Original Research Article

Profile Of Autoimmune Haemolytic Anaemia: analysis of 10 years data from a hematology center in Eastern India

Running head: Autoimmune Haemolytic Anaemia in Eastern India

ABSTRACT

Aims: To study the Clinico-haematological profile and therapy outcome of patients with autoimmune haemolytic anaemia (AIHA)

Study design: Retrospective analysis through the case records of 69 patient treated at the Department of Hematology, NRS Medical College, Kolkata over a period of 10 years from January 2011 to December 2020.

Methodology: Clinico-haematological profile including baseline characteristics and therapy outcome of patients with autoimmune haemolytic anaemia (AIHA) were noted. Presenting symptoms, clinical spectrum, response to therapy and events (relapse, death, dropouts and refractory disease) was analyzed by standard statistical methods.

Results: This study identified 69 (primary-56 and secondary-13) consecutive patients were with a median age of 49 years. The common presentations included pallor (98.5%) and jaundice (84.5%) with the presence of splenomegaly (56.5%) and hepatomegaly (47.2%). Direct antiglobulin test was negative in two patients. Oral prednisolone produced remission in 91.04% patients with a median response duration of 28 days. Among responders, 28 patients relapsed after a median period of 269.2 weeks. The relapsed patients received steroid in most patients. Azathioprine, Rituximab and Splenectomy were given in eight, six and two patients respectively with a overall response rate of 62.65%, 66.6% and 100 % respectively.

Conclusion: Knowledge of clinical and laboratory profile in AIHA help in directing the investigations and HAS effect on the therapy decisions. With the plethora of drugs available as therapeutic options in primary AIHA, steroid still remains the cornerstone of therapy.

Keywords: Autoimmune haemolytic anaemia, clinical presentation, laboratory profile, treatment outcome

Abbreviations

AIHA: autoimmune haemolytic anaemia; RBC: Red Blood corpuscles; NHL: non-Hodgkin lymphoma; SLE: systemic lupus erythematosus; CR: complete response; PR: partial response;

Introduction

Autoimmune haemolytic anaemia (AIHA) is defined as anaemia caused by the destruction of erythrocytes through autoantibodies directed against surface antigens of red blood corpuscles (RBC). It is a heterogenous disorder with a variable demographic, clinical and therapeutic profile. Autoimmune haemolytic anaemia (AIHA) is a heterogenous disorder with a highest incidence of 1 –3 cases/ 100 000 per year in the general population [1]. In one of the studies in children under 18 years of age, the incidence was assessed to be 0.81/100,000 (95% CI 0.76–0.92) per year [2]. AIHA is defined as haemolytic anaemia which is triggered by the destruction of red blood cells (RBCs) through autoantibodies focused against antigens on their surface. The polyclonal immunoglobulin G (IgG) or oligoclonal immunoglobulin M (IgM) autoantibodies present with or without complement fix to the RBC surface causing haemolysis. The physiognomies of the bound antibody i.e. its amount, specificity, thermal aptitude, capability to fix complement and aptitude to bind to tissue macrophages is accountable for the degree of haemolysis [3]. The diagnosis of AIHA is grounded on the evidence for haemolysis, escorted by a positive direct antiglobulin test (DAT) and refuting the alternative causes [4]. In a study from north India, pallor (89%), fever (38%), jaundice (43%) and splenomegaly (81%) were the common presentations and all the patients in this series had a warm antibody of IgG type; no correlation existed between DAT positivity and severity of anaemia[5]. In a study reported from south India, the response rate in primary warm AIHA to corticosteroid was observed in 90% cases (62%, complete response and 28% partial response) [6]. In view of the paucity of reported data from the eastern region of the country, we aim to report a larger series of AIHA cases with the aim of investigating the clinico-haematological profile and treatment efficacy.

