IMMUNE-MEDIATED HEMATOLOGIC DISEASES

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Immune-mediated hematologic disease including hemolytic anemia (IMHA) and/or thrombocytopenia is being reported with increasing frequency in animals and humans. In dogs and occasionally in cats, this disorder can be associated with bone marrow failure (nonregenerative anemia, erythroid dysgenesis, red cell aplasia). Affected animals have one or more of the following signs: lethargy, anorexia, pale mucous membranes, weakness, exercise intolerance, tachycardia, tachypnea, icterus, hemoglobinuria and fever. Prognosis is guarded to poor with reported mortality rates between 28-70%. Laboratory abnormalities may include: red cell auto-agglutination, positive Coombs' test, spherocytosis, thrombocytopenia, and neutrophilia. Anemia may be regenerative or nonregenerative depending on the duration of illness and immunological targeting of red cell precursors in the bone marrow. Some dogs may also have other autoimmune diseases. While many cases may be classified as idiopathic, a recent stress event such as vaccination, drug, chemical or toxic exposure, surgery, hormonal change, infection, or injury within the previous 30-45 days may be identified as a potential trigger. Many breeds are reported to have an increased risk for IMHA, and mixed breed dogs can also be affected.

Hemolytic Anemia

Four recent retrospective studies have addressed the clinical and laboratory findings and compared treatment outcomes of dogs with IMHA. In one study of 70 cases, Cocker Spaniels, English Springer Spaniels, Poodles, Miniature Schnauzers, and Collies were at increased risk. Only 3 dogs had been vaccinated within 2 weeks of the diagnosis. Regenerative anemia was present in 83% and 79% had spherocytosis. Only 37% of the dogs had positive Coombs' test. Thrombocytopenia was also found in 29 dogs. Elevated serum bilirubin concentrations, present in 68% of cases, were significantly associated with decreased survival. A significant difference in survival was found between treatment groups.

The overall mortality was 70%. While 29 dogs died or were euthanized during hospitalization, 41 were discharged but 15 died, most within 3 months of discharge. Dogs with IMHA were four times more likely to die than dogs in the general hospital population.

The second study involved 60 cases. Cocker Spaniels had a 3.3 times increased relative risk for IMHA. Unlike an earlier study, no seasonal incidence, or correlation between vaccination and onset of disease or survival times was found. Positive Coombs' test and auto agglutination were seen in 89% of cases, and 75% had spherocytosis. The anemia was regenerative in 42% and nonregenerative in 58%. Increased bilirubin concentrations were present in 80% of cases, but hyperbilirubinemia was not associated with higher mortality. The median survival time was only 21 days. Dogs receiving prednisone, cyclophosphamide, and azathioprine had a median survival time of 370 days as compared to only 9 days for those given only prednisone and cyclophosphamide. Of the dogs given compatible transfusions, no adverse effects were recorded and the median survival time was better (21 days) versus 2 days for dogs that were not transfused. Overall mortality was 52%. Thirty-three dogs were discharged and followed for at least 2 years; 8 dogs relapsed and in 7 of these, relapse occurred within 21 days of discharge.

The third study included 88 dogs. Twenty-six dogs received only prednisone. Of these, 15 (58%) survived to be discharged, and the mortality rate was 30%. The relative risk of death for dogs treated with prednisone and azathioprine (n=27), prednisone and danazol (n=16), prednisone and cyclosporine (n=24) or prednisone and intravenous gamma globulin (n=7) was not different from dogs treated only with prednisone. With cyclophosphamide, however, there was a significant increased risk of mortality. Although dogs with auto agglutination were twice as likely to be treated with cyclophosphamide, there was no significant relationship between auto

agglutination and mortality. The mean PCV of dogs that were treated with cyclophosphamide was not significantly different from dogs not receiving this drug. The 3 dogs receiving bovine hemoglobin solution did not survive. Overall mortality rate in this study was 50%, but dogs were followed only until discharge.

