IDIOPATHIC AUTOIMMUNE HEMOLYTIC ANEMIA PRESENTING AS MASSIVE SPLENOMEGALY IN AN ELDERLY MALE WHICH RESPONDED TO STEROID THERAPY
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ABSTRACT: Autoimmune hemolytic anemia is uncommon. The estimated overall (not age-adjusted) annual incidence is about 1 case per 100,000 populations; after age 60 years, the annual incidence reaches 10 per 100,000. The disorder can occur at any age, but most patients are older than 40 years. About 65% of patients with primary autoimmune hemolytic anemia are women, and almost all cases that complicate systemic lupus erythematosus occur in women. A 65 years old male presented with generalized weakness, breathlessness on exertion, swelling of lower limbs and pain abdomen of 6 months duration. He was previously admitted elsewhere on several occasions (within past 3-4 months) with similar complaints, and had received multiple blood transfusions. On clinical examination, patient had pallor and bilateral pitting pedal edema. Abdominal examination revealed massive Splenomegaly (12 cm below left costal margin), moderate Hepatomegaly. Investigations revealed Hb% of 8.6 g/dl, Platelet count = 1 lakh/cmm, ESR = 120 mm, retic count -2.2 %. Peripheral smear showed evidence of hemolysis. Serum LDH was high, Serum bilirubin predominantly indirect hemoglobin= 2, S. Haptoglobin below 6.63. These findings suggested hemolysis as a cause of his anemia and splenomegaly. Further evaluation was done to find out the cause of hemolysis in this elderly male. Hb Quantification using HPLC was normal. Serum G6PD activity was normal. ANA was negative. Hams test was negative. Direct & Indirect Coomb’s tests were positive. Bone marrow examination showed erythroid hyperplasia. CT Abdomen showed hepatosplenomegaly. Upper GI endoscopy and colonoscopy were normal. Based on these findings a diagnosis of warm antibody type auto immune haemolytic anaemia (AIHA), probably of idiopathic type, was made and patient was started on steroid therapy. After 2 weeks, repeat haemoglobin was 12.8 gm%, WBC count was 7020/cumm reflecting response to steroid therapy. Severe AIHA can be a medical emergency. Red cell transfusion poses a special problem as the transfused cells are rapidly destroyed, but can be life-saving and in the meantime steroids can exert their effect. This unique situation requires good liaison and understanding between the clinical unit and the serology lab. Thus it is very important to diagnose this condition and treat accordingly. Untreated, chronic auto immune hemolysis progresses to severe anaemia and associated complications. It is important to consider the diagnosis of autoimmune hemolysis in elderly males also, although this condition is usually seen in females. It is important to rule out malignancies and connective tissue disease in elderly presenting with autoimmune hemolysis.

KEYWORDS: Autoimmune hemolytic anemia, massive splenomegaly, steroids.
INTRODUCTION:
Autoimmune Hemolytic Anemia with IgG Autoantibodies:

EPIDEMIOLOGY: Autoimmune hemolytic anemia associated with IgG auto-antibodies (generally referred to simply as autoimmune hemolytic anemia) accounts for about 75% of all autoimmune hemolytic anemias. It has also been called warm antibody autoimmune hemolytic anemia because the IgG auto-antibodies in this disorder react best with red cells at 37° C. Autoimmune hemolytic anemia can be primary (idiopathic), or it can develop in association with another disease (secondary).

Autoimmune hemolytic anemia is uncommon. The estimated overall (not age-adjusted) annual incidence is about 1 case per 100,000 population; after age 60 years, the annual incidence reaches 10 per 100,000. The disorder can occur at any age, but most patients are older than 40 years. About 65% of patients with primary autoimmune hemolytic anemia are women, and almost all cases that complicate systemic lupus erythematosus occur in women.

Autoimmune Hemolytic anemia (AIHA) is the clinical condition caused by autoantibodies, which bind to the red cell surface resulting in extravascular hemolysis. RBC destruction is mediated via the complement system or the reticuloendothelial system.

