Which Underlying Diseases Should I Screen for in Dogs With IMHA?

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Pathogenesis of IMHA

Immune mediated hemolytic anemia (IMHA) is an important cause of morbidity and mortality in dogs. IMHA is characterized by the inappropriate immune-mediated destruction of self red blood cells. This self attack can be triggered by infections, inflammatory disease, neoplasia, toxins and drugs. IMHA is deemed "idiopathic when no underlying cause ids found. Several mechanisms for IMHA have been proposed. Inciting agents may result in a change in the way that self antigens are presented to the immune system so that they are seen as novel and foreign. Alternatively, foreign antigens can be similar enough to self antigens such that stimulation of the immune receptors results in the production of self-directed effector cells and antibody (molecular mimicry). The immune system can also damage red blood cells while attacking agents that directly infect them or by immune complex deposition.

Inciting agents with or without genetic predisposition may also cause an imbalance in immunoregulation that results in a loss of peripheral immune tolerance and unmasking of preexisting self-directed T and B cells. This may come about by several mechanisms. Recent evidence from several mouse models suggests that an insufficient regulatory T cell response plays a central role in the loss of tolerance that occurs in IMHA. In addition, a decrease in complement regulatory proteins that protect host cells from complement mediated destruction has been shown in people with IMHA. Mouse models and studies in people also support the concept that an imbalance in the production of cytokines plays a role in the pathogenesis of IMHA. For example IL-17 has been shown to be an important mediator of autoimmunity in mouse models. IL-17 is also increased in human patients with IMHA and levels are correlated with anti-RBC antibody production, and negatively correlated with hemoglobin. MHCII molecules display antigens in the context of self to immune effector cells so that they may be recognized as foreign. Certain dog breeds such as English cocker spaniels are predisposed to idiopathic IMHA and specific MHCII DNA polymorphisms have been associated with the disease in this breed. It is likely that a combination of environmental factors and genetics contributes to the loss of immunologic self tolerance and that some idiopathic cases may result from unrecognized infections or other environmental triggers.

Determining whether underlying disease is present in dogs with IMHA is important because failure to identify instigating causes can contribute to treatment failure and death. That said, it is impossible to screen for all the diseases that have been or theoretically could be associated with IMHA. Therefore it is necessary to tailor diagnostics to the individual patient to a degree. Clues that underlying disease may have triggered IMHA or that IMHA is idiopathic can be found by considering the signalment, history, physical examination findings and initial diagnostic testing.

Consideration of the signalment helps increase the index of suspicion for idiopathic IMHA and for other potential causes of hemolysis. Some dog breeds have been shown to be at increased risk or are overrepresented in studies of idiopathic IMHA. These include but are not limited to Cocker spaniels, Irish setters, old English sheepdogs, and English Springer spaniels. It is important to note that some cases of reported idiopathic IMHA in the literature may have been triggered by undetected infection that triggered IMHA in a genetically predisposed individual. Breed is also important to consider when ruling out non-immune mediated causes for hemolysis in dogs with IMHA. For example, Phosphofructokinase deficiency is an inherited disorder that also causes hemolysis in English Springer spaniels. Similarly, it is extremely important for clinicians to consider breed when ruling out vector borne disease in patients with IMHA. That is because running a single "tick panel" doesn't rule out the presence of vector borne disease. Tick panels" and "vector borne disease panels" offered by diagnostic laboratories or in house test kits give clinicians the ability to test peripheral blood for multiple agents using serology, polymerase chain reaction (PCR) or a combination of the two. Choosing a panel requires the clinician to consider which organisms to test for and which methodology (PCR or serology) to employ. The sensitivity of serology as compared to PCR varies with characteristics of the host, the assays, and pathophysiologic characteristics of the organism. Therefore, panels using only PCR or serology may overlook the presence of infection. Knowing when clinical signs occur, when and if organisms circulate in peripheral blood, whether they circulate in high or low number helps determine whether serology and/or PCR, or acute and convalescent serologic testing are most appropriate for an individual patient. It is also important to confirm that the lab test will detect the species of organism in question. For example, not all PCR tests that target the genus Babesia detect all species of Babesia that cause IMHA in dogs.

For certain breeds it is important to be aggressive about ruling out underlying infection that may cause IMHA. For example it is very important to rule out Babesia gibsoni in a pit bull with IMHA. Greyhounds and other dogs exposed to Rhipicephalus sanguineus ticks may be at increased risk for Ehrlichia canis or Babesia canis infection. See accompanying proceedings entitled "How to use PCR and serology in vector borne disease testing, which test and when" for more information on this topic.