The last study involved 43 dogs with severe idiopathic nonregenerative anemia. Labrador Retrievers were overrepresented here. While 54% of cases had spherocytosis and 57% had positive Coombs tests, only 5% had auto agglutination. Seven of 31 dogs tested (23%) had positive antinuclear antibody titers. Leukocyte counts were normal, but 22% of the dogs had some degree of thrombocytopenia. All dogs had bone marrow biopsies. Bone marrow aspirates were difficult to obtain in 27 dogs, and core marrow biopsies were performed in 16 of them. Fifty-five percent of dogs had erythroid hyperplasia, 14% had normal erythrogenesis and 26% had erythroid hypoplasia, 37% had erythroid maturation arrest, and 2 dogs had pure red cell aplasia with no red blood cell precursors found. All 16 core biopsies revealed myelofibrosis. Iron stores were moderate in 23% and large in 72% of the dogs. Treatment outcomes varied with responses seen in 1-10 weeks. Follow-up bone marrow biopsy on 2 dogs showed resolution of myelofibrosis. Overall mortality was 28%.

Conclusion ***Prognosis*** for dogs with IMHA is guarded to poor. The various combination drug protocols may not work better than corticosteroids alone. Use of cyclophosphamide to treat dogs with the regenerative form of IMHA may be associated with increased mortality. Dogs with the non-regenerative form of IMHA do not have a worse prognosis than dogs with the classic regenerative form. Myelofibrosis can occur secondary to immune-mediated destruction of red cell precursors and may respond to immunosuppressive therapy.

Treatment	# of Dogs	Median Survival(Days)	Mortality Rate (%)
Prednisone only	**16**	**57**	**62.5**
Prednisone + Cyclophosphamide	**5**	**28**	**67.8**
Prednisone + Azathioprine	**18**	**97**	**80.0**
Prednisone + Azathioprine + Cyclophosphamide	**16**	**15**	**68.7**

Thrombocytopenia

Quantitative platelet defects produce either thrombocytopenia due to: 1) increased platelet destruction, utilization, or sequestration, or decreased platelet production, or 2) thrombocytosis from increased platelet production or release from tissue stores. Of these conditions, immune-mediated thrombocytopenia accounts for the majority of chronic cases. The immunological basis has been examined in humans and in the dog, cat, and horse. Primary immunological thrombocytopenia, of unknown etiology, has been termed idiopathic thrombocytopenic purpura (ITP), although the majority of cases appear secondary to a variety of underlying conditions such as thrombosis, neoplasia, viral diseases, vaccine-associated reactions, and use of estrogens, other drugs and chemicals.

Large platelets (megathrombocytes) are young and generally more active than normal sized platelets. Conversely, the presence of predominantly small platelets (microthrombocytes) in canine blood appears to be a specific indicator of immune-mediated thrombocytopenia. Small platelets (mean platelet volume < 5.4 fl) were found in 17 of the 31 IMT cases in study of 68

thrombocytopenia cases.

Author's Experience

Our experience with these cases indicates that:

1) Autoimmune thyroiditis/hypothyroidism is frequently present and/or affected dogs are often of breeds or cross-breeds susceptible to thyroid disease.

2) Aggressive and more sustained treatment with corticosteroids is needed. Suggested doses are: Prednisone or prednisolone given at 2-3 mg/lb/day divided BID for 5-7 days, or dexamethasone equivalents at 0.25-0.35 mg/l b/day divided BID. Therapy is reduced weekly by 1/2 and maintained for at least six weeks. Alternate day steroid therapy may be needed for some time thereafter on a long-term, low level basis.

3) For severe cases, other immunosuppressive therapy is given. We prefer cyclosporine (Neoral, 100 mg/ml oral syrup, or capsules) instead of cyclophosphamide (Cytoxan) and give it at 10 mg/**kg** for 5 days rest 2 days, then at 5mg/**kg** for another 5 days. The lower dose is repeated after a 2 day rest on a 5 days on, 2 days off cycle as long as is needed (usually 2-3 courses of 5 days). This drug induces rapid T-cell suppression within about 48 hours and has been safe, effective, and well-tolerated at these doses. In cases where sustained more potent immunosuppression is required for clinical stabilization, azathioprine (Imuran) should be instituted along with cyclosporine. Dose is 1 mg/lb/day for 7-10 days initially followed by a downward tapering over several weeks. Azathioprine may be needed every other day or less often, on a long-term basis. As azathioprine takes about 10 days to effectively suppress T-cells, clinical responsiveness will not occur immediately. Cyclosporine is therefore given concurrently in the early stages of the disease to provide rapid immunosuppression until the azathioprine takes hold.

The goal of this immunosuppressive therapy is to stabilize the ongoing immune destructive process. The dosage guideline we use is adjusted to maintain the absolute lymphocyte count as about 1/4 of the normal range (500-7500/ul).

