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Canine Immune-Mediated Hemolytic Anemia

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개의 면역성 용혈성 빈혈

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요 약 : 면역성 용혈성 빈혈증 병견은 임상적으로 허약, 황달, 발열, 침울 및 점막창백을 나타낸다. 본 병의 진단은 구상적혈구증가증, 혈구응집반응 또는 직접적 Coomb시험 양성반응을 확인함과 동시에 용혈성 빈혈의 다른 원인을 배제함으로서 확정하여야 한다. 치료방법에는 적절한 보조적 요법과 함께 면역억압제가 포함된다. 일반적으로 치료에 있어서는 일차적으로 glucocorticoids가 선택되는데 흔히 cytoxan, aziothioprin, vincristine 또는 danazole과 같은 다른 약제와 병용된다. 치료는 그 반응에 근거하여 면역억압제의 용량을 2-4주 간격으로 점차 감량하면서 6개월 또는 그 이상까지 계속한다. 면역억압제에 대한 치료반응은 지연될 수 있고 또 적혈구 보유가 골수로부터 새로운 적혈구를 유리하게될 때까지 병축을 유지시키기에 부적당할 수 있기때문에 병축의 예후판정은 경계하여야 한다. 심급성 내지 급성 예는 일반적으로 예후가 보다더 좋지 않다.

Key words : immune-mediated hemolytic anemia, direct Coom's test, spherocytosis, immunosuppresive agent

Introduction

Immune-mediated hemolytic anemia is a immunohemolytic disorder in which the patient's red blood cell are destryed. This type II immune reaction, in which red cell destruction is antibody or complement mediated, results from the binding of IgG or IgM antibodies to antigenic determinants on the surface of the erythrocyte with complement often participating in this reaction.

Clinical signs of this disease in dogs are related to anemia and red cell destruction. Anorexia, depression, emesis, fever, weakness, plae mucous membranes, icterus, tachycardia and hyperpnea are often seen in affected animals. This review provides a basic overview of the immunology, causes, diagnosis and treatment of immune-mediated hemolytic anemia.

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Immunology

The autoantibodies most commonly involved in canine immune-mediated hemolytic anemia are of the IgG class and are often found in combination with the third component complement (C_3) on the patients red cells[12,29]. Autoantibodies of the IgM class have also been reported, and may occur with IgG and/or C_3. Antibodies may be temperature dependent in attaching to red cell antigens and are classified as warm acting if they attach at room temperature and cold acting if their critical temperature is less than 37°C. Cold acting antibodies are most often of the IgM class[4,11,12,29].

Five immunological subtypes of antibodies cause

RBC destruction found in immune-mediated hemolytic anemia. Subtype I or saline-acting agglutinins cause intravascular hemagglutination. This subtype presents with a peracute onset and agglutination can be observed immediately upon withdrawall of blood into a syringe or when a drop is placed on a slide. This frank slide agglutination eliminated the necessity of performing the direct Coomb's test.

Subtype II are intravascular hemolysin antibodies that fix complement in sufficient quantity to cause massive intravascular hemolysis. They are usually of the IgM class, but may be of the IgG class.

Subtype III is the most common antibody causing immune mediated hemolytic anemia in the dog. This antibody coats the red blood cell but it is incomplete or insufficient to cause hemagglutination or lysis. These red blood cells are the destroyed by the mononuclear phagocytic system. The direct Coomb's test is necessary to detect this antibody.

Subtype IV is composed of cold agglutinins that are most active at temperatures lower than 37°C. They are most commonly of the IgM class.

Subtype V is a cold-acting, non agglutinating immunoglobulin. These antibodies do not cause direct agglutination, but lead to increased RBC destruction at low temperatures.

All five of these subtypes occur in dogs but the classification is not absolute since clinical presentations tend to bridge the subtypes as the disease progresses. Subtypes I and II develop peracutely. Acute cases that result in a 2 to 5 day course are of the subtypes II and III. Chronic cases with a course longer than 5 days are of subtypes III to V and are less severe and slower to respond.

