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Autoimmune Hemolytic Anemia in Children and Adolescents: A 22-Year Single-Center Experience

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Abstract

Introduction: Autoimmune Hemolytic Anemia (AIHA) is characterized by premature destruction of erythrocytes, due to auto-antibodies directed against antigens on their surface, with or without complement involvement. The clinical course of AIHA varies from mild to severe or even life-threatening.

Methods: Medical files of 38 pediatric patients diagnosed with AIHA and followed in a single pediatric hematology center during the period 2002-2023 were retrospectively reviewed.

Results: Median age of diagnosis was 4 years and mean follow up period 5.3 years. Out of 38 patients, 13 (34.2%) presented >1 episodes, with mean number of relapses 3.3. Warm AIHA was diagnosed in 30 (78.9%), PCH in 7 (18.4%) and atypical negative-DAT AIHA in one (2.7%) patient. Secondary AIHA was established in 13 (34.2%) patients and was statistically correlated to relapse occurrence, as well as to the number of relapses. Complete response after first line therapy was reported in 93.1% of patients. In total, 11 (29%) patients received various treatment regimens due to multiple relapsing episodes. All patients were alive, while 36 (94.7%) presented continuous complete response at last follow up.

Conclusion: Even though study results largely agree with previous similar studies, key difference of the present study is the absence of reported mortality. As most immunomodulatory drugs remain off label for use in pediatric AIHA and given the rarity of the disease, national or multi-center registries would offer access to the experience gained in larger centers, and could be used for guideline formation.

Keywords: Autoimmune hemolytic anemia; Direct antiglobulin test; Immunosuppressive treatment; Pediatric patients

Abbreviations: AIHA: Autoimmune Hemolytic Anemia; ANA: Antinuclear Antibodies; CCR: Continuous Complete Response; CVID: Common Variable Immunodeficiency; DAT: Direct Antiglobulin Test; dsDNA: Anti-Double Stranded-DNA Antibodies; IgG: Immunoglobulin G; IQR: Interquartile Range; Hb: Hemoglobin Level; LDH: Lactate Dehydrogenase; PCH: Paroxysmal Cold Hemoglobinuria; wAIHA: Warm Autoimmune Hemolytic Anemia

Introduction

Autoimmune Hemolytic Anemia (AIHA) was recognized as a specific hematological disease in 1951 and is characterized by the presence of autoantibodies directed against antigens on the surface of the erythrocytes [1,2]. AIHA is a rare disorder and the annual incidence is estimated as <1 case per 100,000 children under 18 years of age [3]. The clinical course is heterogenous, varying from mild to severe, acute or chronic hemolytic anemia, in addition to hemoglobinuria in case of significant complement activation [4]. Generally, AIHA is distinguished serologically from other hemolytic anemias due to the presence of positive Direct Antiglobulin Test (DAT). Although DAT is the gold standard for AIHA diagnosis, it results negative in up to 11% of the cases with clinical picture consistent with AIHA [5,6]. The disease is classified as warm or cold (cold AIHA and Paroxysmal Cold Hemoglobinuria- PCH), based on the optimal autoantibody binding temperature, the immunoglobulin class and the affinity

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for complement activation. In approximately 90% of pediatric cases, patients present with warm AIHA (wAIHA). The pathogenic autoantibody belongs to Immunoglobulin G (IgG) subtype and lysis of IgG-coated erythrocytes takes place in the spleen. On the other hand, in cold AIHA the responsible autoantibody is a cold agglutinin, usually IgM, that activates the classical complement pathway and results in extravascular hepatic hemolysis, or even intravascular in case of membrane attack complex formation [7]. In the pediatric population, PCH follows wAIHA in frequency [8]. It is characterized by a biphasic IgG, that causes erythrocyte lysis in body temperature, albeit, after binding in a cold temperature and activating the complement [7].

AIHA pathogenesisis complex, varies between age groups and can be either primary or secondary [9]. Infections are considered the most common cause of AIHA in infants, while underlying systemic conditions, such as autoimmune disorders and immunodeficiencies, are more frequently associated with AIHA in adolescents [10].