Material and methods

This retrospective study included all the patients with AIHA diagnosed between January 2011 to December 2020 and attending the Hematology clinic at NRS Medical College, Kolkata, a tertiary/referral hematology care centre from eastern India. All cases were diagnosed to have haemolytic anaemia after an evidence of peripheral blood spherocytosis and fragmented cell in peripheral smear with reticulocytosis, unconjugated hyperbilirubinemia and a positive direct

antiglobulin test (DAT) were documented. All the other cases of haemolytic anaemia from other causes such as haemoglobinopathies were excluded from the study. All the cases were also investigated to rule out the secondary causes of AIHA by doing antinuclear antibody (ANA), anti-dsDNA and viral screen for HBsAg, Anti HCV, HIV-1&2 in all patients. They were also investigated for the presence of either warm type or cold titre antibodies. Depending on the clinical profile, in selected cases where required, imaging studies like ultrasonography (USG) or computerized tomography (CT) scan were done. Flow Cytometry for chronic lymphoproliferative disorder (CLPD) panel, lymph node biopsy and bone marrow aspiration and biopsy were done wherever indicated.

All the patients of primary AIHA had received a course of steroid in a dose of 1mg /kg/day followed by slow tapering until haemoglobin achieved a normal level. Folic acid (5 mg) supplementation was given to all patients. Patients with symptomatic anemia received packed red blood cell (PRBC) transfusion depending upon the standard guidelines and clinical judgement of treating physician.

The follow up outdoor records were assessed for the response to steroids. They were assessed for the duration of steroid response and duration of maintenance of steroid response. CR was defined as normalization of haemoglobin with no haemolysis as evidenced by absence of transfusion requirement and normalisation of reticulocyte counts, serum bilirubin, lactate dehydrogenase (LDH), haptoglobin. Whereas, PR was defined as increase in haemoglobin by >2 g/dL or normalization of haemoglobin without any biochemical resolution of haemolysis; and absence of transfusion for the last 7 days. No response was defined as failure to achieve at least partial response[4].

All the patients who relapsed were counselled for the various therapy options. Unless contraindicated, all the patients who relapsed were given a repeat course of steroids and subsequently patients were offered therapeutic options as second line and third line therapy. Azathioprine was added (in the dose of 2 mg/kg/day) as a steroid sparing agent for the relapse cases for maintaining response and the response was assessed after 6 weeks [7].Rituximab could not be given to all at initial stage because of prohibitive cost and at this juncture splenectomy was chosen as a treatment choice. For those who could afford, rituximab was given at a dose of 375 mg/m2 per week for consecutive 4 doses and the response were assessed after 3-6 weeks[8]

Statistical methods

Data analysis was done using SPSS version 25.0 (IL, Chicago, USA). A p value of <0.05 were considered significant. The Pearson's correlation coefficient (r) was used to test the correlation between continuous variables.

Results

A total of 69 cases of AIHA consecutive patients were identified with a median age of 49 years (range, 7 – 72 years). **Figure 1** shows the clinical presentations, diagnostic, and therapeutic profile of the patients included in the study.

