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Original Research Article

Autoimmune hemolytic anemia in children during 2004–2014 in the Department of Pediatrics, Hematology and Oncology, Warsaw Medical University

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ABSTRACT

Introduction: Autoimmune hemolytic anemia (AIHA) is a rare disorder in which the immune system produces pathologic antibodies directed against its own red blood cells (autoantibodies). AIHA is most commonly diagnosed by a positive result of the direct antiglobulin test (DAT, Coombs test). Depending on the temperature at which autoantibodies react with red blood cells *in vitro* AIHA is classified as warm-type AIHA with incomplete IgG autoantibodies and cold-type AIHA with cold IgM agglutinins (CAS – cold agglutinin syndrome) or with biphasic hemolysins (PCH – paroxysmal cold hemoglobinuria). In mixed-type AIHA there are simultaneously warm autoantibodies and cold agglutinins.

Aim: The aim of this study was to find the number and types of AIHA diagnosed and treated in the Department of Pediatrics, Hematology and Oncology of Warsaw Medical University during the years 2004–2014.

Material and methods: The authors analyzed 54 children in which AIHA was diagnosed. The age of the child at diagnosis, the result of direct antiglobulin test and the type of autoantibodies in serum were taken into account.

Results and discussion: The most common type of AIHA in a group covered by the survey was cold-type AIHA, including paroxysmal cold hemoglobinuria (37%) and cold agglutinin syndrome (16%).

Conclusions: As far as the results are concerned, avoidance of cold is essential before the serological diagnosis is reached.

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1. Introduction

Autoimmune hemolytic anemia (AIHA) is an acquired hemolytic anemia in which the immune system produces pathologic antibodies against its own red blood cells shortening their survival time, either present in the plasma or completely bound to red cells.

It is a rare disease. The incidence is approximately 1–3 cases per 100 000 of population per year.[1–3] The diagnosis is based on typical clinical symptoms and the analysis of serological and biochemical results. The clinical picture of the disease is varied – from an acute, postinfectious, self-limiting type to chronic, lasting constantly for years or periodic hemolysis, with sudden relapses. During the relapse, severe hemolysis may be life threatening. During the chronic phase the immunosuppressive treatment is not always unreservedly effective; however, side effects are still commonplace.

The diagnosis of the disease is based on clinical features and the results of laboratory tests indicating hemolysis as a cause of anemia. In patients with AIHA, apart from anemia, there is jaundice. The jaundice is caused by an excessive increase of unconjugated bilirubin concentration as a result of red blood cells destruction. The patient's dark-colored urine is related to hemolobinuria or the presence of bile pigments in urine. The results of laboratory tests state decreased concentration of hemoglobin, reduced amount of red blood cells and increased percent of young red blood cells – reticulocytes.[4] The concentration of haptoglobin is reduced, whereas lactate dehydrogenase and aspartate aminotrasferase concentrations are increased.

The direct antiglobulin test (DAT, Coombs test) is the screening test that allows the immune nature of the hemolysis to be identified. However, the negative result of DAT does not exclude AIHA.[5] In some cases, the autoantibodies against red cells cannot be displayed in standard serological tests and AIHA is diagnosed whether from typical clinical features.[6] On the other hand, a positive DAT plus anemia does not necessarily mean that the patient has autoimmune hemolytic anemia.[7]

Precise and insightful analysis of the activity of autoantibodies and their thermal properties allows to distinguish AIHA into groups.[6,8–10] Depending on the temperature at which the autoantibodies react *in vitro* with red blood cells, AIHA is classified as warm-type AIHA with incomplete IgG autoantibodies and cold-type AIHA with cold agglutinins (cold agglutinin disease) or with biphasic hemolysins (paroxysmal cold hemoglobinuria).[2,3,9] Some cases, however, escape this classification, such as the mixed-type AIHA in which the hemolysis is sustained by both warm and cold autoantibodies.[11] Serologic features of autoantibodies in AIHA are presented in Table 1.