There is currently no specific protocol for treating pediatric AIHA and recommendations are based mainly on expert opinions [11]. Due to the lack of universal guidelines, pediatric hematology centers follow different diagnostic and therapeutic approaches, based on their own experience. Approximately one third of patients is considered to have constant remission after initial therapy, however, most of the patients experience chronic, relapsing course [12]. Hemolysis may prove life-threatening in acute cases, and efficacy of immunosuppressive agents might be short-term in chronic cases [13]. The reported mortality rateremains high (3-10%), so that early recognition and appropriate management is of great importance [3,4].

Aim of the present study is to report on long term data of AIHA patients diagnosed and treated in a single pediatric hematologycenter over the past 22 years.

Methods

The present is an observational study on AIHA course in pediatric patients that were diagnosed or referred with AIHA diagnosis for further treatment and follow up at the Pediatric Hematological Center of the 1st Pediatric Department of Aristotle University of Thessaloniki, in Greece, during the period 2002-2023.

The diagnosis of AIHA was based on the presence of anemia, defined as lower than normal for the patient's age and gender Hemoglobin Level (Hb), positive DAT and ≥ 1 abnormal hemolytic marker -including reticulocyte count, Lactate Dehydrogenase (LDH) and indirect bilirubin. Atypical forms, such as patients with clear evidence of AIHA but negative DAT, as well as patients with positive DAT and compensated hemolysis, without anemia, were also included in the study.

At diagnosis patient characteristics were recorded, in terms of age, gender, clinical manifestations and semiotics. Furthermore, the following laboratory data were recorded: Hb, reticulocyte number, LDH (normal value <248 U/L), total and indirect bilirubin, DAT, immunoglobulins (IgG, IgA, IgM), complement components (C3, C4), Antinuclear Antibodies (ANA) and anti-double stranded-DNA antibodies (dsDNA). DAT intensity was evaluated on a scale from 1+ to 4+, with 1-2+ indicating weak to moderate agglutination and 3-4+ strong agglutination.

Additional recorded data included initial therapeutic approach and patient's response (complete, partial or absence of), duration of remission, relapses, treatment and response of relapsing episodes, time on follow up, treatment adverse events, as well as diagnosis of underlying conditions during follow up.

Complete response was defined as normalization of Hb, reticulocyte count and indirect bilirubin value, partial response as an increase of Hb value $\geq$2 g/dl but below normal levels for patient's age and gender, while absence of response was defined as the failure to normalize Hb or to increase >2 g/dl. Finally, complete response that lasted over 12 months was characterized as Continuous Complete Response (CCR), while recurrence of anemia after complete or partial response was considered as disease relapse.

With regards to statistical analysis, descriptive analysis for all variables was performed through mean and standard deviation for normally distributed scale variables, median and Interquartile Range (IQR) for skewed scale variables and absolute (N) and relative (%) frequency for categorical variables. Normality was tested both graphically (histograms, Q-Q plots and boxplots) and with statistical tests (Shapiro-Wilk and Kolmogorov Smirnov). When results did not correspond, normality was decided based on graphical methods as both statistical tests have limitations. Complete case-analysis was used to address missing values.

In the analytical part, univariate logistic regression was used to investigate whether different parameters have an impact on relapse (yes/no) and diagnosis (primary/secondary). Total number of relapses was treated as a scale variable in the models. The level of statistical significance (alpha) was set at 0.05 and all p-values represent two-tailed tests, unless otherwise noted. The analyses were performed with statistical software R 4.1.2.

Results

In total, 38 patients were enrolled in the study. Male: Female ratio was 1.1:1 (20 boys, 18 girls). Median patient age was 4 years (IQR 7) and distinct age groups <1 year, 1-2.9, 3-4.9, 5-9.9 and 10-18 years were represented by 6 (15.8%), 8 (21%), 6 (15.8%), 10 (26.4%) and 8 patients (21%) respectively. Mean time on follow up was 5.3 years (range 3 months-15 years). Consanguinity was reported in 5/38 (13.1%) patients.

