A PROSPECTIVE STUDY ON RESPONSE OF IMMUNOSUPPRESSIVE THERAPY WITH CYCLOSPORINE AND PREDNISOLONE IN ACQUIRED APLASTIC ANAEMIA.

ISLAM MS[1], HOSSAIN MZ[2], AZIZ MA[3], BEGUM M[4], RAHMAN MJ[5], ARA T[6]

Abstract

Context: *A randomized prospective study was done to see the effect of therapy with cyclosporine and prednisolone in patients with idiopathic aplastic anemia.*

Methods: *A total of 40 patients were included in this study .We use cyclosporine 6mg/kg/day and prednisolone 30 mg /day for a period 6 months. The study was done during the period of July 2005 to October 2006. In the study population, out of 40 patients, 26 were male and 14 were female. 2 patients died early in the treatment and 4 patients transformed into acute leukemia. The rest 34 patients completed therapy for 6 months.*

Results: *Among the 34 patients, 22 patients responded to therapy and 12 patients were non-responders. According to severity, population distribution was as follows: 12 (35.30%) patients were non-severe AA, 19 (55.88%) patients were severe AA and 3 (8.82%) patients were very severe AA. Out of 22 patients (responders), 7 (31.82%) patients start to respond at 3rd month, 5 (22.73%) patients at 4th month, 8 (36.36%) patients at 5th month and 2 (9.09%) patients were very late to respond; they respond at 6th month. At the end of 6th month trial, 7 (20.59%) patients had complete response, 15 (44.12 %) patients had partial response and 12 (35. 29%) patients were non-responders; overall response rate was 64.71%. The adverse effects of the therapy were found e.g. weight gain in 100%, hypertension in 26.47%, increased serum creatinine in 38.23%, hyperglycemia in 20.58% and infection due to immunosuppression in 14.71%.*

Conclusion: *Combination of cyclosporine and prednisolone therapy merits further study as primary treatment for aplastic anemia in patients who are not able to undergo bone marrow transplantation.*

Key words: *Aplastic anaemia, immunosuppressive therapy, combination of cyclosporine and prednisolone.*

Introduction:

Aplastic anaemia is a life threatening disease. It is rare in western countries (2-4/million) but its prevalence is more in Asia (6-14/million)[1]. In Bangladesh, we have no nationwide survey and actual prevalence is not known. Aplastic anaemia is defined as pancytopenia with hypocellular bone marrow in the absence of an abnormal infiltrate and with no increase in reticuline[2]. Examination of peripheral blood reveals a decrease in all granulocytic cells as well as monocytes, platelet and reticulocytes with a variable number of lymphocytes counts. Malignant or markedly dysplastic cells are not seen. In bone marrow, all hematopoietic cell lines are reduced,

1. Dr. Md. Sirajul Islam, Assistant Professor, Department of Haematology, Dhaka Medical College, Dhaka.
2. Dr. Mohammad Zaid Hossain, Assistant Professor, Department of Medicine, Dhaka Medical College, Dhaka.
3. Dr. Md. Abdul Aziz, Assistant Professor, Department of Haematology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka.
4. Dr. Masuda Begum, Associate Professor, Department of Haematology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka.
5. Prof. Md. Jalilur Rahman, Professor and Chairman, Department of Haematology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka.
6. Dr. Tasneem Ara, Assistant Professor, Department of Haematology, Dhaka Medical College, Dhaka.

Correspondence:: Dr. Md. Sirajul Islam, Assistant Professor, Department of Haematology, Dhaka Medical College, Dhaka, Cell Phone: +8801711976214