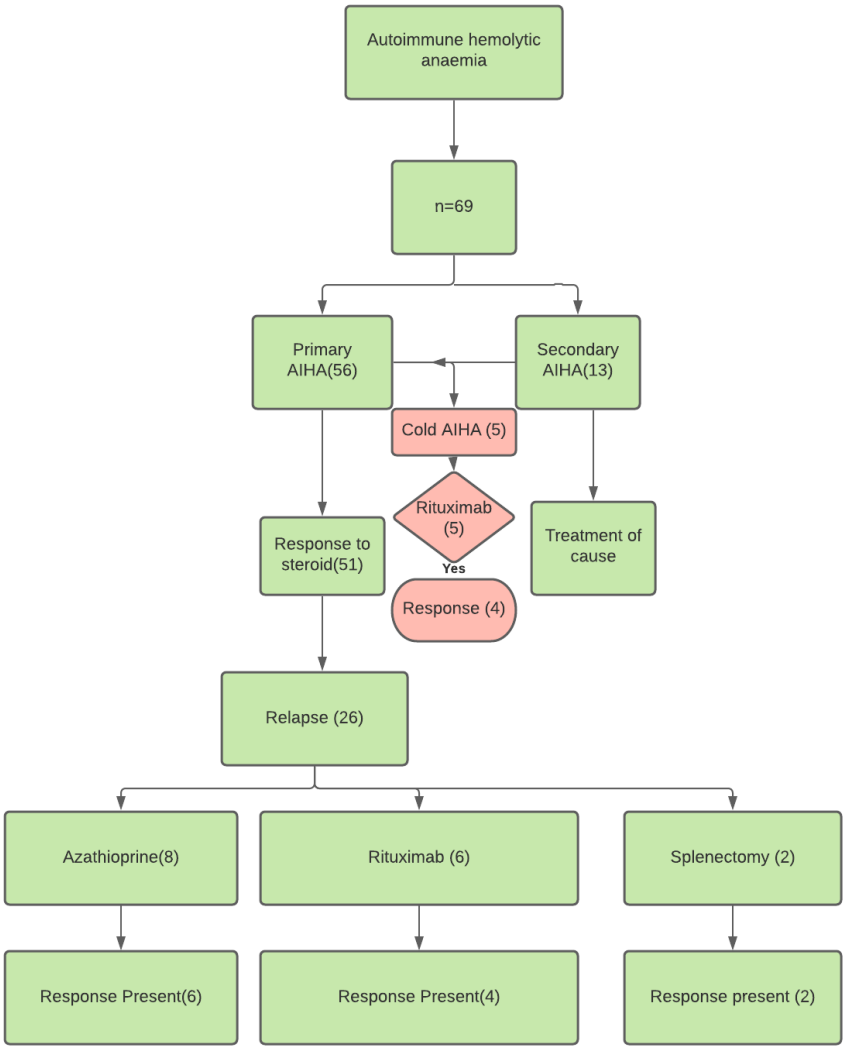

Figure 1: Consort diagram showing the different clinical, diagnostic, and therapeutic profile of the patients.

The presenting manifestations are presented in Table 1 and shows that the incidence of AIHA in this patient cohort was found to be females 49 (71.01%). Symptomatic anaemia and jaundice were seen 68 (98.5 %) and 58(84.05%) patients respectively. Arthralgia, GI symptoms, flu like symptoms, oral ulcers, Raynaud's phenomena, neurological manifestations and vitiligo was observed in 7(10.14%), 1(1.44%), 5(7.2%), 5(7.2%), 4(5.7%), 4(5.7%) and 1(1.4%) patients respectively. Fever

and bleeding manifestations in the form of petechiae, epistaxis and gum bleeding were seen in none of the patients. None of the patients gave a family history of AIHA. No drug was incriminated as cause of haemolysis in our series.

Parameters	**Results**
Age (years); median (range)	49 (7 – 72)
Sex; Female, n (%) : Male, n (%)	49(71.01) : 20(28.98)
Duration of illness before presentation (months)**;** median (range)	4 (0.5-12)
Presenting complaint(s); n (%)	
Pallor-	68 (98.5)
Jaundice-	58 (84.05)
Bleeding-	0 (0)
Arthralgia-	7 (10.14)
GI symptoms-	1 (1.44)
Flu like symptoms-	5 (7.2)
Oral ulcers-	5 (7.2)
Raynaud's phenomena-	4 (5.7)
Neurological manifestations-	4 (5.7)
Vitiligo-	1 (1.4)
Clinical examination;	
Splenomegaly; n(%)	39 (56.5)
Spleen size (cm); median (range)-	1 (0-7)
Hepatomegaly; n(%)	33 (47.82)
Liver size(cm); median (range)-	0 (0-4)
Lymphadenopathy; n(%)-	4 (5.7)
Primary AIHA	*56 (81.15%)*
Secondary AIHA	13 (18.85%)
Secondary causes:-	
Systemic lupus erythematosus (SLE), n (%)	8(61.5%)
Connective Tissue disorders, n (%)	2(15.38%)
Chronic lymphoproliferative disorders (CLPD), n (%)	3(23.07%)

Table 1: *Demographic and clinical profile of the patients (n=69)*

Clinically, on examining the patient, lymphadenopathy was seen in 4 (5.7 %) of the patients and 39 (56.5% of total) with a median size of 1 cm and hepatomegaly in 33 (47.82% of total) with a

median size of 0 cm. Fifty(72.46%) patients were found to have symptoms of less than 6 months duration before starting treatment.