Getting a complete history that includes dates and types of vaccinations, heartworm testing and preventative administration, the administration of other drugs, diet, travel, and possible envenomization such as bee stings should be elucidated. A history of recent vaccination has been associated with IMHA in some, but not all studies. Certain drugs such as cephalosporins and sulphonamides have been associated with IMHA in dogs. Over 100 drugs have been associated with IMHA in people. Therefore any history of recent

drug administration should be investigated as a potential instigator of IMHA. Interestingly a natural commercial diet was been implicated in the induction of IMHA in dogs in 2004. The hemolysis associated with the diet was confirmed to be immune mediated. Certain food additives such as garlic and onions can cause oxidative damage to red cells and induce non-immune hemolysis. Zn toxicity may cause hemolysis through oxidative and possibly immune mediated mechanisms. A thorough investigation by the FDA did not reveal any potential instigating agents for the diet in question. Dietary history and awareness of food recalls and associated clinical signs is therefore important in these patients. Travel history and a familiarity with infectious diseases in the geographic locale of the dogs home is necessary to raise the index of suspicion for diseases such as leptospirosis or vector borne disease associated with IMHA pertinent to the geographical region involved. For example *Babesia conradae* is present in southern California and dogs presenting to clinicians in that region of the country with evidence of IMHA should be screened for the organism.

Physical examination findings can also provide clues as to underlying diseases that may be present in dogs with IMHA. For example, findings such as lymphadenomegaly, uveitis, vasculitis or CNS signs may be associated with or vector borne or other infectious disease or neoplasia. A new heart murmur may be associated with anemia, however endocarditis is also a consideration as it may trigger IMHA.

The minimum database in a patient with IMHA should include diagnostic testing that will detect the presence of underlying infectious, inflammatory or neoplastic disease. This may include CBC with slide review, heartworm testing serum chemistry with electrolytes, urinalysis, urine culture, abdominal ultrasound and thoracic radiographs.

It is important to remember that not all hemolysis is immune mediated. In a patient with icterus and pale mucous membranes and suspected hemolysis, the first step in diagnostic testing is to verify you are dealing with immune-mediated red cell destruction rather than another cause of hemolysis. CBC with evaluation of red cell morphology is extremely important when evaluating a patient with hemolysis. Evaluation of cell morphology is vital and cannot be performed by an automated instrument. For example, the presence of eccentrocytes or Heinz bodies suggests that hemolysis may be due to oxidative damage rather than immune mediated damage, while the presence of spherocytosis, suggests immune mediated destruction is present. Keep in mind however, that the observation of spherocytes is not 100% specific for IMHA. For example, spherocytosis may be observed zinc toxicity and other disease states such as splenic torsion. Furthermore, the absence of spherocytosis does not rule out immune mediated destruction. Coombs testing and a slide agglutination test can help confirm immune mediated destruction, although they too are not 100% sensitive or specific. For example positive Coombs test has been associated with a number of infectious disease agents Evaluation of a blood smear can also aid in the identification of vector borne disease associated with immune mediated hemolytic anemia, such as *Ehrlichia spp, Babesia spp* and *Anaplasma phagocytophilum*.

Serum biochemical abnormalities may help focus the hunt for underlying disease that may have triggered the IMHA. Hyperbilirubinemia is usually presumed to be due to hemolysis. Elevations in ALT and AP are also common, presumably due to hypoxic injury from the anemia. However other neoplastic, infectious or inflammatory disease of the liver may also be associated hyperbilirubinemia and elevated ALT and AP and could be possible triggers of IMHA. Renal azotemia may increase the index of suspicion for leptospirosis. Hypoalbuminemia may suggest the presence of concurrent protein losing nephropathy.

Urinalysis often reveals the presence of bilirubin. An active sediment should prompt investigation into the presence of pyelonephtiris. Often culture is performed even in the absence of an active sediment to avoid overlooking the presence of a silent urinary tract infection. Underlying infectious, neoplastic, inflammatory or immune mediated disease associated with PLN may be more aggressively pursued if proteinuria and an elevated urine protein creatinine ratio is present in the face of an inactive sediment.

Imaging can also allow the detection of underlying disease triggers such as neoplasia or infection. For example an unstructured interstitial pulmonary pattern is associated with some vector borne diseases and lymphoma. Hepatomegaly, splenomegaly and mild effusion are common findings on abdominal ultrasound in dogs with idiopathic IMHA. Abdominal ultrasound allows evaluation of multiple organs to provide clues as to the presence of underlying disease. Abdominal radiographs are indicated in any patient with hemolysis to rule out Zinc ingestion as a cause.

Vector borne disease screening should include heartworm testing, slide review and PCR with or without serologic testing for multiple agents. Particular effort, which may include testing using both serology and PCR, should be made to rule out those agents that have been associated with IMHA including Babesia species, Bartonella species, Anaplasma phagocytophilum, Ehrlichia and Mycoplasma species. See the accompanying proceedings entitled “How to use PCR and serology in vector borne disease testing, which test and when” for more information on this topic. Empiric treatment with doxycycline is prudent while awaiting results of vector borne disease screening.

In summary, screening for underlying disease in dogs with IMHA is important to maximize the chance of a favorable outcome. Considering factors in the signalment, history, physical examination and minimum database can signal the need for and justify more aggressive and thorough diagnostic testing for infectious agents in particular.

References

Semple Autoimmune pathogenesis and autoimmune hemolytic anemia. 2005. Semin hematol 42:122-30.
Xu et al. Critical role of Th 17 cells in development of Autoimmune hemolytic anemia. 2012 Exp. Hematol 2012 Sept 5 epub ahead of print.
Smith AJ et al. Diet associated hepatic failure and immune mediated hemolytic anemia in a Weimarainer. 2006 JVECCS 16(2) S1 S42-47.