AIHA are classified into warm AIHA, cold AIHA, mixed type and drug induced type. Warm antibody type accounts for 70% of all AIHA. It is of IgG type and usually does not fix the complement. AIHA can be a primary disorder (50%) or secondary to lymphoproliferative diseases, other systemic autoimmune diseases, viral infections, immune deficiency states, etc. Direct Coomb's test which detects Ig and/or Complement bound to the surface of the red cell is the diagnostic test for AIHA.1

CASE HISTORY: A 65years old male presented with generalized weakness, breathlessness on exertion, swelling of lower limbs and pain abdomen of 6 months duration. He was previously admitted elsewhere on several occasions (within past 3-4 months) with similar complaints, and had received multiple blood transfusions. There was no history of blood loss / bleeding diathesis, no history of fever, rash and no history of recent drug intake. No history of loss of weight, loss of appetite.

On clinical examination, patient had pallor and bilateral pitting pedal edema. Abdominal examination revealed massive Splenomegaly (12 cm below left costal margin), moderate Hepatomegaly.

Investigations revealed Hb% of 8.6 g/dl, Platelet count = 1 lakh/cmm, ESR = 120 mm, retic count -2.2 %. Peripheral smear showed evidence of hemolysis. Serum LDH was high, Serum bilirubin predominantly indirect hemoglobin= 2, S. Haptoglobin below 6.63. These findings suggested hemolysis as a cause of his anemia and splenomegaly. Further evaluation was done to find out the cause of hemolysis in this elderly male.

Hb Quantification using HPLC was normal. Serum G6PD activity was normal. ANA was negative. Ham's test was negative.

Direct & Indirect Coomb's test was strongly positive. Thus a diagnosis of autoimmune hemolysis was done. Further investigations were done to rule out secondary causes of auto immune haemolytic anemia.

Bone marrow examination showed erythroid hyperplasia. CT Abdomen showed hepatosplenomegaly. Upper GI endoscopy and colonoscopy were normal. Thus malignancy as a cause
of hemolysis was ruled out. ANA profile was completely negative ruling out other autoimmune diseases as a cause of hemolysis.

Based on these findings a diagnosis of warm antibody type auto immune haemolytic anaemia (AIHA), probably of idiopathic type, was made and patient was started on steroid therapy. Patient was started on Prednisolone 1mg/kg/day. After 2 weeks, repeat haemoglobin was 12.8 gm%, WBC count was 7020/cumm reflecting response to steroid therapy.

**DISCUSSION:**

**Causes of Autoimmune Hemolytic Anemia:** The cause of autoimmune hemolytic anemia is unknown. In about a third of cases, the autoantibodies have specificity for an antigen in the Rh system. In another third, the antibodies target proteins in membrane glycoproteins (glycophorins) of the red cell; in other cases, the antibodies have specificity for antigens in the Kell or Duffy blood group system (very rarely for ABO antigens) or for structures in the membrane that are not blood group antigens (e.g. band 3). In all these cases, the patient’s own erythrocytes display the relevant antigen.²

During fetal life, the thymus deletes lymphocytes with receptors that can bind to autoantigen. This effect, one of the mechanisms of immunologic tolerance of endogenous (“self”) antigens, prevents the development of autoantibodies against blood group antigens. The extreme rarity of autoimmune hemolytic anemia secondary to anti-A or anti-B antibodies indicates that the deletion occurs early in ontogeny, because the embryo begins to synthesize A and B substances at 5 weeks of gestation.

A population of CD4+/CD25+ T cells that express the transcription factor Foxp3 restrains immune responses against autoantigens. There is evidence that a deficiency of these regulatory T cells plays a role in the pathogenesis of autoimmune hemolytic anemia, but how this deficiency arises is unknown.