The laboratory findings of the cases included in the study are presented in table 2 as follows:-

Laboratory parameters	**Results**
Haemoglobin (gm/L); median (range)	68.1 (20 -125)
Reticulocyte Counts (%); median (range)	12.09 (1.5 - 29)
Platelet Count($\leq$1.5 x 10^6/µL)	4 (5.7)
Patients with leukopenia ($\leq$4.0 x 10^3/µL), n (%)	6 (8.69)
Pancytopenia; n(%)	2 (2.8)
Serum bilirubin (mg/dl) median(range)	2.56 (0.4-7.3)
Serum LDH (IU/L); median(range)	694.63(210-2000)
Direct Agglutination Test (Positive); n(%)	67 (97.10)
Monospecific Coombs test (IgG, C3d) ; n(%)	2 (2.89)
ANA Positive; n(%)	10 (14.49)
Anti dsDNA Positive; n(%)	5 (7.2)
Hypothyroidism; n(%)	11 (15.94)
Cold Titre Present; n(%)	6 (8.69)
Indirect Agglutination Test (Positive); n(%)	2(2.89%)

Table 2: *Laboratory profile of the patients(n=69)*

Leukopenia was noted in 6(8.69%) patients, thrombocytopenia in 4 (5.7%) patients and pancytopenia was seen in two patients (2.8%). Peripheral smear examination showed spherocytosis in 49 (71.01%) patients. DAT was negative in 2 (2.8 %) patients. Other tests carried out showed positivity for antinuclear antibody in 10 (14.49%) and ds DNA in 5 (7.2%) patients. Finally a total of 56 cases were diagnosed as primary AIHA; rest (13 cases) were diagnosed as secondary AIHA. There was also no correlation observed between the haemoglobin level and DAT positivity.

Eighteen (22.8%) patients were started with treatment in the form of steroid for 3 days to 30 days before attending our centre. The different types of treatment received by the primary AIHA patients and therapy outcomes are represented in Table 3.

Parameters	Results
Front-line Warm AIHA	
Steroid; n(%) 1.Primary Steroid Response	56 (100%)

Response present; n(%)	51(91.07%)
No Response present; n(%)	5 (8.93%)
2. Duration of Steroid Response (days); Median (range)	28 (14 – 60)
3. Patient with First Relapse	
Present; n(%)	26(46.40%)
Absent; n(%)	30 (53.57%)
4. Onset of first Relapse (months)	
Median (range)	31.04 (2-156)
Front-line cold antibody cases	
Rituximab ; n(%)	5
Response ; n(%)	4 (80%)
No Response; n(%)	1 (20%)
Relapse Case	
Azathioprine ; n	8
Response; n(%)	5(62.5%)
No Response; n(%)	3(37.5%)
Rituximab; n	6
Response ; n(%)	4(66.67%)
No response; n(%)	2(33.33%)
Splenectomy; n	2
Response ; n(%)	2 (100%)
No Response ; n(%)	0 (0%)

Table 3: *Therapeutic profile of primary AIHA (n=56)*

Steroids were started in sixty-seven patients and along with this patient received supportive care with Packed Red Blood cells and Folic acid, depending upon there haemoglobin. The Results were adequately estimated, and response was achieved in 61 (91.04%) patients. The median duration of steroid response was 28 days which varied from the range of 14-60 days. Among the patients who achieved remission, 26 patients (38.80%) relapsed and the median period for relapse free survival was 269.2 weeks (=1885 days).