Occurrence

Canine immune-mediated hemolytic anemia occurs predominantly in females, usually young to middle aged dogs[7,16,22]. There has been an increased incidence reported in bitches with a history of reproductive problems[7], since increased estrogen reportedly increases the likelihood of IMHA forming. There also has been a seasonal relationship to it incidence with 40% of the cases being reported during May and June at one institution[18].

Breeds with apparent predispositions include poodles, cocker spaniels, irish setters and old english sheep dogs[7,35]. In a study conducted by Jackson and Kurth[6] other conditions associated with IMHA include recent vaccination, neoplasia, ovariohysterectomy, seizure or professional groomings.

Table 1. Causes associated with immune mediated hemolytic anemia

A. Modification of red cell antigens
1. Drugs-phenothiazines, methylene blue, acetominophen, topical benzocaine, propyl thiouracil, heparin, cephalosporins, dipyrone, quinidine
2. Microorganisma
B. Cross reacting antibodies
1. Microorganisms
C. Failure of autoregulatory function of the immune system
1. Diseases of the lymphoreticular system
2. Depressed T suppresser (C_8) function
D. Genetic predisposition causing failure of self tolerance[6,10]
E. Proliferation of a primary or secondary clone
1. Primary clone-may be idiopathic or reflect decreased autoregulation
2. Secondary clone-may be due to an underlying neoplasm
F. Defective or ineffective erythropoesis-aberrant RBCs with new antigenic determinants

Causes

The onset of immune-mediated hemolytic anemia is an alteration in the surface of red blood cells or immunological control. These changes may be due to any number of causes ranging from drug induced changes on the RBC membrane to genetic predisposition that causes failure of self tolerance[7,9]. Table 1 summarizes the more common causes associated with immune mediated hemolytic anemia.

Clinical features

Patients with immune-mediated hemolytic anemia often present with a history of anorexia, emesis, depression, weakness, fever, red-brown urine, excessive water intake or not drinking, or abdominal pain[16]. Patients with subtypes I and II tend to

present with acute disease, while subtypes III, IV and V have a more chronic history.

Findings commonly seen on physical exam include pale mucous membranes, iterus, hepatosplenomegaly, fever, systolic heart murmur, tachycardia, dehydration and back pain[16]. Petechia, ecchymosis and skin lesions (especially if IgM is involved) may be seen[6,25,33].

Diagnosis

Hemogram

There is usually moderate to severe anemia, marked reticulocytosis, spherocytosis, and some degree of agglutination when red cells cool to room temperature. A striking morphological feature on blood film is extreme variation in RBC diameter, due to the presence of spheres and polychromatophilic cells with increased diameters[8]. On automated systems the presence of agglutination may cause a false high MCV and false low hematocrit values. Reticulocytes (large polychromatic red blood cells) may be present to varying degrees. Peripheral reticulocyte responses take 4 to 5 days after acute loss of circulating RBCs [13]. If the onset is acute or peracute, the reticulocyte response may not yet be evident[20].

Spherocytosis is often seen in patients with immune mediated hemolytic anemia. Spherocytes are small, dense, uniform-sized RBCs that have lost the characteristic central pallor of the normal biconcave shape as the result of cell membrane damage. The degree of spherocytosis depends on the modes of destruction. In complement mediated hemolysis, spherocytes may not be evident because RBCs may be totally destroyed[31,33]. IgG mediated diseases may more commonly involve spherocytosis.

There is usually a leukocytosis seen with mature neutrophilia often accompanied by a left shift[30] and microcytosis[16]. The leukocytosis may be due to an active bone marrow, stress, or chemostatic byproducts of the complement cascade. When the neutrophilia is moderate to marked, there is an indication of an underlying inflammatory lesion which is frequently associated with immune-mediated hemolytic anemias[8]. Thrombocytopenia may also occur in conjunction with the immune-mediated hemolytic anemia.