Out of 38 patients, 25 (65.8%) presented with a sole AIHA episode, while 13 (34.2%) experienced>episodes, with mean number of relapses 3.3 (range 1-11) and mean time between relapses 13.5 months (range 1-36 months). No statistically significant correlation was found between presentation of relapse and patient age (P=0.06), however, for every year of age relapse risk decreased by 20%.

The clinical presentation at diagnosis included fever in 10 cases (26.3%), acute signs of anemia such as pallor and/or jaundice in 16 (42.1%) and dark urine in 5 (13.1%) cases. Splenomegaly was reported in 10 (26.3%) patients. Furthermore, 10 (26.3%) patients presented with mild bleeding manifestations (petechiae, ecchymosis and/or gum bleeding), the laboratory work-up revealing thrombocytopenia in addition to hemolytic anemia and the patients receiving a diagnosis of Evans syndrome. Evans syndrome was established also in 4 (10.5%) patients with thrombocytopenia and a positive DAT and increased hemolytic markers, without presence of anemia (Table 1).

At diagnosis, the vast majority of patients (37/38, 97.4%) presented with a positive DAT, with one (2.6%) patient presenting with a negative DAT but parameters consistent with hemo-

Table 1: Patient characteristics enrolled in the study.

	Patients, N = 38
Male: female, n	20:18
Median age at diagnosis (IQR)	4 (7)
Age groups, n (%)	
<1 year	6 (15.8)
1-2.9 years	8 (21)
3-4.9 years	6 (15.8)
5-9.9 years	10 (26.4)
10-18 years	8 (21)
Mean time on follow up (range)	5.3 (3 months- 15 years)
Clinical manifestation at diagnosis, n (%)	
Fever	10 (26.3)
Pallor and/or jaundice	16 (42.1)
Dark urine	5 (13.1)
Bleeding	10 (26.3)
Splenomegaly	10 (26.3)
None	4 (10.5)

Table 2: Patient laboratory findings at diagnosis.

	Study Entry (N = 38)
Hemoglobin level, n (%)	
<6 g/dl	11 (32.4)
6-8 g/dl	5 (14.7)
>8 g/dl	18 (52.9)
Elevated hemolytic markers, n (%)	
Reticulocyte count	25 (65.8)
Total and indirect bilirubin	18 (47.3)
Lactate dehydrogenase	26 (68.4)
Direct antiglobulin test, n (%)	
Negative	1 (2.6)
Positive	37 (97.4)
Weak to moderate (1-2+)	20 (54)
Strong (3-4+)	17 (46)
IgG	19 (51.3)
IgG+C3	11 (29.7)
C3	7 (19)
IgM	0 (0)
IgA	0 (0)

Table 3: AIHA course, type classification and form.

Autoimmune hemolytic anemia	**Patients, N=38**
AIHA episodes, n (%)	
One episode	25 (65.8)
>1 episodes	13 (34.2)
Classification, n (%)	
Warm	30 (78.9)
Cold	0 (0)
Mixed	0 (0)
Paroxysmal cold hemoglobinuria	7 (18.4)
Drug-induced	0 (0)
Atypical (negative DAT)	1 (2.7)
AIHA type, n (%)	
Primary	25 (65.8)
Secondary	13 (34.2)

lytic anemia non-responsive to red blood cell transfusions. In 20 out of 37 (54%) patients DAT had a weak to moderate positivity (1 to 2+), while in the rest 17 (46%) a strong positivity (3 to 4+). DAT intensity was statistically correlated to subsequent relapses (P=0.02). DAT characteristics included positive IgG in 19 (51.3%), IgG and C3 in 11 (29.7%) and C3 (19%) in 7 cases. No clinically significant cold agglutinins were identified in the study group. Based on clinical and laboratory findings, 30 (78.9%) patients were diagnosed with wAIHA, 7 (18.4%) with PCH and one (2.7%) with an atypical negative-DAT form of AIHA. No history of antibiotics administration or other drug related to AIHA manifestation was reported.