The anti-red cell autoantibodies in autoimmune hemolytic anemia constitute a polyclonal population of IgG antibodies—a typical feature of an antigen-driven immune response. Evidence that cultured T cells from patients with autoimmune hemolytic anemia proliferate in the presence of certain synthetic Rh peptides supports the idea that the patient’s own Rh antigens, or exogenous cross-reactive antigens, drive the autoimmune response.

These peptides are cryptic—they reside in regions of the Rh protein that are not normally exposed on the red cell surface. By contrast, T cells from Rh-negative persons who were alloimmunized by transfusion of Rh-positive blood respond to peptides in exposed regions of the Rh protein. The weak response of T cells from normal subjects to allogeneic Rh peptides suggests that cross-reactive environmental antigens may prime T cells for an anti-Rh response.

T cells from patients with chronic lymphocytic leukemia complicated by autoimmune hemolytic anemia also respond in vitro to self-Rh proteins. Notably, the leukemic B cells are highly effective in presenting Rh protein to T cells.

In some cases of chronic lymphocytic leukemia with autoimmune hemolytic anemia, the leukemic cells display surface immunoglobulins with light chain isotypes that differ from those of the patient’s anti-red cell autoantibodies, indicating that the source of the autoantibodies in these patients is not the leukemic clone.

**Drug-Induced Immune Hemolytic Anemia.**
Drug induced hemolytic anemias are rare. Many drugs or drug metabolites have the potential to elicit antidrug antibodies. Drugs that form covalent bonds with proteins in the red cell membrane can bind antidrug antibodies to the red cell surface, causing a positive direct antiglobulin test (see later) and, in some cases, initiating antibody-mediated destruction of red cells. Other drugs, such as the cephalosporins, can bind to red cell membranes and take up IgG nonspecifically from plasma. In these cases, there is no antidrug antibody.

The diagnosis of drug-induced immune-mediated hemolytic anemia should be considered if the patient has a history of taking a suspected medication, there is acute complement-mediated hemolysis, only complement components are detectable on the red cell surface, or the patient’s serum reacts with red cells in the presence of the suspected drug.

Some drugs can induce true autoantibodies against red cells. Fludarabine, a purine nucleoside analogue used in the treatment of chronic lymphocytic leukemia, and monoclonal antibodies against tumor necrosis factor-α (infliximab and adalimumab), T cells (alemtuzumab), α4 integrin (natalizumab), and interleukin-2 receptor (daclizumab) also have this property, the cause of which is unknown.

Notably, there is no sure way of distinguishing drug-induced autoimmune hemolytic anemia from primary autoimmune hemolytic anemia.

Clinical Manifestations

Autoimmune hemolytic anemia usually unfolds insidiously, but in some cases it begins abruptly with overt symptoms of severe anemia. When the disease is secondary to chronic lymphocytic leukemia, systemic lupus erythematosus or some other disorder, the primary condition generally brings the patient to the physician.

The symptoms are nonspecific and varied. They depend on the age of the patient; the presence of co-morbid conditions, especially cardiovascular disease; whether the autoimmune process is primary or a complication of another disorder; and the degree of anemia.

In primary autoimmune hemolytic anemia, symptoms of anemia predominate; asthenia and easy fatigue are typical. Dyspnea suggests coexisting heart disease; angina or myocardial infarction can occur in patients with severe anemia and coronary artery disease.

Differential Diagnosis of Hemolytic Anemia:

**HEREDITARY HEMOLYTIC ANEMIA**

The thalassemias

- β-Thalassemia
- α-Thalassemia

Combined thalassemia and hemoglobinopathy

The hemoglobinopathies

- Sickle cell disease (hemoglobin S)
- Hemoglobin C disease
**Hemoglobin E disease**

**Combined hemoglobinopathies (hemoglobin SC disease)**

**Defects of the red cell membrane**

- Hereditary spherocytosis
- Hereditary elliptocytosis
- Acanthocytosis

**Deficiencies in red cell enzymes**

- Glucose-6-phosphate dehydrogenase deficiency
- Pyruvate kinase deficiency
- Defects of the Embden-Meyerhof pathway

