Immune-mediated Hemolytic Anemia: Review and Update on Splenectomy as Adjunctive Therapy

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Introduction:
Immune mediated hemolytic anemia (IMHA) is one of the most common immune mediated conditions in dogs treated in both the primary care and referral settings. Despite decades of knowledge and research on IMHA, it remains to be a frustrating and very deadly disease with reported mortality rates range from 22-70%. Although the highest mortality rates were seen in the earlier studies, the frequency of poor outcomes is still unacceptably high. Most veterinarians are familiar with the foundations of IMHA treatment, mainly immunosuppression with corticosteroids and thromboprophylaxis, however, it is also important for veterinarians to be aware of adjunctive therapies that may need to be utilized in more severe or refractory cases.

IMHA results in hemolysis of red blood cells that are targeted by the body’s own immune system. The complexity of this immune mediated process is beyond the scope of this presentation. Simplistically, red blood cells are coated with immunoglobulin, complement, or both, leading to hemolysis. The hemolysis may occur intravascularly or extravascularly, although both routes are occurring simultaneously in most cases. Indicators of intravascular hemolysis include free hemoglobin in the plasma or urine. Clinically these can be identified by finding hemolized or red colored plasma after centrifugation, and red colored urine (pigmenturia) that does not separate out with centrifugation. Extravascular hemolysis is mediated by red cell removal via macrophages within spleen and liver. Extravascular hemolysis results in icterus, or bilirubin found in the plasma or urine.

IMHA may also be classified as either a primary or secondary process. This talk will mostly focus on primary or idiopathic IMHA, in which no identifiable trigger is found. Certain secondary triggers should always be ruled out including any neoplasia, infections, and drug reactions. The most commonly implicated infections are the tick-borne, including Erlichia spp., Anaplasma spp., and Babesia. Reported drug-related causes of secondary IMHA include cephalosporins, sulphonamides, and carprofen. The discontinuation of these drugs may resolve the IMHA. Vaccination was associated with IMHA in an early study, however, this has not been confirmed by more recent reports.

Clinical Presentation:
Patient signalment may be an important aspect when considering IMHA. IMHA is typically seen in middle-aged to older dogs, although it has been reported in dogs of all ages. Spayed females are overrepresented in our IMHA population at MSU as well as other studies. Although it may occur in any breed, a number of breed predispositions have been reported including Cocker Spaniels, Shih Tzus, Lhasas, Old English Sheepdogs, Springer Spaniels, Collies, and Dobermans.
A majority of the clinical signs are secondary to anemia and the resulting tissue hypoxia. Common signs include pale gums, icterus, lethargy, anorexia, vomiting, and diarrhea. Some owners may also notice discolored urine as the initial sign. The clinician should then inquire about recent medications, vaccinations, or travel, as well as toxins known to cause hemolysis such as onions and heavy metals (i.e. pennies or hardware).

Physical exam findings often relate to the severity of the anemia. In addition to obviously pale or icteric mucous membranes, the anemia will cause tachycardia, tachypnea, bounding pulses, and occasionally a physiologic heart murmur. Cranial organomegaly may also be palpated and this is secondary to liver and spleen enlargement from extramedullary hematopoiesis. Dogs with IMHA may also have a fever due to systemic inflammation and pyrogenic cytokines.

Diagnosis:
A confident diagnosis includes an anemia (HCT <30%) along with at least 2 of 4 of the following criteria:

1) Regenerative anemia as evidence by reticulocytosis or moderate to marked polychromasia.
2) Spherocytosis
3) Positive saline autoagglutination
4) Positive Coomb’s test

As with any suspected anemic patient, initial diagnostics should include PCV/TS, blood smear evaluation, and slide-agglutination test with saline dispersion. Since the anemia is due to hemolysis rather than blood loss, the PCV will be low with a normal to high TS. The plasma from the spun PCV should be evaluated for hemolysis or icterus. Not all IMHA patients will have obvious agglutination but its presence is essentially pathognomonic. It is important to differentiate agglutination from rouleaux. This is best done by adding saline to a drop of blood in a ≥5:1 ratio. True agglutination should not disperse with saline. The importance of the blood smear evaluation cannot be overemphasized. The smear should be evaluated for evidence of regeneration (polychromasia, anisocytosis). Spherocytes are typically seen in large numbers in dogs with IMHA and are the result of partial phagocytosis or partial loss of the red blood cell membrane due to antibody labeling.