4) Those breeds most often affected in our case population are cocker spaniels, poodles (all varieties), golden retrievers, Doberman pinschers, dachshunds, miniature schnauzers, akitas, beagles, rottweilers, Lhasa apsos, German shepherds, shih tzus, terriers, and mixed breeds of these backgrounds. Any of the nearly 50 breeds predisposed to thyroid disease are at risk for an immune-mediated condition. Thyroid supplementation at 0.1 mg/10lb given twice daily is essential for cases with concomitant thyroid disease and is helpful to stimulate the bone marrow whether or not thyroid tests indicate hypothyroidism. It also enhances platelet function.

5) Anabolic steroid (nandrolone decanoate, Deca Durabolin, 2-5 mg/**kg** given once a week or 4-6 doses) can be given to stimulate the marrow.

6) Hematinics containing iron and vitamin B12 have been helpful.

7) In poorly responsive immune thrombocytopenias (ITP), an initial dose of vincristine (Oncovin, 0.01 mg/lb IV) may be helpful to release remaining platelet stores, and danazol (Danacrine, 2.5-5 mg/lb BID initially and then tapered to SID) has been effective along with steroids and thyroid for long-term maintenance.

8) The most severe cases with auto agglutinating red cells or profound thrombocytopenia may recover completely with the aggressive therapeutic approach outlined above, although a subset of these dogs convert to having a chronic low-grade nonresponsive anemia over the long-term.

9) Cases with the best overall prognosis tend to be younger animals in which the underlying primary "trigger" of the immune-mediated disease was hypothyroidism, a drug which is withdrawn, or a recent vaccination/toxic exposure. Correction of the thyroid disease with serial monitoring of thyroid function to establish the appropriate maintenance dose of hormonal supplement is important.

References

Burgess K, Moore A, Rand W et al. (2000), Treatment of immune-mediated hemolytic anemia in dogs with cyclophosphamide. J Vet Int Med 14: 456-462
Carr AP, Panciera DL, Kidd L. (2002), Prognostic factors for mortality and thromboembolism in canine immune-mediated hemolytic anemia: a retrospective study of 72 dogs. J Vet Int Med 16: 504-509.
Dodds WJ (1983), Immune-mediated diseases of the blood. Adv Vet Sci Comp Med 27:163-196.
Dodds WJ (1997), Holistic approaches for immune-mediated disease. Proc Am Hol Vet Med Assoc, 96-100.
Duval D, Giger U. (1996), Vaccine-associated immune-mediated hemolytic anemia in the dog. J Vet Int Med 10:290-295.
Grundy SA, and Barton C (2001), Influence of drug treatment on survival of dogs with immune-mediated hemolytic anemia: 88 cases (1989-1999). J Am Vet Med Assoc 218: 543-546.
Klag AR, Giger U, Shofer FS. (1993), Idiopathic immune-mediated hemolytic anemia in dogs: 42 cases (1986-1990) J Am Vet Med Assoc 202: 783-788.
Northern J Jr, Tvedten HW. (1992), Diagnosis of microthrombocytosis and immune mediated thrombocytopenia in dogs with thrombocytopenia: 68 cases (1987-1989). J Am Vet Med Assoc 200: 368-376.
Pesillo SA, Freeman LM, Rush JE. (2004), Assessment of lipid peroxidation and serum vitamin E concentration in dogs with immune-mediated hemolytic anemia. Am J Vet Res 65: 1621-1624.
Reimer ME, Troy GC, Warnick LD. (1999), Immune-mediated hemolytic anemia: 70 cases (1988-1999). J Am An Hosp Assoc 35: 384-391.
Scott-Moncrieff JC, Treadwell NG, McCullough SM et al. (2001), Hemostatic abnormalities in dogs with primary immune-mediated hemolytic anemia J Am An Hosp Assoc 37:220-227.
Scoot M. (2000), Immune-mediated thrombocytopenia, Ch 68. In, Schalm's Veterinary Hematology, 5th Ed, Lippincott, Williams & Wilkins, Philadelphia, pp. 478-486.
Stokol T, Blue JT, French TW. (2001), Idiopathic pure red cell aplasia and nonregenerative immune-mediated anemia in dogs: 43 cases (1988-1999). JAm Vet Med Assoc 216: 1429-1436.
Wilkins RJ, Hurvitz AJ, Doods-Laffin WJ. (1973), Immunologically mediated thrombocytopenia in the dog. J Am Vet Med Assoc 163: 277-282.