediated hemolysis, anemia of chronic disease, or bone marrow disease. Erythrocytes stick together in clumps to cause slide agglutination, and this is a pathologic finding supportive of immune mediated hemolytic anemia. This is due to antibodies that bind the erythrocytes together. Causes of non-pathologic agglutination include EDTA pseudo agglutination, and hereditary spectrin deficiency which also leads to hemolysis.[6,9]

Pathophysiology

Erythrocyte destruction from IMHA is a type two hypersensitivity where antibodies that can be immunoglobulin G or immunoglobulin M coat the erythrocytes and target them for destruction.[5] Intravascular hemolysis is associated with IgM and is generally more severe with acute erythrocyte destruction whereas extravascular hemolysis is associated with IgG. IgG is not as effective as IgM at activating the complement system since it takes many molecules of IgG to be bound to an antigen to activate the complement system.[5] There is much variability between

different IMHA cases of immunoglobulin and complement system involvement. [5] In addition, antibodies are not consistent in their attachment to an erythrocyte but seem to prefer attaching to glycophorins, a sialogycoprotein of an erythrocyte membrane. [5] When antibodies are attached to an erythrocyte and complement system is activated, the erythrocyte swells and ruptures while in circulation. When complement is not activated, the erythrocytes are removed by macrophages in the mononuclear phagocytic system and this occurs typically in the liver and spleen. In cases where there are significant numbers of antibodies produced against an erythrocyte, antibodies can attach to two different erythrocytes and cause agglutination and are then removed through extravascular hemolysis.[5]

Most commonly, antibodies in IMHA target mature circulating erythrocytes and these patients have a regenerative anemia. The onset and development of primary IMHA are not well understood though the complications of IMHA associated with severe anemia are well documented. With low circulating erythrocytes, tissue hypoxia can lead to an increased risk of mortality. Thrombosis is another risk factor with multifactorial causes including damage associated molecular patterns (DAMPS) from tissue hypoxia triggering the neutrophil extracellular trap that promotes thrombosis.[3]

Diagnostic Approach

There is not a gold standard for the diagnosis of IMHA though there is a diagnostic algorithm released by the ACVIM Consensus Statement on IMHA. A history and physical exam that includes common clinical signs of anemia such as lethargy, weakness, vomiting, inappetence, heart murmur, pale mucus membranes, polyuria, polydipsia, tachycardia, tachypnea, bounding pulses, and pyrexia along with a low packed cell volume indicate that a disease-causing anemia is present. After confirming anemia, positive signs of immune mediated

destruction through a positive saline agglutination test, a direct antiglobulin test, or flow cytometry of which at least two is present or a positive saline agglutination test with washing make a strong diagnosis of IMHA.[2] Following evidence of immune mediated destruction, if there is more than one sign of hemolysis it is diagnostic for IMHA.[2]

Baseline tests and tests to rule out secondary causes of IMHA should be performed as well. Baseline tests include a complete blood count with a reticulocyte count. A complete blood count most consistent with IMHA is a moderate to severe regenerative anemia. Leukocytes are often increased from both stimulation of bone marrow and from the inflammatory response. To look for an underlying cause of IMHA to rule out secondary IMHA, tick borne panels, abdominal radiographs, and a serum chemistry panel can be performed to rule out blood borne disease, an abdominal foreign body, and neoplasia. When there is no obvious cause of IMHA, primary IMHA is suspected.

In Gracie's case, primary IMHA was suspected following her diagnostic workup. Anemia was confirmed with a severely low PCV. Immune mediated destruction of erythrocytes was confirmed with a positive slide agglutination test and a blood smear that revealed spherocytes. Evidence of icterus with lack of hepatic disease was supportive of IMHA and established as the diagnosis for Gracie's cause of anemia. Further workup included a complete blood count revealing a moderate leukocytosis characterized by neutrophilia. Serum chemistry revealed a mild ALT elevation. Abdominal and thoracic radiographs were unremarkable. Abdominal ultrasound revealed heterogeneity of the spleen that was likely consistent with extramedullary hematopoiesis or extravascular hemolysis. There was no history of medications that are linked to IMHA. A 4DX Snap test was performed and negative for blood borne disease. A vector borne disease panel was sent out to North Caroline State Veterinary Hospital which was slightly

seropositive for Rickettsia rickettsii though there was not strong enough evidence to support Rickettsia being the cause of IMHA as convalescent titers were not pursued. Given the lack of evidence for an underlying cause of hemolytic anemia, primary IMHA was suspected.