Among 34 patients presenting with anemia mean Hb was 7.7 g/dl (range 3.1-11 g/dl). Very severe anemia with Hb<6 g/dl was recorded in 11 (32.4%) patients, Hb 6-8 g/dl in 5 (14.7%) and Hb>8 g/dl in 18 (52.9%) patients. As for hemolytic markers, 26 (68.4%) patients had abnormal LDH values (mean value 557 U/L, range 178-1990 U/L), 18 (47.3%) increased total and indirect bilirubin level (mean total bilirubin level 2.8 mg/dl, range 0.21-13.2 mg/dl - mean indirect bilirubin 2.3 mg/dl, range 0.13-7.5 mg/dl) and 25(65.8%) elevated reticulocyte count (mean Retis7.3%, range 0.2-36.5%). Reticulocytopenia was present in 13 (34.2%) cases at diagnosis (Table 2). No correlation was found between Hb at diagnosis, reticulocyte count, LDH or bilirubin and disease relapse (P=0.09, P=0.09, P=0.9 and P=0.13 respectively.

With regards to immunological work-up, immunoglobulin levels were pathological in 17 (44.7%) cases at diagnosis, either increased or reduced for the patient's age (IgG or/and IgA or/and IgM), without being consistent with the diagnosis of a specific immunological disorder. Complement components values, C3 or/and C4, were abnormal in 9 (23.6%) cases. Positive ANA were identified in 8 (21%) patients, while dsDNA was negative in all patients. Laboratory confirmation of an infection was reported in 6 (15.7%) cases at diagnosis (3 patients with Epstein Barr Virus and 3 patients with Parvovirus).

The presence of an underlying condition was already known at AIHA diagnosis in 3 (7.9%) patients (2 giant cell hepatitis/ 1 Wiskott-Aldrich syndrome). Post-infection AIHA was diagnosed in 6 (15.8%) cases, while the rest 29 (76.3%) patients were initially considered as presenting with primary AIHA. During follow up, however, an underlying condition was recognized in another 10 (26.3%) cases. More specifically, 5 (13.2%) patients were diagnosed with autoimmune lymphoproliferative syndrome (ALPS), 2 (5.2%) with systemic lupus erythematosus and 3 with primary immunodeficiency (common variable immunodeficiency-CVID, selective IgG deficiency and activated PI3Kδ syndrome). Furthermore, 5 (13.2%) patients presented with a constantly mild deviation from normal immunoglobulin level, with 3 showing additional lymphoid hyperplasia and slightly increased double negative T-cells in flow cytometry, and were still under investigation at study end for primary immunodeficiency.

In total, secondary AIHA was established in 13 out of 38 (34.2%) patients. With regards to the post-infection AIHA, in 4 out of 6 (66.7%) patients, an underlying immune disease was diagnosed at follow up. (Table 3) Male: female ratio among patients with secondary AIHA was 1.6:1 (8 boys/ 5 girls). As for relapse episodes, 7/13 (53.9%) patients with secondary AIHA experienced at least one relapse with median number of episodes 2.2 per patient (range 0-11), while 6/25 (24%) patients with primary AIHA presented at least one relapse, with median number of episodes 0.5 per patient (range 0-4). Secondary AIHA was statistically correlated to relapse occurrence, as well as to the number of relapse episodes (P= 0.04 and P=0.05 respectively).

With regards to treatments, 7/38 (18.4%) patients required no therapy at diagnosis, 21 (55.2%) received combination thera-

py with intravenous Immunoglobulin (IVIg) and corticosteroids, while 8/21 (38.1%) additionally received blood transfusion due to the severity of anemia. Monotherapy with corticosteroids was administered in 4 (10.5%) patients and monotherapy with IVIg in another 4 (10.5%). The rest 2 (5.4%) patients received second line therapy at diagnosis due to a known underlying disease (azathioprine in a patient with giant cell hepatitis and cyclosporine in patient with chronic immune thrombocytopenia).

