

Clinical Profile and Outcome of Childhood Autoimmune Hemolytic Anemia: A Single Center Study

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Received: May 16, 2020;
Initial review: August 01, 2020;
Accepted: December 13, 2020.

Objective: To analyze clinical and laboratory parameters, and treatment outcomes of children with autoimmune hemolytic anemia (AIHA). **Methods:** Retrospective analysis of 50 children aged 0-18 years. Monospecific direct antiglobulin test (DAT) and investigations for secondary causes were performed. Disease status was categorized based on Cerevance criteria. **Results:** Median (range) age at diagnosis was 36 (1.5-204) months. AIHA was categorized as cold (IgM+, C3+/cold agglutinin+) (35%), warm (IgG+ with/without C3+) (28%), mixed (IgG+, IgM+, C3+) (15%) and paroxysmal cold hemoglobinuria (4%). Primary AIHA accounted for 64% cases. Treatment modalities included steroid (66%), intravenous immunoglobulin (IVIg) (4%), steroid+IVIg (4%), and steroid+rituximab (4%). Treatment duration was longer for secondary AIHA than primary (11 vs 6.6 months, $P<0.02$) and in patients needing polytherapy than steroids only (13.3 vs 7.5 months, $P<0.006$). During median (range) follow-up period of 73 (1-150) months, 29 (58%) remained in continuous complete remission, 16 (32%) remained in complete remission. **Conclusion:** Infants with AIHA have a more severe presentation. Monospecific DAT and a thorough search for an underlying cause help optimize therapy in most patients of AIHA.

Keywords: Cerevance criteria, Direct antiglobulin test, Rituximab, Treatment.

Published online: January 2, 2021; PII: S097475591600296

Autoimmune hemolytic anemia (AIHA) is caused by the presence of auto-antibodies directed against antigens on the surface of red blood cells, leading to premature destruction [1,2]. AIHA is the main cause of acquired extra corpuscular hemolysis in children [1]. AIHA can be subdivided into primary (or idiopathic) and secondary. AIHA presenting with thrombocytopenia (Evans synd-rome) tends to have a more chronic and relapsing clinical course [3-5]. There is scarcity of data on Indian children with AIHA and their treatment outcome. We present data on children with AIHA from a single center in India.

METHODS

This is a retrospective analysis of data from January, 2007 to April, 2019 from our unit's database. Fifty children less than 18 years of age diagnosed with AIHA were enrolled in the study. AIHA was diagnosed based on the clinical presentation, positive direct anti globulin test (DAT) and at least one of the following: reticulocytosis, haptoglobin <10 mg/dL, and total bilirubin >1 mg/dL [7]. DAT test was done by gel card method (Bio-Rad). Infants were evaluated for TORCH profile. Based on clinical suspicion, hepatitis B,

hepatitis C, HIV serology, Epstein-Barr virus (EBV) PCR, cytomegalovirus (CMV) PCR, *Mycoplasma pneumoniae* antibody test and antinuclear antibody (ANA) were done. For children with repeated infections, immunoglobulin profile, Lymphocyte subset analysis (CD3, CD4, CD8, CD19, CD16, CD56) was performed. Monospecific DAT test routinely was performed for all children with suspected AIHA after the year 2013. AIHA was categorized based on types of serological antibodies (IgG, IgM, IgA, C3, and/or combinations) found in monospecific DAT test, cold agglutinin test and Donath Landsteiner test. History of drug intake and recent blood transfusion was obtained for all patients.

Glucocorticoids were used as the first line therapy in both warm and cold AIHA. In cold AIHA, additionally, treatment of the underlying disease was prioritized. The patient was kept warm and in cases with very severe anemia, packed red cell transfusion was given with a heat generator inside the tubing. Intravenous methylprednisolone (2 mg/kg 8 hourly for 3 days followed by oral prednisolone, 2mg/kg/day for 4 weeks, then tapered gradually) was used for patients who were sick, unable to take orally or had very severe hemolysis.

If there was complete remission after 4 weeks, tapering of prednisolone was done by 10% with each dose change over a period of 6 months. If there was no remission/steroid dependence with prednisolone dose of 0.2 mg/kg/day, second line treatment was used. Second-line therapy comprised of either intravenous immunoglobulin (IVIg), rituximab, cyclosporine, mycophenolate mofetil (MMF) or azathioprine. In steroid dependent cases, one of the immunosuppressants (cyclosporine, MMF, azathioprine) was used. In common variable immunodeficiency (CVID), IVIg was additionally used. Packed red blood cell transfusion as supportive therapy was given if the child had a hemoglobin value less than 3 g/dL or 3-6 g/dL with cardiac failure or respiratory distress and needing intensive care unit (ICU) care. All patients received folic acid and vitamin B12 during treatment to support hematopoiesis.

Patients were followed every month till complete remission and then 3-monthly till 1 year. Clinical and lab parameters at last follow-up were classified based on Cerevance criteria [6] into 4 categories: No remission (NR), partial remission (PR), complete remission (CR) and continuous complete remission (CCR).