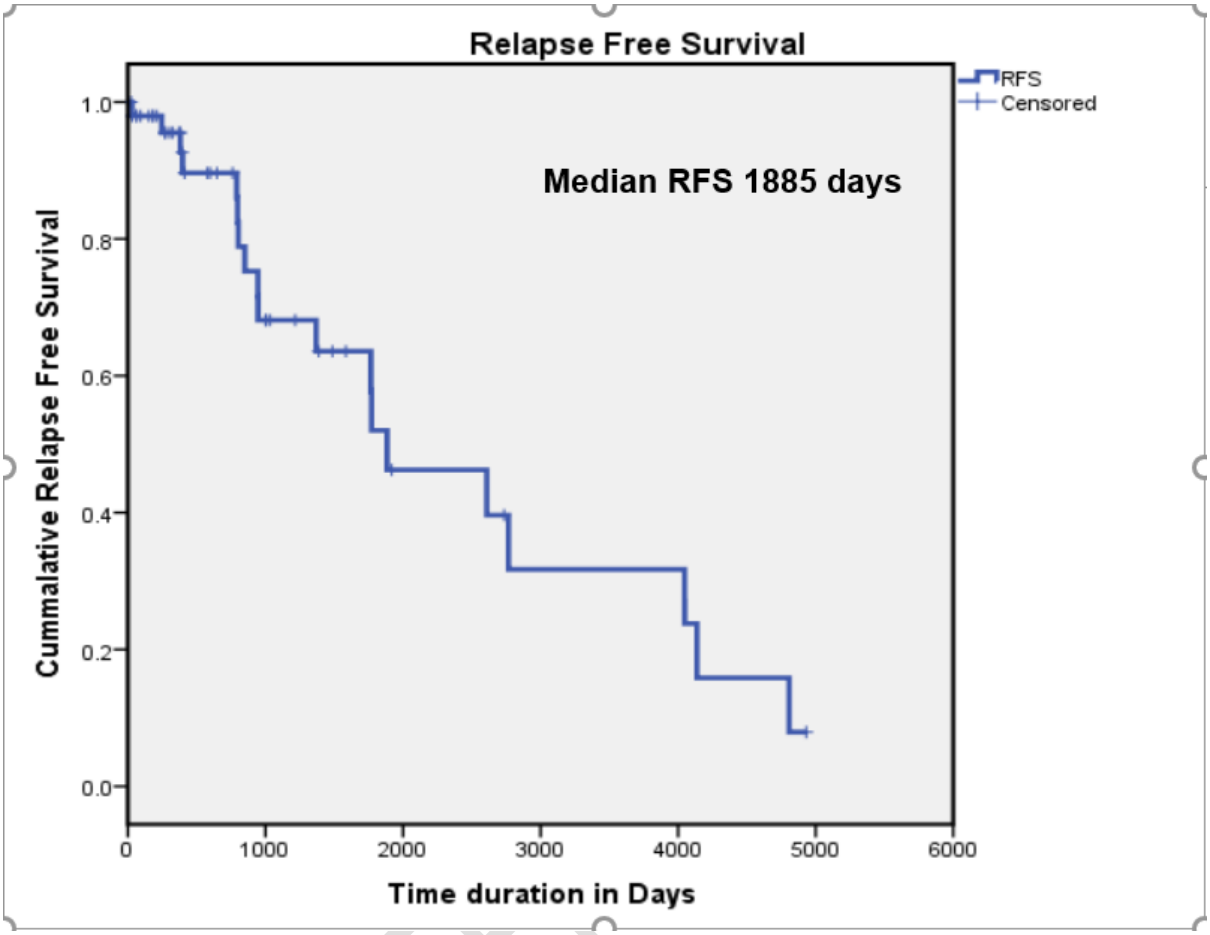

Figure 2: Survival analysis curve for median relapse free survival in AIHA

The relapsed patients received steroid in most patients. Azathioprine, Rituximab and Splenectomy were given in eight, six and two patients respectively with a overall response rate of five (62.65 %), four (66.6 %) and two (100 %) respectively.