Complete response after first line therapy was reported in 27 out of 29 (93.1%) patients. In cases where partial or no response was reported second line therapy with azathioprine and later on with rituximab was administered in one case, while cyclosporine and supportive treatment with erythropoietin in another case, finally achieving complete response. Out of 38 patients, 24 (63.2%) presented CCR after AIHA diagnosis.

Out of 13 (34.2%) patients who experienced the first relapse, 10 (76.9%) received combination therapy with IVIg and corticosteroids, while 2 additionally received blood transfusion. Second line therapy at first relapsing episode was administered in 3 (23,1%) patients (2 cyclosporine, 1 rituximab). In total, 11/38 (29%) patients received various treatment regimens due to multiple relapsing episodes. More commonly used second line therapy was cyclosporine (9/11, 82%), followed by rituximab (3/11, 27.3%), azathioprine (2/11, 18.2%), sirolimus (2/10, 18.2%), vincristine (1/11, 9.1%), and cyclophosphamide (1/11, 9.1%). Second line therapy was administered for 2-12 months, with the exception of two patients required long-term treatment with sirolimus (66 and 48 months, respectively, at last follow up). Splenectomy was performed in 2 (5.3%) patients.

All patients were alive, while 36 (94.7%) presented CCR at last follow up. Treatment adverse events were reported in 3 (7.9%) patients: hypogammaglobulinemia in one patient after rituximab administration, recurrent viral infections and chronic otitis in a patient on sirolimus, and nephrotoxicity after cyclosporine, in addition to neuropathy after vincristine administration, in a third patient. In the first case substitution IVIg therapy and in the second chemoprophylaxis were decided. In the third case discontinuation of each therapy resulted in symptom resolution.

Statistical analysis revealed no correlation between gender, Hb, reticulocyte count or splenomegaly at diagnosis with disease relapse, in contrast to intensity of DAT at diagnosis and presence of an underlying condition.

Discussion

AIHA is characterized by an unpredictable course, presenting with mortality as high as 4% in pediatric patients. Clinical manifestation varies, with patients being at risk of rapid deterioration, so that treatment delay may prove detrimental [14]. In specific, pediatric patients with Evans syndrome are reported to have a mortality rate of up to 10% according to a large French study and a 22 times higher rate compared to the general pediatric population according to a more recent Danish study [13,15]. In the present study, zero mortality was reported among the 38 pediatric patients with AIHA, including the 14 patients with Evans syndrome, that were diagnosed and treated during the past 22 years.

Similar to all but one previous report, in the present study boys and girls were almost equally affected, although there was a male predominance in terms of secondary AIHA [16-18]. AIHA was more frequent in school age children in the present report however mean age at diagnosis varies in most relevant studies [19,20]. In the present study, younger age was not statistically correlated to a higher risk for relapse, however for every year of increasing age relapse risk decreased by 20%.

With regards to AIHA classification, the most commonly reported in the present study was wAIHA (78.9%), while several patients presented with PCH (18.4%). There is a wide range of PCH reported frequency in pediatric patients, varying from 0-5%to 32% [16,21,22]. PCH may be underdiagnosed because of its benign, uncomplicated course, as well as the difficulties in timely performing the appropriate diagnostic test. In the present study, as Donath-Landsteiner test was not available, PCH was diagnosed by exclusion, taking into account the presence of hemoglobinuria, DAT positivity for C3, transient symptomatic and unproblematic clinical course and absence of need for intervention.

The initial clinical symptoms reported were related to the hemolytic anemia, with severe anemia being present in over one third of the study population. Currently, hemoglobin level is reported as the only hematological parameter correlated to the risk of relapse. More specifically, Hb reduction per 1g/dl under the normal for age and gender level is reported to increase the risk for relapse by 14%. On the other hand, reticulocytopenia at diagnosis, indicating an insufficient compensatory activity of the bone marrow, has been considered as predictor of poor disease outcome [11]. In the present study reticulocytopenia was reported in over one third of patients, a finding similar to the study by Aladjidi et al., reporting a mean duration of reticulocytopenia of 6 days, with a maximum of 70 days [13]. The present study found no correlation between Hb or reticulocyte count and subsequent disease relapse. There was, however, a statistically significant correlation between DAT intensity and the risk for relapse. An earlier study by Das et al. also reported a correlation between DAT intensity and a more severe disease course of the disease [23].