The method of treatment depends on the type of AIHA and the severity of clinical symptoms and these are presented in Table 2.

1.1. *Warm-type AIHA*

Warm-type AIHA is the most common type of AIHA, observed in about 75% of cases.[8] The disease can be distinguished into primary (idiopathic) and secondary forms, the last predominately in association with lymphoproliferative disorders, infections, immunodeficiency states and autoimmune disorders such as systemic lupus erythematosus. In this type of AIHA, Coombs test is usually positive and reveals autoantibodies IgG associated with C3d component of complement system.[12] In the majority of patients incomplete IgG autoantibodies which bind to erythrocytes most avidly at 37°C[3,12–16] are detected in plasma. In this type of AIHA the hemolysis is extravascular – red blood cells are destroyed by phagocytosis in the reticuloendothelial system, primarily in spleen, and later in liver.[6,17] Warm-antibody AIHA is characterized by great variability in terms of onset, grade of severity and clinical course. The onset of idiopathic forms is often insidious, although in some patients it may be sudden, with rapidly worsening anemia and jaundice. Moreover, we can observe enlargement of spleen or liver and spleen. Life-threatening hemolytic episodes are more common in AIHA cases secondary to infections. Massive, usually short-lasting hemoglobinuria may also occur in acute cases, albeit rarely. Some patients may be not significantly anemic and indeed may be symptomless. Purpura is not commonly found, except in Evans' syndrome, in which AIHA is associated

Table 1 – Serologic features of autoantibodies in AIHA.

Classification	Warm type	Cold type	Paroxysmal cold hemogobinuria	Mixed type
Antibodies (Ig)	IgG	IgG	IgM	IgG+IgM/IgA
DAT	IgG, IgG+C3d	C3d	C3d, IgM	IgG, IgM, IgA, C3d

Table 2 – Characteristics of main clinical features of AIHA.

	Clinical manifestations	Treatment
Warm-type AIHA	Mild to severe anemia, sometimes acute hemolysis	Good response to corticosteroids, immunosuppressive therapy, blood transfusions
Cold agglutinins disease	Moderate, dependent of exposure to cold	Avoidance of cold, immunosuppressive therapy, intravenous IgG, blood transfusions only when necessary
Paroxysmal cold hemoglobinuria	Acute hemolytic anemia, often with significant reduction of hemoglobin concentration, hemoglobinuria	Supportive care (avoidance of cold, antibiotics if signs of infection), blood transfusions if needed, a short course of corticosteroids if hemolysis is severe

with idiopathic thrombocytopenic purpura.[14] As regards the clinical course, the disease may be in some cases, particularly in children, of short duration, but more often it is a chronic disease extending over years, with unpredictable course.

The main aim of warm-type AIHA treatment is to reduce the production of antibodies. To achieve this aim, corticosteroids, immunosuppressive drugs or monoclonal antibodies anti-CD20 (rituximab) are used.[1,7,18] Blood transfusions should be performed when necessary, even though the compatibility test might reveal gross incompatibility of all units. Special compatibility test procedures are necessary prior to selection of the most appropriate unit for transfusion.[7]

1.2. *Cold-type AIHA*

Paroxysmal cold hemoglobinuria (PCH) is a type of AIHA recognized by the presence of biphasic hemolysins in plasma (Donath-Landsteiner antibodies). The causative in PCH antibody is an IgG immunoglobulin which has an ability to activate the complement.[13,19] DAT reveals the component of complement. Many reports emphasize that paroxysmal cold hemoglobinuria is an unusual disease; in adults it is found in 1%–2% of cases,[18] while in children it occurs more often, in about 40%, and is connected with past diseases, such as viral infections, measles, mumps, chickenpox, influenza or after measles vaccination.[19–21] In this type of AIHA the hemolysis is intravascular. The antibody is best detected *in vitro* by its ability to cause hemolysis of normal red blood cells in a two-step procedure, which requires incubation in the cold followed by incubation at 37°C in the presence of complement.[22,23]