Direct antiglobulin testing (direct Coomb's testing)

The direct Coomb's test is utilized to detect antibodies or complement attached to the RBC membrane. A positive Coomb's test is regraded as supportive of the diagnosis of immune-mediated hemolytic anemia when other essential features are present. The indirect Coomb's test detects anti-RBC antibody in the patient's serum. Peracute cases usually have positive direct Coomb's test and negative indirect Coomb's test because all antibody molecules are actively bond and not free in circulation. The chronic forms (III, IV, and V) can be positive for the direct and indirect Coomb's test because free antibodies exist. The acute form bridges the spectrum. A positive direct Coomb's test for IgG and a positive indirect Coomb's test for IgM are most common[30].

A commom misconception is that a positive Coomb's test is required to diagnose immune-mediated hemolytic anemia. One of the most common reasons for false negative reactions is elution immunoglobulins during washing of the RBCs for preparation for the test. If a negative titer is obtained but the clinical picture is suspicious, further diluting should be requested to rule out a strong prozone effect[30]. Other causes of false negative Coomb's test include use of corticosterodis, disease in remission, antisera inadequate is strength or dilution[33], immunoglobulin M elution at high temperatures, poor technique or insufficient IgG on the RBC membrane [30]. False positives on the Coomb's test may also occur due to antibodies being present on the RBCs from other reasons such as drugs or parasites, nonspecific absorption of serum proteins by damaged RBCs, some malignancies and post transfusion[33].

Bone Marrow Aspirate

Bone marrow aspirates are useful as part of the diagnostic workup in suspected immune-mediated hemolytic anemia, especially if the disease is suspected but there is a nonregenerative response on

the hemogram. Aspirates help in diagnosing red blood cell aplasis caused by maturation defects or immune destruction of precursors and can indicate the presence of all red blood cell precursors without erythroid hyperplasia (which is common in long standing chronic cases)[18]. Immunoflourecense with anti-red blood cell antibodies should also be done to help confirm the diagnois.

Differential diagnosis

Unless there are strong supportive findings of immune-mediated hemolytic anemia, such as spherocytosis, agglutination or a positive Coomb's test, other causes of regenerative anemia should be suspected. Blood parasites and bleeding tumors, such as hemangiosarcoma or gastrointestinal neoplasia, can cause signs similar to immune-mediated hemolytic anemia. False positive Coomb's tests have been associated with hemangiosarcomas of the the spleen and liver[12]. The red blood cells in these patients are thought to be injured passing through the neoplastic microcirculation beds.

Other causes of hemolytic anemia in dogs include postcaval syndrome of canine dirofilariasis[16], Heinz body anemia due to oxidant chemicals[20], microangiopathic hermolytic anemia[4,5,15] and congenital red cell disorders, such as pyruvate kinase deficiency of Basenji's[28] or hereditary hemolytic anemia and chondrodysplasia of Alaskan Malamutes.

Treatment

Glucocorticoids are often used as first line of defense in the treatment of immune-mediated hemolytic anemia. Glucocorticoid administration often yields a rapids response because of the steroid response on the clearance mechanism[5]. There is interference with the FRC (constant fragment of IgG) and C_3b receptors on macrophages[2,26,27,31] and depressed immunoglogulin affinity for the red blood cell membrane[2,27]. Glucocorticoid use has also been proven to increase the magnitude of the reticulocyte response[16] by a marrow sensitive mechanism. Glucocorticosteroids also inhibit the amplification pathways of the complement cascade[19,32]. Prednisone at 1 mg/kg twice day is often used, but doses up to 2 mg/kg twice daily may be needed. Prednisilone, methylprednisilone and dexamethasone may also be used. Glucocorticosteroids are not tapered until a therapeutic response is noted[35] which may take up to 2 to 4 weeks. Tapering should be done slowly in 2 to 4 week blocks with close monitoring, with therapy continuing up to 6 months.

Antimetabolite therapy should be considered in peracute or acute cases of IMHA, especially if there is autoagglutination. Cyclophophamide (Cytoxan) is an alkalating agent that works by cross-linking the DNA of dividing and resting cells. The agent inhibits normal primary and secondary immune responses, diminishes antigen trapping in lymph nodes, inhibits local inflammatory responses, kills slowly proliferating antigen reactive cells and rapidly proliferating stimulated cells[23]. The dose is usually 50 mg/m^2 given on the first four days of the week or every other day. The durg should be administered in the morning and should be used no longer than 4 to 6 weeks.