malignant cells are not present ,and macrophages, lymphocytes, mast cells and fibroblast may be prominent but the striking characteristics is the expansion of fatty marrow, which replaces the hematopoietic marrow without an increase in reticuline[2]. The high incidence of aplastic anaemia in countries of South-east Asia including Bangladesh (3 to 4 times than Europe) does not reflect a genetic or racial predisposition. More likely, the high incidence is related to the greater prevalence of viral infection particularly hepatitis, more widespread use of potentially toxic antibiotic and perhaps to the wide use of industrial and agricultural chemicals including pesticides without adequate protection[3,4,5]. So, this geographic variation is probably mainly environmental rather than genetic. Aplastic anaemia may be acquired or inherited and acquired aplastic anaemia is again subdivided into secondary and idiopathic aplastic anaemia[2]. The course of the disease is usually fatal without treatment and high mortality is due to bleeding or infection. Fatality depends on severity; severe and very severe aplastic anaemia cause life threatening medical emergencies, chronic disability disorder for families and a major drain on health resources[3,6.] Aplastic anaemia results from immune mediated bone marrow suppression. Hematopoietic stem cells are markedly reduced in aplastic anaemia. The interferons secreted by lymphocytes are known to be potent inhibitors of hematopoietic colony formation. Cytotoxic lymphocytes also appear to be pathophysiologic in aplastic anaemia[7,8,9]. Bone marrow transplantation and immunosuppressive therapy are two modes of curative treatment of this devastating disease. In our country, we have no bone marrow transplant (BMT) center but have the option of immunosuppressive therapy. The immunosuppressive therapy (IST) is the only available satisfactory method of management in our country. Among IST, ATG is the treatment of choice. The response rate in horse ATG ranges from 70-80% with 5 years survival of 80-90%[7]. But its cost is unbearable for most of the patients. In the late 1980s, interest was centered on cyclosporine as an alternative immunosuppressive agent to ATG for management of patients with aplastic anaemia. Addition of cyclosporine with ATG increases the rate of response. The response rates to cyclosporine alone were 45% overall, 16% for VSAA, 47% for SAA, and 85% for NSAA[9]. The percentages of complete and partial response in SAA were 11.6% at 3rd month and 30% at 12th month respectively with cyclosporine[10]. As aplastic anemia is a T-cell mediated immunologic disease, its specific treatment should be a combination of immunosuppressive drugs directed to T-cell mediated immunologic injury[11,12]. Chronic GVHD in post transplant is also an immunologic disorder like aplastic anaemia. For its specific treatment, commonest protocol used in the Seattle regimen is Cyclosporine and prednisolone[12,13]. So, it was assumed that the combination of CSA and prednisolone would be a readily available and cost effective management of aplastic anemia in a poor country like Bangladesh.

Materials and Methods:

A prospective study conducted in the Department of Haematology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from July 2005 to October 2006 on patients with aplastic anaemia, where all the patients, irrespective of age and sex, were included. The purpose of the study was clearly explained to each of subjects and they were included in the study only after they gave full informed consent, the detailed clinical history was recorded in a pre-designed case record form. The patients were categorized as non-severe, severe and very severe aplastic anemia according to severity assessment criteria. The patients who fulfilled the inclusion and exclusion criteria, were considered to undergo screening investigations e.g. CBC, PBF, bone marrow study and biopsy, vit-B_{12} and folate, liver function test, renal function test, viral markers, ANA and anti-ds DNA, x-ray of chest, abdominal ultrasonography to exclude the other causes of pancytopenia.

In this study, a total of 40 patients were included. The mean age (±SD) was 32±16 years. Each patients was given cyclosporine 6 mg/

kg/day and prednisolone 30 mg/day for 6 months .Initially patients were supported with antibiotic and blood and blood products .A structured and planned follow up schedule was maintained for the evaluation of hematologic response and drugs toxicities . Initially patients were monitored in out patients department every ten days for one month, then every fifteen days for next one month, every month for 2 months and every 2months for 2 months (after responding). Examination findings, hematologic responses and drugs toxicities parameters were recorded at 3rd, 4th, 5th and 6th month. Complications arising during the study period and any adverse effects of drugs were managed properly.

The total duration of study was 16 months. A structured and planned educational (patient information) class of one hour was taken. The emphasis of the class was on providing general education about aplastic anemia, healthy life style and appropriate use of medication, adverse effects of drugs and treatment outcome. In each follow up, the patients was thoroughly examined including blood pressure monitoring and investigated with complete blood count, peripheral blood film, serum creatinine, SGPT, random blood sugar and routine urine test. The haematological response was evaluated.

Results:

Among the 40 patients, 26 were male and 14 were female. 2 patients died earlier in the study, 4 patients turned into acute leukemia; the rest 34 patients completed the 6 month therapy with follow up. The results of the study are shown below in table-I, II, III, IV and V.

Table-I

Age distribution in study population.

Age group	No. of patients	% of patients	X^2 value	P value
10-30 yrs	24	60	12.95	0.002
31-50 yrs	09	22.5	12.95	0.002
51 -70 yrs	07	17.5	12.95	0.002

Table-II

Hematologic parameter before treatment

Hematological features	Range	Mean ±SD
Hemoglobin conc. gm/dl	2-9	5.1±1.8
Total count of WBC $x10^9$/L	1.5-4.5	2.7±1.2
Neutrophil (%)	10-60	20±11
Marrow cellularity (%)	20-40	22±9
Platelet count $x10^9$/L	20-35	27±1

Table-III

Grading of aplastic anaemia among the patients who completed 6 month trial

Severity assessment	Number	Percentage	P value
Non-severe AA	12	35.30	0.003
Severe AA	19	55.88	0.003
Very severe AA	03	8.82	0.003