### ACQUIRED HEMOLYTIC ANEMIA

**Immune-mediated hemolytic anemia**

- Autoimmune hemolytic anemia
- Cold agglutinin disease
- Paroxysmal nocturnal hemoglobinuria
- Paroxysmal cold hemoglobinuria
- Drug-induced immune hemolytic anemia
- Hemolytic transfusion reaction
- Hemolytic disease of the newborn secondary to maternal alloantibodies
- Systemic lupus erythematosus

**Microangiopathic hemolytic anemia**

- Disseminated intravascular coagulation
- Thrombotic thrombocytopenic purpura
- Hemolytic-uremic syndrome
- Infections (e.g., Clostridium, malaria, babesiosis, Bartonella)
- Deficiency of factor H
- Giant hemangioma
- Disseminated carcinomatosis
- Chemotherapy (e.g. mitomycin C)
Laboratory Findings: The blood smear reflects the marrow's attempt to compensate for accelerated hemolysis and the effects of the interaction between antibody-coated red cells and macrophages. Two populations are evident: large, blue-tinged red cells, which correspond to reticulocytes, and small, dark red spherocytes (microspherocytes). Such a dimorphic population is characteristic of hemolytic anemia but is not diagnostic of any particular type of hemolytic anemia.

The white blood cell count is often moderately elevated, a reflection of a bone marrow stressed by severe hemolytic anemia. In uncomplicated cases, platelet counts are normal or occasionally increased slightly. Platelet counts may be low in systemic lupus erythematosus or in Evans syndrome, a rare combination of autoimmune hemolytic anemia and immune thrombocytopenia.

The characteristic finding in bone marrow is erythroid hyperplasia. However, examination of marrow is necessary only if there are unexpected findings or suspicion of lymphoma.

Elevated serum levels of prehepatic (indirect) bilirubin and lactate dehydrogenase (LDH) and a reduced serum haptoglobin concentration are signs of hemolytic anemia. The indirect bilirubin in hemolytic anemia usually does not exceed 4.0 mg/dl unless there is coexisting liver
disease. Serum LDH can be useful, especially in cases with intravascular hemolysis, but neither LDH nor the haptoglobin level is specific for hemolytic anemia. Moreover, the presence of inflammation negates the value of measuring haptoglobin, which is an acute phase protein. Oral contraceptives, estrogen replacement therapy, and tamoxifen all increase haptoglobin levels.

Laboratory signs of intravascular destruction of red blood cells (hemoglobinemia, hemoglobinuria, and hemosiderinuria; see later) are unusual in autoimmune hemolytic anemia.\(^6\)

The Antiglobulin (Coombs) Test

The antiglobulin test is central to the diagnosis of autoimmune hemolytic anemia and to an understanding of the antibody-mediated mechanism of red blood cell destruction in this disorder.

The complete and incomplete antibodies refers to the ability (or inability) of antibodies to cross-link adjacent red cells, thereby building the lattice needed for the macroscopic clumping of red cells. The strong negative charge of red blood cells suspended in saline keeps them apart, even if they have a coating of antibodies—the average distance between cells is 24 nm. IgM antibodies, being pentamers, are efficient agglutinins; with five antigen-binding sites, they can bridge this distance. In contrast, bivalent IgG antibodies usually cannot cause the clumping of saline-suspended erythrocytes.

The test that reveals antibody-coated red blood cells is the direct Coombs (antiglobulin) test. The indirect Coombs test was devised to seek the presence of incomplete antibodies in the patient’s serum. As presently used, the standard antiglobulin reagent contains antibodies against all four classes of IgG and components of complement (usually C3 and C4).

A positive Coombs test requires cautious interpretation when there are no other features of autoimmune hemolytic anemia. False-positive test results are not unusual. The reported incidence of positive antiglobulin tests in normal blood donors and general populations of hospitalized patients varies widely—from 1 in 100 to 1 in 15,000. Differences in the technique used to perform the test account for this variation. The usual reason for a false-positive direct antiglobulin test is nonspecific, low-avidity adherence of IgG to red cells.