A more thorough diagnostic work-up should be performed in dogs with suspected IMHA to rule out other causes of anemia, and exclude secondary triggers. A CBC, chemistry panel, and urinalysis should be performed as a minimum database. Tick-borne illness testing should be performed in cases with a moderate thrombocytopenia, or in dogs from high risk regions. Full chest radiographs and abdominal ultrasound should be performed to rule out cancers and the more common neoplastic triggers include hemangiosarcoma, lymphoma, and histiocytic cancers.

The foundations of IMHA treatment include improving oxygen delivery (fluids and blood transfusions), suppressing the immune system, and preventing thromboembolic complications. When presented with emergent anemic patient, improving oxygen delivery should be prioritized. If the patient is hypovolemic in addition to the anemia, do not hesitate to administer an IV crystalloid fluid bolus, which will improve cardiac output and oxygen delivery. The notion of harmfully diluting out the red cells in an anemic patient is flawed since adequate perfusion is necessary to deliver the blood cells to the tissues. Many IMHA patients will require one or more transfusions. There isn’t a set transfusion trigger for PCV or HCT. Transfusion requirements are...
Immunosuppressive therapy should be started as soon as a diagnosis of IMHA has been established and the patient’s oxygen delivery needs have been addressed. Prednisone at 2-4 mg/kg/day PO, or dexamethasone at 0.28 mg/kg/d IV, are currently the mainstay therapies. Some studies have suggested improved outcome with using an additional agent, such as azathioprine (Immunuran), however, all data has been retrospective. To date, no data has proven that two immunosuppressive drugs are better than steroids alone. Blood clot prevention should be instituted as soon as possible since thromboembolic disease, often in the form of pulmonary thromboemboli, is a leading cause of death in IMHA. Multiple options are available including antiplatelet drugs such as aspirin (0.5-1 mg/kg PO BID) or Plavix (clopidogrel) (1-3 mg/kg/d PO), or antithrombotics such as heparin. To date, evidence for the best clot prevention strategy is lacking. The results of a recent randomized controlled trial performed at our hospital comparing aspirin to heparin in IMHA dogs are still pending. Currently, we most commonly start patients on heparin sodium with a 100U/kg IV bolus followed by 30-50 U/kg/hr CRI. Baseline coagulation testing should be performed first and the heparin is adjusted daily to prolong the aPTT to 1.5-2 times baseline. At the time of discharge we most commonly prescribe either aspirin or clopidogrel, although Fragmin, a low molecular weight heparin, can also be used. Clinicians should watch for upcoming recommendations regarding the use of newer antithrombotics in dogs such as anti-Xa drugs like rivaroxaban (Xarelto), and low molecular weight heparins such as enoxaparin (Lovenox). These may be attractive options for IMHA in the near future.