Statistical analysis: Chi square test and Student *t* test (two tailed, unpaired) were used to compare variables between primary and secondary AIHA. *P* value less than 0.05 was considered significant. SPSS version 20.0 was used for the analyses.

RESULTS

Data of 50 children [median (range) age at diagnosis, 36 months (1.5 months-17 years)] were analyzed. Commonest clinical feature at diagnosis was pallor (100%) followed by fever (68%) and jaundice (60%). Hepatomegaly (90%) was seen more often than splenomegaly (38%).

Mean (SD) hemoglobin at presentation was 4.7 (1.6) g/dL. Out of 50 children, 72% children presented with very severe anemia (hemoglobin <3 g/dL, *n*=6) or severe anemia (hemoglobin 3-6g/dL *n*=33, 66%); only 1 child had mild anemia (>9g/dL). Admission to intensive care unit (ICU) was needed in 28% children. Leukocytosis (after correction for nucleated RBCs) was noted in 27 (54%) patients, and leucopenia in 4 (8%) patients. Only 3 children had thrombocytopenia at diagnosis. Reticulocytosis was seen in 37 cases (74%), whereas reticulocytopenia was seen in 13 cases (26%). Elevated lactate dehydrogenase (LDH) was seen in 86% children with median (range) LDH level of 521.5(163- 12858) U/L.

Direct anti-globulin test was 4+ positive in 26 children, 3+ positive in 11 children, 2+ positive in 5 children, and 1+ positive in 5 children. In three DAT-

negative children, the diagnosis was based on clinicopathological suspicion after ruling out other causes of hemolytic anemia and on the basis of response to treatment. Two out of three DAT-negative patients were positive for Donath Landsteiner test. Monospecific DAT test was performed in 24 children after its availability from the year 2013; of which, IgG ± C3 was present in 10 (41%) children, IgM and C3 were present in 3 children (13%) and both IgG and IgM with C3 were present in 4 children (17%). Cold agglutinin testing was performed in 21 children and was positive in 13 children. Based on above results cold, warm, mixed AIHA and PCH was seen in 35%, 28%, 15% and 4% children, respectively. In the other 18% children seen prior to 2010, AIHA was unclassified.

Secondary AIHA was identified in 36% cases with etiology being infection in 5 (10%) (*M.pneumonia*, 3; cytomegalovirus infection, 1; *Plasmodium vivax* malaria, 1), autoimmune diseases in 5 (10%) (autoimmune hepatitis, 2; systemic lupus erythematosus (SLE, 2; and giant cell hepatitis, 1). Other causes leading to secondary AIHA were Evans syndrome (6%); childhood malignancies (6%) (Hodgkin lymphoma, 2 and precursor B cell acute lymphoblastic leukemia, 1); CVID, 1(2%); and Wiskott Aldrich syndrome, 1 (2%).

Among infants, hemolysis was found to be much severe than those who developed AIHA after infancy (mean (SD) hemoglobin, 3.96 (1.18) vs. 5.13 (1.65) g/dL, *P*=0.01). In primary AIHA, the mechanism of hemolysis was more often IgM and combined antibody mediated than in children with secondary AIHA wherein it was mainly IgG-mediated hemolysis.

Steroids alone were used in 33 (66%) children; other medications used were IVIg in 2 children, steroid and IVIg in 2, steroid and rituximab in 2, steroid, rituximab and cyclosporine in 1, and steroid and other drugs (three or more) in 7 children. Other immunosuppressive medications used were mycophenolate mofetil and azathioprine. Among three patients of Evan syndrome, two patients responded to first line glucocorticoid therapy and one responded to second line therapy with IVIg followed by rituximab. One patient improved spontaneously and was not given any therapy.

Treatment duration was longer for children with secondary AIHA than primary (11 vs 6.6 months, *P*=0.02) and in patients needing polytherapy than those who improved with steroids only (13.3 vs 7.5 months, *P*=0.006). Median (range) follow up duration was 73 (1-150) months; 29 (58%) remained in CCR, and 16 (32%) in CR (**Fig.1**). Despite relapse in 26% cases, 61.5% still showed good response to steroids. One patient with

WHAT THIS STUDY ADDS?

- Managing AIHA in infants and those with secondary AIHA is challenging, with almost one-third patients at our center needing additional agents to the steroid backbone.

secondary AIHA and underlying Hodgkin lymphoma died due to fulminant fungal sepsis and hemophagocytosis.

DISCUSSION

We present our institutional data on pediatric AIHA and underscore the preponderance of AIHA in younger children; although, the median age at diagnosis in our study was higher than that in previous studies (10.8-16 months) [6,7]. Patients younger than one year required ICU care in view of severe anemia and hypoxia, similar to the report by Fan, et al. [7].

In our study, 94% cases had positive DAT, similar to another Indian study by Naithani, et al. [8]. Negative DAT in some patients may be due to low titer of IgG antibodies or IgA or IgM auto antibody mediated hemolysis. Reticulocytopenia seen in 26% cases was probably due to destruction of erythroid progenitors by autoantibodies [9]. A French national study [6] also observed a high incidence of reticulocytopenia (39%) indicating that although reticulocytosis is an important marker of hemolysis, its absence alone should not rule out AIHA.