Treatment and Management

Immunosuppressive drugs are the mainstay of treatment but supportive care through blood transfusions to address the anemia while the bone marrow takes time to produce mature erythrocytes is a necessity. Cross matching or blood typing are first performed for the dog erythrocyte antigen (DEA). The most commonly found blood type in dogs is DEA 1.1 but in Gracie's case she was DEA 1.1 negative which meant she could only receive blood that was DEA 1.1 negative. The decision to perform a blood transfusion is dependent on the patient and include clinical signs, normal packed cell volume for the patient, onset of anemia and monitoring availability. There is no set PCV number where a transfusion is definitively indicated.[7] In Gracie's case, because the onset of her clinical signs was acute and her PCV had dropped from her referring veterinarian to her presentation at MSU-CVM Emergency Services, she received a blood transfusion of DEA 1.1 negative packed red blood cells on presentation. While packed red blood cells is the ideal blood product for transfusion, whole blood is still a good alternative. There is a chance of volume overload with whole blood transfusion since these dogs are not hypovolemic and the plasma component can increase the risk of transfusion reactions. There is also an association with the age of packed red blood cells and complications from transfusion reactions.[7] The recommendation is to provide fresh packed red blood cells that are not older than 7-10 days.[7] Patients diagnosed with IMHA typically require more than one transfusion and in Gracie's case, she received two more transfusion of packed red blood cells on May 26nd and May 29th.

Along with blood transfusions, antithrombic treatment was initiated since there is strong evidence of increased risk of thrombosis with IMHA. This risk is further increased if glucocorticoids are part of the immunosuppressive therapy since glucocorticoids increase clotting factors and fibrinogen.[9] Antithrombic treatment is not recommended with platelet counts less than 30,000/uL.[7] Mortality from thrombosis is highest in the first two weeks after starting immunosuppressive treatment and can be discontinued as immunosuppression begins to combat the disease.[7] In Gracie's case, she was administered subcutaneous enoxaparin while in hospital, a low molecular weight heparin and concurrently received clopidogrel at 2.4mg/kg/day given orally which was continued after discharge.

To combat the immune system, immunosuppressive drugs are used to get the patient into remission. There are two different options for immunosuppressive therapy for when to add in a second or third immunomodulating drug after starting prednisolone or dexamethasone. The recommendation is to start with an immunomodulating drug with a steroid at disease diagnosis for severe diseases.[7] Another benefit of starting a second immunomodulating drug is to attenuate the polyuria and polydipsia that occurs from glucocorticoid administration by being able to transition the patient off glucocorticoids sooner. The starting dose for prednisone is not to exceed 2mg/kg/day to avoid risk of adverse side effects of higher dosages of prednisone.[7] Intravenous dexamethasone is not shown to be more effective than prednisone.[7] In Gracie's case she was given 1.6mg/kg/day of prednisone to start.

Other immunomodulating drugs that are preferred options for IMHA include azathioprine, cyclosporine and mycophenolate mofetil. All three of these drugs have been shown to be effective in treating IMHA. Gracie was started on mycophenolate at 11mg/kg twice a day. Mycopehnolate mofetil is a purine inhibitor that inhibits B and T cells and is shown to have a

more rapid onset than azathioprine. Gastrointestinal side effects can occur with mycophenolate mofetil, but this drug is well tolerated and Gracie never showed gastrointestinal signs while in hospital.[7] She was also given doxycycline prior to her vector borne disease panel results for potential diseases in case there was an underlying cause for her IMHA that needed to be addressed.

While in hospital Gracie had her PCV checked at least once a day for evidence that the steroids were working, and she did not need another blood transfusion. Gracie was discharged from the hospital on May 30th when her PCV was 32%. She was advised to have her PCV rechecked 2-3 days following discharge to ensure her IMHA was responding appropriately. In terms of drug tapering, when the PCV is stable and above 30% for two weeks, the prednisone dose can be reduced by 25% and if the PCV remains stable, prednisone dose can be decreased by 25% every 3 weeks. High glucocorticoid administration carries many adverse side effects including polyphagia, polydipsia, polyuria, panting and gastrointestinal bleeding which Gracie did exhibit symptoms. Gracie gained weight and showed signs of polyuria and polydipsia. Gracie had nurse rechecks with MSU-CVM Internal Medicine on June 7th and June 16th and had a PCV of 25% and 33% respectively and her prednisone dose was decreased by 30% at that time. At her full recheck on June 23rd with a PCV of 44% her clopidogrel was discontinued. When she developed diarrhea with melena prior on September 14th her prednisone was discontinued and she received sucralfate and omeprazole.