Occasionally dogs with IMHA have more severe hemolysis, and may require more transfusions or longer hospitalization than typical. Unfortunately, no clear-cut guidelines exist that guide when adjunctive treatments should be instituted. Multiple studies report that the average length of treatment to onset of remission is 5 to 6 days, and the average number of transfusions required is 1 to 2. Therefore it is reasonable to consider an adjunctive therapy in cases that are clearly requiring more transfusions or longer duration of transfusion dependency than average. Some of the adjunctive therapies that have been tried in dogs with IMHA include human intravenous immunoglobulin (hIVIG) infusions, plasmapheresis, and splenectomy. Human intravenous immunoglobulin (hIVIG) is an infusion product that contains over 90% of biologically active IgG, and trace amounts of IgA and IgM. It is thought that the predominant mechanism of action is IgG blockade of Fc receptors on the macrophages. As blockade of Fc receptors is immediate, it is postulated that hIVIG may allow for rapid reduction in hemolysis, thus allowing for more time for the other immunosuppressive drugs to work. Anecdotal reports of rapid resolution of various immune-mediated conditions in dogs treated with hIVIG exist. But at this juncture, hIVIG has not routinely proven to reduce transfusion requirements or shorten hospitalization in IMHA, compared with standard immunosuppressive therapies alone. Adverse effects of hIVIG include acute hypersensitivity (most common), thromboembolism, renal failure, hypotension, and fluid overload.

Therapeutic plasmapheresis has been successfully used in a select few dogs with IMHA. Plasmapheresis involves the use of specialized equipment, either a hemodialysis unit with a specialized filter or a cell separator machine, to separate and discard the plasma from other blood components. Then the blood components are returned to the patient. Fresh frozen plasma from donors is typically transfused to the patient to replace the removed plasma. The goal of this therapy is to rapidly reduce the circulating autoantibodies contained within the plasma which may allow for rapid cessation in hemolysis. The effect with a single session is transient, but may
be sufficient to allow more time for the standard immunosuppressive drugs to work. Adverse events with plasmapheresis in people at least are uncommon, but may include difficulty in obtaining vascular access for the dialysis catheter, coagulopathy from the anticoagulants used for the dialysis circuit, and reactions to the plasma transfusions administered. Although this technique has been successful, specialized equipment, expense, and very limited availability restrict its use.

Splenectomy appears to be a promising adjunctive therapy. The spleen plays a major role in both the immune and hematologic systems. Additionally, the spleen largely facilitates removal of abnormal red blood cells, including RBC’s targeted with autoantibodies. In people with autoimmune hemolytic anemia, a condition similar to IMHA in dogs, splenectomy is widely used as a second-line therapy when steroids alone fail. In people failing steroid therapy, splenectomy allows for prompt remission in approximately 70% of cases. Successful use of splenectomy in difficult canine IMHA cases has been described in two previous publications, and it is an active area of research at the MSU VMC.

We recently evaluated the use of splenectomy in selected IMHA cases at our institution. We specifically selected for dogs with IMHA that had higher transfusion dependency than is typically reported. Specifically, dogs with IMHA were eligible if they required one or more blood transfusion for $\geq 3$ days, or if they were still being transfused after 7 or more days of standard therapy (steroids, antithrombotic therapy, +/- an additional immunosuppressive agent). Previous experience with splenectomy at our hospital revealed that the PCV (or HCT) often stabilized and increased within 24 hours of the procedure, suggestive of a positive response. We also evaluated the safety of a rapid reduction in steroid dose in those that responded favorably to the splenectomy. The steroid dose was cut in half the day after the splenectomy if the PCV increased within 24 hours of the procedure. Overall, we have included 9 dogs, and found that 7 of 9 (78%) dogs had an immediate rise in PCV within a day of the splenectomy and continued to remain in remission despite having their steroid dose immediately reduced by 50%. Additionally, there were significantly fewer transfusions given after the splenectomy compared to before (median of 0 vs 4, $p<0.001$). At our hospital we use a minimally invasive splenectomy technique and the median surgical time was only 17.5 minutes and no surgical complications were reported. These dogs were on antithrombotic therapy prior to surgery and no surgical complications were seen. If using heparin, we recommend discontinuing the heparin at least 4 hours prior to surgery, and restarting the following day.

Splenectomy appears to be a useful and safe adjunctive therapy in appropriate cases. It can be considered in dogs with primary IMHA requiring daily transfusions for $\geq 3$ days, or needing transfusions after 7 days of standard therapy. However prospective randomized controlled studies are warranted to better test the effectiveness of this therapy.

*References available upon request.*