Clinical course of autoimmune hemolytic anemia: an observational study

**Maria Christina L. A. Oliveira,[1] Benigna M. Oliveira,[1] Mitiko Murao,[2]
Zilma Maria Vieira,[3] Letícia T. Gresta,[4] Marcos B. Viana[5]**

Abstract

Objective: Autoimmune hemolytic anemia is characterized by the production of autoantibodies against erythrocyte membrane antigens. This study was carried out to identify the clinical, immunological and outcome characteristics of autoimmune hemolytic anemia patients treated at the (HC-UFMG) Pediatric Hematology Unit and the *Hemocentro de Belo Horizonte*.

Methods: We evaluated 17 patients younger than 15 years old admitted from 1988 to 2003 were evaluated. Autoimmune hemolytic anemia diagnosis was based on the presence of acquired hemolysis and confirmed by positive direct Coombs polyspecific test results. Clinical, laboratory, and outcome data were obtained from patient records.

Results: The median age at diagnosis was 10.5 months. The direct Coombs polyspecific test was positive in 13 and negative in four patients. Monospecific testing was performed for 14 patients. The most frequent red cell autoantibody was IgG (five patients), followed by IgM in two. Thirteen patients had severe anemia and needed blood transfusions. Underlying diseases were identified in four patients: systemic lupus erythematosus, Hodgkin's lymphoma, autoimmune hepatitis and Langerhans cell histiocytosis. The remaining patients were classified as having primary disease. The median follow-up period was 11 months (5 to 23 months). Three children died, two after splenectomy and one with complications of the underlying disease.

Conclusion: Autoimmune hemolytic anemia is rare in children and adolescents. Although patients usually respond to corticosteroids and/or immunoglobulin, fatal cases can occur. Prognosis is worse in patients with chronic underlying diseases.

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Introduction

Autoimmune hemolytic anemia (AIHA) consists of a group of diseases which have as their common factor the presence of autoantibodies that bind to red blood cells and lead to its premature destruction by promoting their removal from the circulation by macrophages from the reticuloendothelial system.[1] This is one of the most common autoimmune events in humans.[2] The occurrence of AIHA in children and adolescents, however, is rare. The exact incidence is unknown, but it is estimated that the rate is around 0.2 per 1,000,000 individuals under 20 years old. Peak incidence is among pre-school aged children.[3] It is more common among males, although during adolescence female patients predominate.

The causes of AIHA remain unknown. Some hypotheses include immune system depression by viral activity, changes to the balance between helper and suppressor T cells, alteration of red blood cell surface antigens by viruses or drugs or possibly a cross reaction between antibodies induced by infectious agents against red blood cell surface antigens. The clinical presentation of AIHA is heterogeneous, but hemolytic anemia stands out.[4]

Autoimmune hemolytic anemias are classified as primary or secondary. In primary AIHA, hemolytic anemia is the only clinical finding and no underlying systemic disease is observed that could explain the presence of autoantibodies. Secondary AIHA occurs against the

background of a systemic disease, and the hemolytic anemia is just one manifestation of that disease. It can affect patients with autoimmune diseases such as systemic lupus erythematosus, or other autoimmune inflammatory diseases, such as ulcerative colitis.[5,6] It is also observed in patients with neoplasms such as Hodgkin's and non-Hodgkin's lymphomas, chronic lymphocytic leukemia, myelodysplastic syndromes, immunodeficiency, infection by *Mycoplasma*, *Epstein-Barr* virus*, Cytomegalovirus,* or drug usage.[7]

Diagnosis is based on a positive direct Coombs test in the presence of hemolysis. The direct Coombs test can, however, be negative in 2% to 4% of cases and has 8% false positives.[3] Progression is extremely variable. There may be acute onset with short duration and resolution within six months, or there may be insidious onset and a tendency to become chronic, which is what generally occurs with infants and adolescents.[8] Occasionally, chronic cases will resolve spontaneously, after months or years of deterioration.[3]

The objective of this retrospective study was to assess the clinical and laboratory characteristics, and the management of patients with diagnoses of AIHA seen at the HC-UFMG Hematology Service from 1988 to 2003.