In rare cases, however, the result is not a false-positive but a harbinger of the development of autoimmune hemolytic anemia. False-negative direct antiglobulin tests are usually due to low-affinity autoantibodies that spontaneously elute from the red cell in vitro or to amounts of erythrocyte-coating antibodies that are below the limit of detection by the antiglobulin test.

The distinction between a true-positive and a false-positive direct antiglobulin test can be made by eluting the antibody from the red cells and testing its ability to bind to normal red cells. In a false-positive reaction, the eluted antibody does not bind to normal red cells, whereas binding does occur in a true-positive test.

### Treatment

There are no controlled trials of the treatment of autoimmune hemolytic anemia. A corticosteroid, usually prednisone, is the standard initial treatment. Splenectomy is indicated in patients who fail to attain or sustain a remission. If splenectomy does not result in improvement, one or more immunosuppressive agents can be tried. The physician should not withhold red cell transfusions from symptomatic patients.
Initial Therapy

Corticosteroids:

In the initial management of the disease, the standard of practice is to administer prednisone at a dose of 1.0 to 1.5 mg/kg/day. The duration of treatment at this dose is an unsettled question, but a response—manifested by a rise in the hematocrit and a fall in the reticulocyte count—is usually evident within 3 to 4 weeks. A patient who fails to improve within this time is unlikely to respond to further treatment with prednisone. In a patient who does respond, slow reduction of the dose of prednisone is essential to avoid a relapse. The usual tapering schedule is a weekly reduction of the initial dose by 10 mg/day to a dose of 30 mg/day, followed by a weekly reduction of 5 mg/day to 15 mg/day. Thereafter, slow, cautious tapering over at least 4 months is the rule. A rise in the reticulocyte count or a fall in the hematocrit should prompt an increase in the dose, usually to the previous level.

About 25% of patients treated with corticosteroids in this manner enter a stable, complete remission; half of patients require continuous, low-dose prednisone; and the remaining 25% respond only transiently or not at all or are unable to tolerate continuous corticosteroid treatment. There is no reliable evidence that alternate-day maintenance treatment is superior to daily treatment, but some patients tolerate this schedule better than daily prednisone. Very high doses of intravenous methylprednisolone have been advocated for stubborn cases, but such treatment is risky and should be considered experimental.

Transfusion:

Red blood cell transfusions are indicated in patients with disabling symptoms of anemia: marked fatigue, reduced exercise tolerance, or an inability to work. These symptoms often develop when the hemoglobin concentration falls below 10 g/dL, but the decision to administer red cell transfusions should not depend primarily on laboratory tests—the patient's clinical status is the dominant factor. Some patients can tolerate a stable hemoglobin level as low as 8 g/dL; however, symptoms of coronary artery disease or heart failure may force the decision to transfuse before the hemoglobin level falls below 10 g/dL. Regardless of the patient's clinical status, rapid transfusion of large volumes of red cells can have serious adverse consequences. The blood should be administered at a rate that does not exceed 1 mL/kg/hour.

The risk of reactions to blood transfusions in patients with autoimmune hemolytic anemia is always present because of the destruction of transfused blood by the patient's autoantibodies. This hazard is increased if the patient also has alloantibodies that were induced by pregnancy or previous transfusions. For these reasons, the blood bank should be alerted to the diagnosis and should be informed if the patient was ever pregnant or has ever had transfusions.