Prior to reducing the second immunomodulatory drug, PCV should be assessed every 1-3 weeks and monitoring of responsiveness to treatment through agglutination tests, serum bilirubin concentration and blood smears can also be performed.[7] Gracie had good response to her treatment and she had her mycophenolate dose tapered to 8.3mg/kg twice a day on September

22nd which was further reduced on October 4th to 5.6mg/kg twice a day. While tapering the immunosuppressive drugs, there is always a chance for relapse so monitoring for signs of anemia is prudent. When a relapse is detected, triggers should be identified, but if not, the drug dosage of either prednisone or second immunomodulating drug can be increased in dose.[7] On January 15th, 2022 Gracie did present to MSU-CVM Emergency Services when she was noticed to have pale mucus membranes but her PCV was within normal limits (47%). Another complication from immunosuppressive drugs is the chance for drug associated myelosuppression, which if there is evidence, the drug is to be discontinued and switched to a different immunosuppressive drug. With a neutropenia count of less than 1000 cells/uL prophylactic antibiotics are recommended.[7] Luckily, Gracie never experienced these adverse side effects.

Case Outcome

Approximately10 months since Gracie was first diagnosed with primary IMHA she has been on a low dose mycophenolate at 5.6mg/kg given every 12 hours. Gracie visited MSU-CVM Internal Medicine on March 7, 2022 where it was revealed that she did not receive mycophenolate for the 7 days prior to presentation as she did not have access to mycophenolate. During her appointment, she had a normal PCV and her mycophenolate was refilled at 2.5mg/kg given every 12 hours for the next two weeks at which time, if her PCV is stable, she will have her mycophenolate dose discontinued. Relapse of IMHA is relatively uncommon with a rate between 11%-15%.[4]

Conclusion

Primary IMHA can be a devastating disease due to its enigmatic cause and lead to an owner questioning whether their dog is going to relapse from being given a topical drug or a dental chew even after PCV is stable on medications. However, it can also a very rewarding

disease to treat when blood transfusions, long term medications and monitoring are an option. With this being a common disease, there are studies currently looking for other options to treat IMHA such as targeting complement activation.[1] Hopefully with more research focusing on IMHA it will be possible in the future to find dogs that are at risk for this autoimmune disease prior to presenting with clinical signs of anemia.

References

1. Borchert, C., Herman, A., Roth, M., Brooks, A. C., & Friedenberg, S. G. (2020). RNA sequencing of whole blood in dogs with primary immune-mediated hemolytic anemia (IMHA) reveals novel insights into disease pathogenesis. *PLOS ONE,* 15(10), e0240975. https://doi.org/10.1371/journal.pone.0240975

2. Garden, Oliver A et al. "ACVIM consensus statement on the diagnosis of immune-mediated hemolytic anemia in dogs and cats." *Journal of veterinary internal medicine* vol. 33,2 (2019): 313-334. doi:10.1111/jvim.15441

3. Janeway CA Jr, Travers P, Walport M, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. The distribution and functions of immunoglobulin isotypes. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27162/

4. Johnson, Carla."Possible IMHA Dog – Now What?" *DVM360*, https://www.dvm360.com/view/possible-imha-dog-now-what.

5. Mackin, Andrew. "Immune-Mediated Hemolytic Anemia: Pathophysiology and Diagnosis" *IMMUNE-MEDIATED HEMOLYTIC ANEMIA: PATHOPHYSIOLOGY AND DIAGNOSIS*, Mississippi State University , 2014, http://delawarevalleyacademyvm.org/pdfs/oct14/Autoimmune.pdf.

6. Slappendel, Robbert J et al. "Hereditary spectrin deficiency in Golden Retriever dogs." *Journal of veterinary internal medicine* vol. 19,2 (2005): 187-92. doi:10.1892/0891-6640(2005)19<187:hsdigr>2.0.co;2

7. Swann JW, Garden OA, Fellman CL, Glanemann B, Goggs R, LeVine DN, Mackin AJ, Whitley NT. ACVIM consensus statement on the treatment of immune-mediated hemolytic anemia in dogs. J Vet Intern Med. 2019 May;33(3):1141-1172. doi: 10.1111/jvim.15463. Epub 2019 Mar 7. PMID: 30847984; PMCID: PMC6524099.

8. "Pattern Changes." *EClinpath,* https://eclinpath.com/hematology/morphologic-features/red-blood-cells/patterns/#:~text=Agglutination.one%20blood%20cell%cC%20agglutinates%20for

9. "Thrombosis." *EClinpath*, https://eclinpath.com/hemostasis/disorders/thrombosis/.