With regards to first line treatment, including corticosteroids and IVIg, complete response was reported in the vast majority of patients in the present report (93.1%), similarly to previous literature data [24-27]. However almost 35% of patients presented with one or more relapsing episodes. The finding is in agreement with the study by Fan et al., reporting one third of patients requiring second line therapy [17]. As second line therapy, different strategies were applied, with cyclosporine most commonly being used – especially at the earlier years of the study. More recent national guidelines, referring to the adult population, recommend rituximab as second line therapy. Published data from cohorts or case series are either lacking details on the selection of second line therapy or include individualized treatment regimen, so that conclusion extrapolation seems difficult [10,19,20,26-28]. Nevertheless, the use of all currently available second line treatments remain off label in pediatric AIHA, requiring approval by local health authorities. A lack of recommendation also exists regarding the maximum duration of second line treatment use. In the present study, the vast majority of patients were on continuous complete response at last follow up, however there were two patients that still required long term therapy.

It is well known that immune cytopenia could be the first manifestation of an underlying immunodeficiency or rheumatological disorder, especially in the presence of chronic and resistant to treatment cytopenia and/or coexistence of lymphoid hyperplasia [19]. According to previous studies in children with

AIHA, secondary AIHA is diagnosed in 55-60% of cases and Evans syndrome represents a considerable percentage [13,16-18]. The present study also reported on a considerable percentage of Evans syndrome in the cohort, however, the total incidence of secondary AIHA was lower. Confirmation of an underlying immunological disease was reported in 34.2% patients, with another 13.2% presenting with suspicious, if not pathognomonic findings. On top of that, progress in molecular characterization of primary immunodeficiencies is expected to result in diagnosis of such, still unrecognizable cases. In general, secondary AIHA is characterized by more severe clinical course and prognosis [14]. In the present study, indeed, there was a statistically significant correlation between the presence of an underlying condition and relapsing disease course, with patients with secondary AIHA experiencing more relapses than those with primary AIHA (2.2 vs 0.5 episodes/patient). Patients with no relapse were 4.5 times more likely to be diagnosed with primary AIHA, while every relapse increased the risk for diagnosing an underlying condition by 68%. Vice versa, patients with primary AIHA had an 80% lower risk for disease relapse.

Diagnosis of the underlying disorder is crucial for the selection of suitable therapy options, as some immunosuppressants have been associated with more side effects in patients with specific immunological disorders, such as rituximab in ALPS patients [19]. The less frequent administration of rituximab as second line therapy in the present study could be attributed to the high incidence of ALPS, compared for example to the previous large French study (13.2% vs 1.1%) [13].

Of interest, the present study reported no case of AIHA and underlying malignancy, similarly to other pediatric studies and in contrast to adult studies [10,13,18]. Mannering et al. have reported on 21 pediatric patients with Evans syndrome presenting with 4 cases of malignancy, highlighting the need for close observation [15].

Overall mortality of pediatric AIHA, even though still high, is more rarely reported over the years, indicating availability of more targeted treatments, earlier administration of more aggressive therapy and timely management of disease and treatment complications. Evolution of molecular diagnosis of immunological disorders results in the shrinkage of the patient group diagnosed with primary AIHA, optimizing long-term management.

Conclusions

Results from the present study are encouraging, reporting zero mortality in 38 AIHA pediatric patients over 22-year period. With regards to risk of disease relapse, a significant correlation was found between DAT intensity, as well as presence of an underlying condition. The availability of both classic as well as newer immunomodulatory agents is undoubtedly a positive step towards avoiding long-term corticosteroid use, and allows for a more individualized patient management. Given the rarity of the disease, national or multi-center registries would offer access to the experience gained in larger centers and could be used for guideline formation.

Author Statements

Conflicts of Interest

The authors declare no conflict of interest.

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