Patients and methods

Seventeen children aged less than 15 years were studied. They had all been referred to the Pediatric Hematology Service at the HC-UFMG or the *Hemocentro de Belo Horizonte* (*Fundação Hemominas*), between 1988 and 2003, with clinical status suggestive of acquired hemolytic anemia. Children were excluded if they had congenital hemolytic anemias, such as hemoglobinopathies, congenital spherocytosis and erythrocyte enzyme deficiencies. Data were obtained retrospectively from patient medical records by three researchers and reviewed by the study coordinator. Possible issues of disagreement were discussed by the whole team. Variables from clinical history were: drug usage, previous immunization and symptoms related to viral conditions. Special attention was given to initial hematological clinical status, for screening for the presence of other diseases known to be associated with AIHA. The progress of the disease and the treatment needed were also assessed. Laboratory parameters investigated included blood test and reticulocyte assay, direct Coombs polyspecific test, bilirubins, lactic dehydrogenase and antinuclear antibody assay (ANA). Serology infectious/contagious diseases (hepatitis A, B and C; cytomegalovirus, mononucleosis, and acquired immunodeficiency) was not uniformly available for all patients. Direct Coombs monospecific test for IgG, IgM, IgA and C3d were undertaken for 14 patients. Patients were initially given prednisone,

2 mg/kg/day. Treatment failure was defined as no response to the corticosteroid after 21 days.[9] For severe cases and cases of treatment failure methylprednisolone pulse therapy was given for 3 to 5 days, at a dose of 30 mg/kg/day and/or immunoglobulin, 400 mg/kg/day, for 5 days. Splenectomy was reserved for those cases that remained hemolytic with risk of death, despite the above measures.[10]

Statistical analysis

The database was compiled on the public domain software Epi-Info, version 6.[11] This is an observational study in which only frequency distributions have been employed.

Ethical considerations

This study was approved by the Ethics Committee at UFMG.

Results

Ten (58.8%) of the 17 patients studied were male and seven (41.2%) were female. The median age at diagnosis was 10.5 months (1-162 months). Clinical histories did not report drug usage, immunization or viral infection prior to hemolytic conditions. Median hemoglobin level was 5.9 g/dl (2-11 g/dl) and reticulocytes were at 11.8%. Laboratory evidence of hemolysis was found in all cases. One patient exhibited thrombocytopenia as the hemolytic condition progressed. Patient clinical characteristics, on admission and during follow-up, are summarized in Table 1.

The direct Coombs polyspecific test was positive in 13 cases and negative in four. The direct Coombs monospecific test was run for 14 patients. The most common antibody class observed among these patients was IgG (31.3%), followed by IgM (12.5%). Three (18.9%) patients exhibited a mixed antibody pattern. The antigens IgG and IgM were isolated from one of these patients, IgG and IgA from the second and all three immunoglobulin classes were observed in the third. These three patients had complement fixation. The test results for four patients were inconclusive.

Thirteen patients were classed as suffering from primary AIHA, while in four children the hemolytic condition was associated with an underlying disease whose initial clinical manifestation was AIHA. Autoimmune hepatitis, Hodgkin's lymphoma, systemic lupus erythematosus, and Langerhans cell histiocytosis. This last was diagnosed at autopsy. The median time from diagnosis of AIHA to diagnosis of underlying disease was 7 months (2-44 months).

Median follow-up for all 17 patients was 11 months (5-23 months). In 13 (76%) patients anemia was defined as moderate to severe, with blood transfusion indicated during the course of the disease. Treatment response and progression can be observed in Figure 1. All 13 patients

Table 1 - Clinical and laboratory data of 17 patients diagnosed with autoimmune hemolytic anemia

Patients	Gender	Age (m)	Hemoglobin (g/dL)	Reticulocytes (%)	Direct Coombs polyspecific	Direct Coombs monospecific	Underlying disease	Follow up (m)	Death
1	M	10	5.90	3.0	+	IgG, IgM, C3d	Langerhans cell histiocytosis	10	Yes
2	M	15	11.90	1.3	+	–	–	5	No
3	M	5	5.20	10.0	+	IgG	–	7	No
4	M	4	7.00	6.7	+	IgG	–	9	No
5	M	1	6.20	12.0	–	–	–	11	No
6	F	108	11.00	1.4	+	IgM	–	23	Yes
7	M	39	5.10	20.0	+	IgG	–	24	No
8	F	10	4.50	60.0	+	–	Autoimmune hepatitis	85	Yes
9	M	2	5.90	4.4	–	–	–	68	No
10	M	1	4.30	10.4	+	IgG	–	10	No
11	F	16	2.00	–	+	IgG, IgA, C3d	–	54	No
12	F	8	9.00	12.3	–	–	–	59	No
13	M	123	6.80	1.6	+.	IgM	Hodgkin's lymphoma	62	No
14	F	88	4.30	16.1	+	IgG	–	4	No
15	M	10	4.50	16.0	–	–	–	36	No
16	F	161.7	8.80	11.5	+	IgG, IgM, IgA, C3d	Systemic lupus	2	No
17	F	35	3.38	16.1	+	–	–	22	No

defined as having primary AIHA were initially given prednisone. As their diseases progressed, two patients (12%) also required pulse therapy with methylprednisolone. Six (35%) patients responded satisfactorily and recovered within 3 weeks. Three of these patients presented IgG autoantibodies, one had a mixed antibody pattern and C3d and in two cases the direct Coombs test was positive, but it did not prove possible to identify the antibody class.