Aziothioprin is a thiopurine that is metabolized to 6-mercaptopurine and competes with adenine in synthesis or nucleic acids. This results in prevention of the proliferation of rapidly dividing cells and therefore primary and secondary lymphocytic dependent antibody synthesis is strongly inhibited[3,23]. The dosage of aziothioprin is 2 mg/kg/day orally.

Vincristine is a vinca alkaloid that prevents cellular division and decreases phagocytosis by inhibiting formation of the cytoplasmic microtubular network and the formation of mitotic spindles. It also stimulate megakaryocyte division and therefore is useful in treatment of immune mediated thrombocytopenia. The dosage is 0.05 to 0.75 mg/m^2 every week, as needed.

Danazol is a synthetic androgen derived from ethisterone that is being used more frequently to treat immune-mediated hemolytic anemia. Danazol decreases IgG production, cell-bound IgG and complement. A large decrease in cell bound compliment indicated the main mode of activity may be inhibition of complement activation and complement binding to the cell membrane[1,21,26,34]. Danazol use

should start simultaneously with glucocorticoids. If the patient is stable, the glucocorticoids should be tapered[7,21]. The dose in the dog is 5 mg/kg three times daily. It should be tapered gradually and may be administered exclusively.

Blood transfusions are not advised in most cases since they accelerate hemolysis[14,36], but they may be useful therapy if done appropriately. They should be done is the disease will become more severe before a rise is hematocrit is expected or is supportive care and oxygenation are necessary for therapy to be effective. It is important to use the best possible crossmatched blood. Packed, washed red blood cells are best because they avoid adding additional complement[22,25,31]. It is best to warm the blood in the cold type disease[5]. Providing blood can result in increased antibody production, hemolysis, hemoglobin induced renal damage[14], volume overload and bone marrow suppression.

Splenectomies are a controversial treatment utilized in the treatment of immune-mediated hemolytic anemia. Splenectomies directly remove B cells and primed macrophages and thus decreases antibody production and red cell damage and destruction[2,25]. This procedure also allows a decreased steroid dose and more time for immunosuppressive drugs to be effective[26].

The following are some guidelines in the treatment of IMHA.

1. Initial treatment involves glucocorticoids. Only 5 to 10% of IMHA cases are refractory to steroids[7].

2. If there is no response to glucocorticoids in 3 to 4 days or if the presentation is peracute, cyclophosphamide should be added.

3. Use vincristine if thrombocytopenia occurs simultaneously and hemorrhage is present and if bone marrow aspirates demonstrate megakaryocytes[16,35].

4. Stable, chronic IMHA warrants a 4 week glucocorticoid trial before other modulators are used.

5. Danazol administration started with glucocorticoid administration lowers maintenance prednisilone doses or ultimately allows for prednisilone free maintenance with danazol.

6. Prognositically, it has been noted that decreased reticulocyte counts, leukopenia, and negative indirect antiglobulin tests may be related to a poor prognosis[22]. Cases with subtype I or II mediated disease currently have the worst prognosis[33].

Summary

Dogs presented with immune mediated hemolytic anemia are often weak, icteric, febrile depressed and have pale mucous membranes. Diagnosis must be based on the elimination of other causes of hemolytic anemia as well as the presence of spherocytosis, hemagglutination or a positive direct Coomb's test.

Treatment includes immunosuppressive agents in conjuction with apportive care. Glucocorticoids are generally considered to be the first choice in treatment and are often used with other agent such as cytoxan, aziothioprin, vincristine or danazol. Treatment is continued based on response, with dosages of immunosuppressives tapered at 2 to 4 week intervals for 6 months or longer.

Prognosis should be guarded since response to the immunosuppressive agents may be delayed and reserves of RBCs may inadequated to maintain the patient until new RBCs are released from the bone marrow. Peracute to acute cases usually have a more guarded prognosis.

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