The last components of complement – C6, C7, C8, C9 – form membrane attacking complex (MAC), which destroys erythrocytes.[6] The most typical clinical symptom of PCH is an acute, severe hemolysis, usually transient. Moreover, jaundice and dark urine caused by hemoglobinuria can be observed. As a treatment, often only supportive care is sufficient. Frequent monitoring of the patient's hematologic status is necessary because the hemoglobin and hematocrit can drop precipitously. Meticulous attention should be given to keep the patient warm. In some cases, the severity of hemolysis could dictate the need for blood transfusion and the short course of corticosteroid therapy is warranted empirically if hemolysis is severe. Moreover, if signs of infection are present, appropriate antibiotics should be provided.[7,13,19,24]

Cold-type AIHA, also known as cold agglutinin syndrome (CAS), is more common in adults, about 15%–20% of cases.[8] It can be primary (idiopathic) or secondary.[25] The acute form of the disease may be caused by past viral infection, especially *Mycoplasma pneumoniae* and Epstein–Barr Virus.[22] Chronic form is usually associated with lymphoproliferative disorders. CAS is generally caused by IgM autoantibodies which exhibit maximal reactivity at 4°C, but the thermal amplitude reaches 30°C–32°C and sometimes can be extended up to 37°C.[8] Autoantibodies coat erythrocytes in low environmental temperature and activate complement in visceral temperature.[22] Autoagglutination of anticoagulated blood samples that occurs quickly as blood cools to room temperature is characteristic and is frequently the first observation made to suggest the diagnosis. Autoagglutination is intensified by cooling the blood to 4°C and is reversed by warming to 37°C. The temperatures of 30°C and lower are normally attained in the superficial skin vessels of those parts of body exposed to cold (ears, hands, feet). The exposure to cold air or water intensifies hemolysis, while avoidance of cool temperatures prevents activity of antibodies and causes gradual reduction of their activity. The clinical manifestations vary greatly from patient to patient, depending on the thermal range of the cold antibodies. Moderate hemolytic anemia and hemoglobinuria can be observed. Patients with CAS usually require no therapy other than avoidance of cold. If the hemolysis is more severe, immunosuppressive drugs or rituximab can be effective. Corticosteroids usually are ineffective.[22]

1.3. *Mixed-type AIHA*

Mixed-type AIHA is not common, and its incidence is about 2%–8%,[8] especially as a secondary autoimmune hemolytic anemia in patients with non-Hodgkin lymphoma, systemic disease of connective tissue or after organ transplantations. Patients satisfy the serologic criteria of both warm AIHA and CAS, so warm IgG antibodies and cold IgM are simultaneously found in patients serum.[11,26] Coombs test is usually positive, and it detects IgG and C3d. The diagnosis of combined warm and cold AIHA is sometimes made on the basis of inadequate serologic studies. Unless one can document a cold autoantibody with a high thermal amplitude (>30°C) in association with a warm antibody, a diagnosis of cold and warm (''mixed'') AIHA is not warranted. The treatment includes therapeutic management appropriate for warm AIHA and avoidance of cold at the same time, which prevents activation of cold agglutinins.

2. Aim

The aim of this study was to find the number and types of AIHA diagnosed and treated in the Department of Pediatrics, Hematology and Oncology of Warsaw Medical University during the years 2004–2014.

3. Material and methods

Case histories of 54 children who were diagnosed and treated for AIHA during 2004–2014 in the Department of Pediatrics, Hematology and Oncology of Warsaw Medical University were analyzed. The age of the child at diagnosis, the result of DAT and the type of autoantibodies in serum were taken into account. Specialistic serological tests were performed in the Department of Immunology in the Institute of Hematology and Transfusion Medicine, Warsaw.

4. Results

PCH was the most common type of AIHA recognized in children in years covered by the survey. It was established in 20 of 54 new diagnoses (37%). In 18 cases Coombs test was positive. Most cases were reported in younger age group, *i.e.* 1–6 years old.