Immunoglobulin was administrated as an alternative for those patients who had not responded to corticoid. Four (80%) of the five children who were given intravenous immunoglobulin responded well. Two of these had had a mixed IgG pattern – IgG and IgA – and the immunoglobulin involved was not identified in the other two. Three patients whose hemolysis had not been controlled by medication underwent splenectomy. Two of these exhibited remission
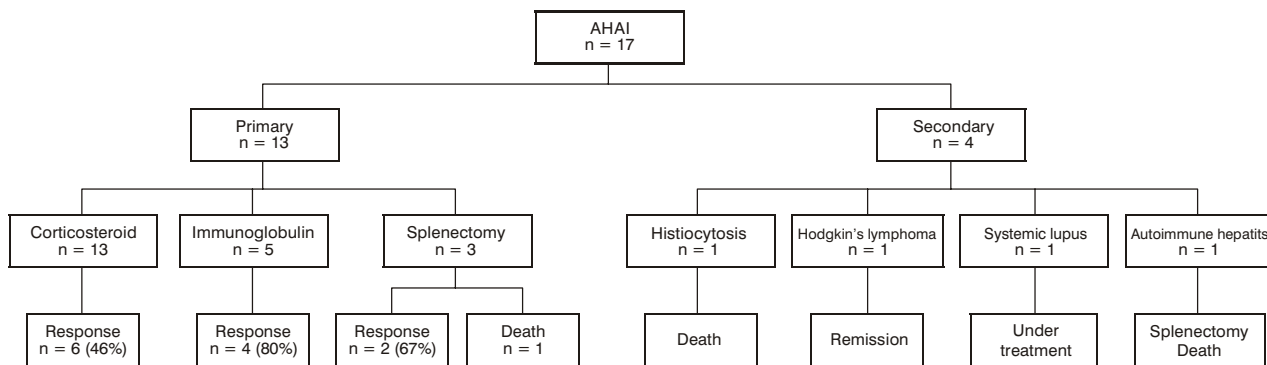

Figure 1 - Treatment response and progression of 17 patients with autoimmune hemolytic anemia

from the hemolytic condition. The median age at splenectomy was 53 months (36-117 months).

In two of the secondary AIHA patients the hemolytic condition was controlled once the underlying disease had been treated (Hodgkin's lymphoma and systemic lupus erythematosus). The other two patients died, one from the underlying disease (Langerhans cell histiocytosis) and the other from post-splenectomy septicemia (autoimmune hepatitis).

Discussion

Autoimmune hemolytic anemia should be considered as one component of a complex multisystemic disease secondary to immune system dysfunction. It is characterized by early destruction of red blood cells due to bonding by immunoglobulin and/or complement with the erythrocyte surface membrane. The hemolytic condition is generally preceded by acute infections or immunization.[12] This relationship was not detected in our study, in contrast with other series in which such situations are frequently reported.[9] Notwithstanding, any causal relationship with acute infections, in particular those of the upper respiratory tract, should be treated with reservations since recurrent infections are common among small children.

The habitually recognized method of testing for AIHA diagnosis is the direct Coombs polyspecific test. The Coombs sera contain antibodies against human IgG and fractions of C3 complement, but exhibit little activity against IgM and IgA. Furthermore the test has low sensitivity, since it returns positive only when the number of IgG molecules per red blood cell is above 200.[13] This being so, if the polyspecific test is negative, workup should be supplemented with monospecific Coombs sera for IgG, IgM and IgA.[3] Even after having employed both test methodologies, four (23%) patients from the present sample presented a negative Coombs test result, similar to data reported in another Brazilian article,[13] but a little above the 2 to 10% described in other studies.[14,15] The antibodies most often responsible for AIHA in children are from the IgG class.[15] The results from our patient sample confirmed this.

In from 5 to 43% of cases an underlying systemic disease can be identified as responsible for the hemolytic condition.[2,9,16] Years may pass between onset of the hemolytic process and the first signs and symptoms of the underlying disease.[4] Four patients from our sample (24%) were classed as having AIHA secondary to a systemic disease.

