

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

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Objective: To analyze clinical, 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. AIH Awas 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%), 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.

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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 syndrome) 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 northern 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, antinuclear antibody (ANA) was 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. IV Methyl prednisolone (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 0.2mg/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 3-6g/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 analyses: 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)] was 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. Leuko-cytosis (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. 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

WHAT THIS STUDY ADDS?

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

CR (Fig.1). Despite relapse in 26% cases, 61.5% still showed good response to steroids. One patient with secondary AIHA and underlying Hodgkin lymphoma died due to fulminant fungal sepsis and hemophagocytosis.

DISCUSSION

We present Indian 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.

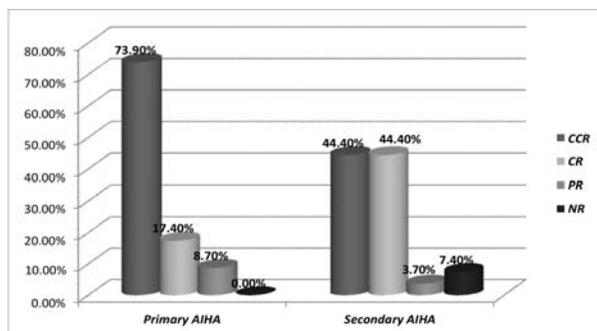
Ethics clearance: EC-SGRH; No.EC/09/20/1715, dated September 30, 2020.

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.

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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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