It is important for the managing physician to understand that no patient with symptomatic autoimmune hemolytic anemia should be denied blood transfusions because of an “incompatible cross match.” The patient's positive antiglobulin test always interferes with compatibility testing. Communication and cooperation between the patient's physician and specialists in transfusion medicine are essential in reducing the risks associated with transfusion in patients with autoimmune hemolytic anemia.
Splenectomy:
Because the spleen is the major site of red cell destruction in autoimmune hemolytic anemia, splenectomy should be considered for patients who have not responded to corticosteroids or who have maintained a stable but corticosteroid-dependent remission. A complete, durable remission follows splenectomy in one half to two thirds of cases. Attempts to predict responsiveness to splenectomy by measuring the splenic sequestration of $^{51}$Cr-labeled erythrocytes have not been reliable. The only way of determining the effectiveness of splenectomy in a given patient is to perform the procedure. Laparoscopic splenectomy, a safe method of removing the organ, is now the preferred surgical technique. A major risk is post-splenectomy sepsis secondary to encapsulated bacteria, particularly pneumococci and especially in children. Splenectomy also increases susceptibility to babesiosis, ehrlichiosis, and malaria. Preoperative immunization with polyvalent pneumococcal and Haemophilus influenzae vaccines reduces the risk of post-splenectomy sepsis. In children, a prophylactic antibiotic, generally penicillin or amoxicillin is essential after splenectomy. Evidence for the effectiveness of (or need for) prophylactic antibiotics in splenectomized adults are inconclusive. Education of the patient concerning the risk for serious infection after splenectomy is also important.
A rise in the platelet count occurs in almost all patients after splenectomy. The increase rarely exceeds 500,000/mL and usually subsides within 3 to 5 months. The low risk for thromboembolism related to post-splenectomy thrombocytosis argues against the need for routine antithrombotic prophylaxis.

Therapy in Refractory Patients
Rituximab:
Rituximab is a chimeric monoclonal antibody with a high affinity for the CD20 antigen on the surface of normal and malignant B lymphocytes. The antibody consists of murine variable region sequences and human constant region sequences. The usual dose is 375 mg/m$^2$ by intravenous infusion once a week for four or eight doses, but it may be continued weekly or biweekly if necessary. Rituximab rapidly depletes the circulation and lymphoid tissue of B cells; it can cause allergic reactions and increase the risk for infection.

Other Monoclonal Antibodies:
Monoclonal antibodies against components of the immune system or cytokines have been used in the treatment of autoimmune hemolytic anemia, but most of the literature on this topic consists of small, uncontrolled series. Among the monoclonal antibodies that have been used are alemtuzumab (anti-CD52, a T-cell marker) and natalizumab (α4 integrin). These and similar monoclonal antibodies should be reserved for experimental use in refractory, transfusion-dependent cases.

Immunosuppressive Drugs and Other Modalities:
Immunosuppressive drugs other than corticosteroids can be useful in stubborn cases of autoimmune hemolytic anemia, but no head-to-head trials have compared the efficacy of these drugs. The choice usually depends on safety and familiarity with the agent. Azathioprine has
the least toxicity; cyclosporine is nephrotoxic; and cyclophosphamide damages the bone marrow, ovaries, and bladder and impairs spermatogenesis. Mycophenolate has also been tried, with some success in refractory cases. In general, these drugs should be administered only by specialists and should be reserved for patients who have failed to respond to splenectomy or who, because of comorbidities, are not suitable candidates for splenectomy. A variety of other treatments have been used in refractory cases of autoimmune hemolytic anemia, including plasma exchange, vinca alkaloids, danazol (a synthetic androgen), and intravenous IgG. None of these forms of therapy is reliably effective, and none of them has been tested for efficacy in a randomized trial.

CONCLUSION: Severe AIHA can be a medical emergency. Red cell transfusion poses a special problem as the transfused cells are rapidly destroyed, but can be life-saving and in the meantime steroids can exert their effect. This unique situation requires good liaison and understanding between the clinical unit and the serology lab. Thus it is very important to diagnose this condition and treat accordingly.

Untreated, chronic autoimmune hemolysis progresses to severe anaemia and associated complications. It is important to consider the diagnosis of autoimmune hemolysis in elderly males also, although this condition is usually seen in females. It is important to rule out malignancies and connective tissue disease in elderly presenting with autoimmune hemolysis.
REFERENCES:


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