The well-known link between AIHA and Hodgkin's lymphoma[17,18] can be observed in one patient from the present series. He initially presented with infection by *Mycoplasma pneumoniae* and, after 4 years, was diagnosed with Hodgkin's lymphoma. On that occasion the patient presented accentuated hemolysis, and remission from this condition only occurred after treatment of the underlying disease. It is believed that around 50% of patients with infections by *Mycoplasma pneumoniae* exhibit elevated IgM antibody titers, which cause the hemolysis.[15] Disease due to cold agglutinin, which are antibodies from the IgM class, or very rarely IgA or IgG, can strike after infectious diseases.[19] It can also be detected in lymphoproliferative diseases and other infectious diseases such as rubella, varicella, mononucleosis and cytomegalovirus.[19,20]

The link between AIHA and systemic lupus erythematosus (SLE), although infrequent, is also well-established in the medical literature. The primary clinical manifestation may be AIHA, which may precede by months or years the other clinical manifestations of SLE.[21] One of our patients exhibited a hemolytic condition with acute onset as the only manifestation of autoimmune disease. The collagenosis diagnosis was established later with a positive serological result for the presence of antinuclear antibodies.

There are few reports in the literature on autoimmune hemolysis among children with liver disease. Some authors have, however, demonstrated a link between AIHA and giant cell hepatitis. This liver disease can be associated with the acquired immunodeficiency virus, hepatitis B virus, drug usage or cholestatic syndrome and in around 40% of cases its etiology will be defined as autoimmune.[22] One patient from the present sample developed hepatomegaly and had abnormal liver function test results after presenting an acute hemolytic condition. An histological study was performed of a liver specimen and autoimmune hepatitis diagnosed.

No reports of AIHA associated with Langerhans cell histiocytosis were found in the literature consulted. The etiology of this pathology has not been explained, but recent studies suggest a clonal or immunological origin. Although immunological studies indicate that the presence of immunoresponse abnormalities or dysfunction may play a role in the pathophysiology of this disease, there is no evidence that Langerhans cell histiocytosis derives from a primary immune system defect.[23]

In general, patients with AIHA exhibit normal granulocyte and platelet series. The combination of AIHA with immune thrombocytopenia immune, concurrently or sequentially, can, however, occur. This was first described by Fisher (1947) and later became known as the Evans syndrome.[24] Pui et al.[25] described 11 patients with AIHA in association with thrombocytopenia, three of whom had systemic lupus erythematosus, one with aplastic anemia and the remaining were defined as having primary Evans syndrome. One patient from the present sample presented thrombocytopenia during an acute hemolysis exacerbation, six months after AIHA was diagnosed.

Some patients undergo spontaneous remission of the hemolytic condition. This outcome was not observed in the present sample. Corticosteroid and immunoglobulin are considered first-line treatments. Splenectomy should be reserved for those cases that do not respond, with intense hemolysis and risk of death.[10] Good responses to corticosteroids have been observed primarily in acute cases,[26] with around 80% of patients whose AIHA is induced by IgG responding to corticosteroids. Cases mediated by IgM, in contrast, do not generally respond well.[3] In the present sample, just 46% of the patients responded well to corticosteroids, while 80% of those given immunoglobulin achieved remission from the hemolytic condition.

The mortality rate in this study of 16% (three patients) is similar to what has been described by other authors – 10 to 31%.[27,28] Deaths are generally linked to a chronic underlying disease, although post-splenectomy septicemia is a common cause.[16,29] Additionally, severe hemolysis itself can prove fatal.[28]

It is necessary to recognize the limitations inherent in the apparently small sample size and the retrospective design of the present study. The possible failings of retrospective studies include lack of protocol uniformity and the lack of researcher control over variables. On the other hand, AIHA is extremely rare in the pediatric age group, and studies with larger samples are scarce.[26]

In conclusion, a diagnosis of AIHA should alert pediatricians to the possibility of an associated systemic disease. Despite the normal response to conventional treatment (corticoid therapy and immunoglobulin), fatal cases have been described. It appears that prognosis is worse when there is an underlying disease. Thus, faced with a patient with AIHA, clinical and laboratory investigations are indicated with the objective of identifying subjacent pathologies such as infectious diseases, autoimmune diseases and neoplasms as early as possible.

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Correspondence:
Maria Christina Lopes A. Oliveira
Rua Engenheiro Amaro Lanari 389/501, Anchieta
CEP 30310-580 – Belo Horizonte, MG –Brazil
Tel./Fax: +55 (31) 3285.1056
E-mail: eduolive@medicina.ufmg.br