Table 3 – DAT results in various types of AIHA.

AIHA	DAT positive	DAT negative	DAT not done	Total
Warm type	16	3	0	19
Paroxysmal cold hemoglobinuria	18	1	1	20
Cold agglutinin disease	5	4	0	9
Combined (warm and cold antibodies)	1	0	0	1
Not established	2	1	2	5
Total	42	9	3	54

Table 4 – Age of patients with various types of AIHA.

AIHA	<1 y	1–3 ys	3–6 ys	6–10 ys	10–14 ys	>14 y
Warm type	1	7	2	0	3	6
Cold agglutinin disease	0	3	3	2	0	1
Paroxysmal cold hemoglobinuria	0	9	9	2	0	0
Combined (warm and cold antibodies)	0	0	1	0	0	0
Not established	0	1	3	0	1	0
Total	1	20	18	4	4	7

CAS was diagnosed in 9 of 54 children (16%). In this group, negative Coombs test was obtained in almost half of the responders, or in 4 patients. The disease was found in children aged 1–10.

Warm-type AIHA was diagnosed in 19 of 54 children of the study group (35% of patients with AIHA). Among them positive DAT was found in 16, and negative in 3 patients. This type of AIHA was the most common in children aged 3–6 and adolescents.

Mixed-type AIHA with the presence of warm autoantibodies and cold agglutinins, with positive DAT, was found in 1 patient, aged 5 (Table 3).

In the case of 5 children from the group covered by the survey, no anti-erythrocytes antibodies were found in serological tests so the type of AIHA was not established. In 4 of them the C3d component of complement was detected on the surface of erythrocytes. In 2 cases DAT was positive, in next 2 the test was not done. In 1 child DAT was negative, serological tests found no antibodies but the diagnosis was established based on typical clinical manifestations.

Analyzing the age of the patients at onset of the disease it was found that 70% of the new cases were recognized in younger age group – 39 children aged 1–6 years old (Table 4).

5. Discussion

AIHA in children are relatively rare conditions.[27,28] Between 1955 and 1975, 47 children with AIHA were observed in the Institute of Pediatrics in Warsaw. Warm autoantibodies were detected in about 70% of cases. In 50% of cases AIHA was followed by a viral infection or vaccination.[27,28]

According to our results, 54 new cases of AIHA were diagnosed during the last 10 years. The most frequently recognized type of AIHA in the group covered by the survey was PCH. It was found in 20 of 54 children (37% cases). This result emphasizes the management of a patient. It is extremely important that every patient observed for AIHA should be protected from cold until the diagnostic process is complete with the result of anti-erythrocytes antibodies. In practice, this involves to provide the child with warm room and clothing and avoiding cold fluids and meals. Intravenous drip infusions and blood products should be administered warm, after heating with a special device or, if not available, in a slow infusion, with the long drain, in a well heated room.

Likewise, such management is necessary in CAS, which was diagnosed in 16% of cases.

Among the patients from the group covered by the survey, 70% of new cases were established in younger age group. Indeed, 39 children were at the age of 1–6 at onset. Younger children are more susceptible to infections, which may precede AIHA.

The occurrence of anemia associated with the symptoms of hemolysis requires confirmation of its congenital or acquired nature. In order to exclude congenital hemolytic anemia, the history of the patient including family history should be taken properly and laboratory test should be planned precisely. Therefore, the weight of DAT is significant.

In the study, positive DAT was found in 42 of 54 children with AIHA; however, in 9 cases the result was negative. Although the broad spectrum antiglobulin test (Coombs' test) is the screening test that allows the immune nature of the hemolysis to be identified, the negative result does not exclude AIHA.

6. Conclusions

Every patient suspected of AIHA requires careful history taking and detailed laboratory tests. The correct diagnosis based on specialist serological test provides appropriate treatment of the disease.

Conflict of interest

None declared.

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