The side effect profile related to long term steroid therapy has been summarised in Table 4 with incidence of acid peptic disease is seen in 15 patients (26.78%), hypertension in 12 (21.42%) and drug induced diabetes in 7 patients (12.5%).

Complications	**Number (%)**
Drug Induced diabetes mellitus	7(12.5%)
Hypertension	12(21.42%)
Cushingoid (moon) Facies	5(8.9%)
Acid peptic disease	15(26.78%)
Steroid Induced cataract	3(5.35%)
Tinnitus	1(1.7%)

Table 4: Adverse effects related to long term steroid therapy (n=56)

Discussion

There is paucity of data in AIHA from developing countries with most of it being case reports and small case series but none of the series have been reported from eastern India [5, 6]. Awareness of the demographics and clinical presentation patterns help in directing the investigations and too an extent has an effect on the therapy compliance. Of the 69 patients reported here with AIHA, 27 (34%) had secondary AIHA. Other series have described incidence of secondary AIHA in between 30 to 50% [5, 6, 9] which is quite similar to the etiological attributes in our study population. Median age at presentation was 49 years (range, 7–72), which is similar to the data published from western world with range of 3–90 with a mean of 53.5 years and is different from the data published in data from northern India where they reported the median age to be 20.5 and 30 years respectively [5, 7, 10,11].

The presenting complaints in our series were fever, anaemia, jaundice and bleeding manifestations, which is in contrast to the western data where AIHA is commonly detected secondary to some other disease process [14]. The incidence of idiopathic AIHA in our patients was higher in females 71.01% and similar findings have been reported from other part of the country. [1, 5, 7]. On examining the patient, lymphadenopathy, splenomegaly and hepatomegaly was seen in 4 (5.7 %), 39 (56.5% of total) and 33 (47.82% of total) patients respectively where as a study reported by Choudhry et al, hepatomegaly and splenomegaly was seen in 76% and 81% cases respectively, which was as many as two times of what we could elicit from our study and this disparity is possibly because of the difference in the size of the study population. The median duration of presentation of symptoms in our study group was 4 months and a study from North India found to have duration of presenting symptoms to be less than 6 months in 38 % of the patients in their study group [5].

Diagnosis of the disease entity and eliciting the cause remains the major crunch for the therapy. The median haemoglobin at presentation in our series is 68.1 gram/L (20 -125). Though the granulocytes and platelets are usually maintained in AIHA patient. These patients usually exhibit mild leucocytosis with neutrophilic predominance, but patients may present with leukopenia, neutropenia, or thrombocytopenia due to associated immune-mediated neutrophil and/or platelet destruction [2]. In our series the reported incidence of leukopenia, thrombocytopenia and pancytopenia was 8.69%, 5.7% and 2.8% respectively.

The DAT positive status was seen in 97.1 % patients, but mere positivity of DAT alone does not define AIHA. Many healthy subjects may be DAT positive as well. These patients may have no evidence of haemolysis, so to diagnose AIHA it becomes primarily important to demonstrate haemolysis. In our study group the bilirubin median (range) was 2.56 mg/dl (0.4-7.3) and LDH median being 694.63 IU/l (range, 210-2000). DAT status and the laboratory parameters observed in patients include reduced serum haptoglobin level, increased indirect bilirubin level, and elevated

lactate dehydrogenase (LDH) level aides the diagnosis of AIHA [2]. DAT negative statuses were seen in 2 patients and in literature up to 10% of patients with clear evidence of AIHA were reported as DAT negative. DAT may remain positive in patients with AIHA in remission. When ever DAT testing is done by the tube method, for the test to be positive the number of IgG molecules sensitizing each RBCs must be around 200-250 molecules [4, 15, 16]. Various other studies give the threshold of IgG molecules to be detected by antiglobulin test to be anywhere from 50 to 500 [4, 17, 18] and thus in this context, AIHA with low titres of antibodies may present as a DAT negative AIHAs. Though these shortcomings are overcomed by using techniques that detected smaller amounts of autoantibodies which includes flow cytometry, enzyme linked or mitogen stimulated DAT which can amplify the autoimmune reaction in culture [4]. In our study group there was also no correlation between the haemoglobin level and DAT positivity.