We found that hemolysis was severe whenever combined or IgM coated antibody mediated hemolysis occurs. This observation was similar to previously published study by Sokol, et al. [10], which showed that compared to IgG mediated hemolysis alone, IgG along with IgM or IgA leads to more severe hemolysis. Secondary AIHA was due to infection in 10% whereas

Fan, et al. [7] showed that infection accounted for 97.6%. In contrast to this, a French study [6] showed that secondary forms of AIHA were mainly due to immunological cause (53%) and infections contributed to a very small portion (10%). This observation may be due to increased burden of infection and early exposure of viruses like EBV in low or middle in-come countries.

Aladjidi, et al. [6] showed 90% remission rates with 39% achieving CCR and 51% attained CR. This may be due to prolonged usage of steroids (median duration 8 months). Two patients with PCH had early disease remission. This may be due to the self-limiting nature of the condition; however, as per unit policy they were also treated with short course of steroids. If there was no remission/steroid dependence with prednisolone in a dose of 0.2 mg/kg/day, second line treatment was used [11]. We needed immunosuppressants as second line of treatment in 26% cases. Rituximab was used in the standard dose of 375 mg/m² per day [12].

Our study is limited by the fact that it is a retrospective study, comprises of a small cohort of patients and lacks protocol uniformity. Treating AIHA in children can be challenging and may need prolonged and complicated therapy, especially in secondary AIHA. We suggest that relapsed or refractory cases of AIHA should be cared by pediatric hematologists in a tertiary care center.

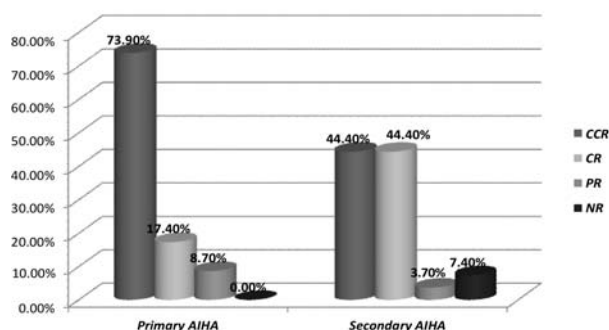
Ethics clearance: EC-SGRH; No.EC/09/20/1715, dated September 30, 2020.

Contributors: KBT: collection of data, analysis of data, writing of manuscript, revising it for important intellectual work; PS: collection of data, analysis of data, writing of manuscript; TP, DS: collection of data, analysis of data, writing of manuscript; AD, MK, AS: contributed patients, final editing of manuscript. All authors approved the final version of manuscript.

Funding: None; *Competing interest:* None stated.

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AIHA: autoimmune hemolytic anemia; CCR: continuous complete remission; CR: complete remission; PR: partial remission; NR-no remission.


Fig. 1 Comparison of remission status among primary and secondary autoimmune hemolytic anemia (AIHA).

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CLIPPINGS

 **Acute kidney injury in pediatric patients hospitalized with acute COVID-19 and multisystem inflammatory syndrome in children associated with COVID-19** (*Kidney Int.* 2021 Mar 3; S0085-2538(21)00268-4)

This retrospective study was conducted in patients 18 years of age or below admitted to study the incidence, clinical presentation and outcome of acute kidney injury (AKI) in children with COVID-19 and Multisystem Inflammatory Syndrome. Total 152 children were included, out of which 97 had acute COVID-19 and 55 had COVID -19 and MIS-C. AKI occurred in 11.8% of children with acute COVID-19 and MIS-C. They had increased WBC counts and lower serum albumin levels on admission and decreased intravascular volume and distributive/cardiogenic shock. In addition, pediatric COVID-19-related AKI was associated with poor outcomes, such as increased PICU and hospital length of stay. Their limitations were small sample size, retrospective study design, and most COVID-19 and MIS-C patients had a basic metabolic panel on presentation and during their hospital stay to assess for AKI. Utilization of serum creatinine without urine output and back-calculation of baseline creatinine values may have also underestimated the incidence of AKI. Therefore, further research in larger cohorts is needed to characterize AKI risk factors in children with acute COVID-19 and MISC

 **Long term renal survival of pediatric patients with lupus nephritis** (*Nephrol Dial Transplant.* 2021 Apr 7: gfab152)

This retrospective study conducted from 2000 till 2020 at Hacettepe University, tried to find out the clinical presentation, treatment options and renal prognosis in children with lupus nephritis. Data collected from medical charts and electronic records of 53 lupus nephritis children who had kidney biopsy at diagnosis. Overall, 52% had lupus nephritis (LN); class IV LN (54.7%) was most common followed by class III LN (22.6%). Around 77.3% and 73% of children received complete and partial remission at 6 and 12 months, respectively. Five-and ten-year renal survival rates were 92% and 85.7%, respectively. This study demonstrated that male gender, failure to achieve remission within 1 year after induction treatment and requiring dialysis at the time of diagnosis were the best predictors of poor renal outcome. Limited sample size, retrospective study design, and bias in starting CYC for patients with more severe disease were some of the limitations reported. Prompt recognition and aggressive management of paediatric LN are essential to achieve and maintain remission

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