In all cases of AIHA which necessitate therapy steroids remains the first choice and response to corticosteroids is seen in 70% - 85% of AIHA cases the first 3 weeks of treatment [19,20]. In this study group, 91.04 % of patients responded to oral corticosteroid therapy with a median response time in our patients was 28 days. After one year of the disease onset 30% of the patients remain in remission and 20% are deemed cured by steroids [20]. Among our study population, 38.80% of them relapsed within a median duration of 269.2 weeks. Due to a long follow period the proportion of patients needing second-line therapy are usually poorly assessed but among the patients who relapsed the overall response rates according to the treatment with azathioprine, rituximab and splenectomy were 62.65%, 66.6%and 100% respectively. However, the response rates which have been reported , similar results have been reported from previous reports and was a preferred second-line option, with a sustained response rate of 60% to 70% [20]. Though with the advent of Rituximab the trends are drifting towards a non-surgical approach. The optimal rituximab dose in AIHA has not been established and mainly used in dose of 4 weekly doses of 375 mg/m2 per week [4, 21,22]. In a meta-analysis of 21 studies, the overall response rate with Rituximab in WAIHA was 79% for patients and in this approximately half the patients received concomitant corticosteroid [23]. However, given the fact that the amount of B cells disturbed in AIHA are considerably lower compared with lymphoproliferative B-cell disorders, lower doses of rituximab have been explored in the treatment of autoimmune cytopenia's but still we need more studies to explore. Azathioprine has been a third line therapy for AIHA with response rates have been 56-71% and the cost and ease of availability with a response rate of 62.5% in our study and it seems to be an affordable option for patients with AIHA as a second line agent[4]. Few case series and studies for the management of warm autoimmune haemolytic anaemia supporting our study results have been summarised in table no 5.

Sl. No.	Therapeutic agents/options	Study	Outcomes
1	Prednisolone as first line agent	Birgens H et al[24]	In Prednisolone treated group alone, 50% of patients achieved response at 3 months and nearly half of the

	in warm AIHA		responders relapsed within a year.
		Barcellini W et al[25]	Higher prednisone-based response rate of 80% with half of the patients at a median follow-up pf 33 months did not require subsequent treatment.
		Choudhry VP et al[5]	Among 56 patients , response was achieved in 49 (87.5%) patients. However, after a median period of 2 months (2 months– 2 years) after response six patients relapsed.
2	Rituximab	Busone et al[26]	A response rate of 100 % was reported with rituximab for primary warm AIHA
		Reynaud et al[27]	In this series, primary and secondary warm AIHA treated patients 79% responded and with CR seen in 42% .
		McLaughlin P et al[23]	In a meta-analysis of 21 studies, the overall response rate with Rituximab in WAIHA was 79% for patients and in this approximately half the patients received concomitant corticosteroid
3	Splenectomy	Barcellini W et al[25]	Approximately 70% respond and 40% achieve complete remission following splenectomy
4	Azathioprine	Hill QA et al[28]	Around 60 % response rate

Table 5: Data on different therapeutic options and Relapse free survival from other studies and recommendations

Longer follow-up with low-dose Rituximab and more studies with experience with Azathioprine are required to enrich the data for their use in management of AIHA.

Conclusion:

There is paucity of data in AIHA from eastern India most of it being case reports and small case series. Awareness of the demographics and clinical presentation patterns help in directing the investigations and too an extent has an effect on the therapy compliance. With the plethora of drugs available as therapeutic options in primary AIHA, steroid still remains the cornerstone of therapy. Rituximab in standard dose or in low-dose regimen is an important therapeutic alternative or additive.

CONSENT

As per international standard or university standard and hospital OPD/IPD registry, patients' written consent has been collected and achieved by the Institute.

ETHICAL APPROVAL

The present study is an analysis of retrospective data from the hospital/institute archive. All the patients had given written consent for OPD/IPD attendance that is preserved by the Institute.

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