Initiation of response among the responders: After initiation of the therapy, 7 (31.82%) patients started to respond at 3rd month, 5 (22.73%) patients at 4th month, 8 (36.36%) patients at 5th month and 2 (9.09%) patients begin to respond at 6th month. At the end of 6 month trial, 7 (20.59%) patients had complete response, 15 (44.12%) patients had partial response and 12 (35. 29%) patients were non-responders. So, the overall response rate of combination therapy with cyclosporine and prednisolone in this study was 64.71% (partial+complete response). In complete response group (a total of 7), 6 patients were from non-severe AA and 1 patients was from severe AA. In partial response group (a total of 15), 4 patients were from non-severe AA, 10 patients were from severe AA and 1 patient was from very severe AA. Among non-responders (a total of 12), 66.66% (2 patients) of VSAA, 42.2% (8 patients) of SAA and 16.6% (2 patients) of NSAA did not respond to combination therapy with cyclosporine and prednisolone within 6 month. So among the study population, non-responders were mainly from severe and very severe AA.

Adverse effects: During the study period, all the 34 (100%) patients had weight gain, 9 (26.47%) patients developed hypertension, 13 (38.23%)

Table-IV

Association of response with disease severity

Disease severity (n)	Complete response	Partial response	Non responder	P value non severe vs severe
Non severe AA (12)	6	4	2	0.02
Severe AA (19)	1	10	8	severe vs very severe
Very severe AA (03)	00	01	02	0.02

Table-V

Comparison of treatment outcome at 6th month

Parameter	Before treatment Mean±SD	At 6th month of treatment Mean±SD	t value	P value
Haemoglobin conc. gm/dl	5.1±1.8	10.9±2.4	10.58	<0.001
Total count of WBC $x10^9$/L	2.7±1.2	5.5±2.9	4.88	< 0.001
Platelet count $x10^9$/L	27±1	138±48	15.71	<0.001

patients had raised (upto 2.8mg/dl) serum creatinine, 7 (20.58%) patients developed hyperglycemia and 5 (14.71%) patients had infection e.g. UTI and pneumonia, skin and soft tissue infection which was easily controllable by antibiotics and there was no therapy related mortality.

Discussion:

Idiopathic aplastic anaemia is an immunological disorder characterized by peripheral pancytopenia and bone marrow hypocellularity without any atypical cell. This study focused on the response of immunosuppressive therapy with cyclosporine and prednisolone in cases of aplastic anaemia. This is, in fact, the first comprehensive study conducted in Bangladesh regarding evaluation of the response of cyclosporine and prednisolone. It is a prospective study (single study). In this study, out of 40 study samples, only 2 (5%) patients had died, none beyond one month after therapy. All 2 early deaths occurred in patients with very severe and severe AA. 4 (10%) patients were transformed into acute leukemia within 2 months of enrollment. So, rest 34 (85%) patients completed 6 months therapy with cyclosporine 6 mg/kg/day and prednisolone 30 mg/day. Of them, 36 (90%) patients presented with anaemia, 32 (80%) patients with fatigue and weakness, 24 (60%)

patients with fever, and 18(45%) patients with bleeding of any form (cutaneous, mucosal and internal).

Among the 34 (85%) patients who completed 6 month therapy of cyclosporine and prednisolone, 12 (35%) patients were non-severe AA,19 (55.88%) were severe AA ,and 03 (8.82%) were very severe AA. In non-severe group, 6 (50%) had complete response, 4 (33.33%) patients had partial response and 2 (16.66%) patients were non-responders. In German Aplastic Anaemia Study Group, the response rate with cyclosporine (CSA) in NSAA was 75% (40% complete response+35% partial response)[3]. In severe aplastic anaemia group, 1 (5.26%) patient had complete response, 10 (52.6%) patients had partial response and 8 (42.08%) patients were non-responders. The response rate in multicenter randomized study with CSA in SAA was 52% (10% complete response+42% partial response)[11]. In very severe AA, 1 (33.33%) patient had partial response and 2 (66.66%) were non-responders; no one had complete response.

Initiation of response: Among the responders (22 patients), 7 (31.82%) patients started to respond at 3rd month, 5 (22.73%) patients at 4th month, 8 (36.36%) patients at 5th month and 2 (9.09%)

patients were very late to respond; they started to respond at 6th month. The percentages of complete and partial response in SAA were 5% and 11.6 % at 3rd month and 16% and 30% at 12th month respectively with cyclosporine[10]. In another multicenter study, the percentages of complete and partial response in SAA were 7% and 18% at 3rd month; 15% and 35% at 6th month respectively treated with cyclosporine[14]. So, at the end of 6th month therapy with cyclosporine and prednisolone, and of 34 patients, 7 (20.59%) patients had complete response, 15 (44.12%) patients had partial response and 12 (35.29%) patients were non-responders. Therefore, the rate of total response (both complete and partial) was 64.71%. In international study, the response rate to cyclosporine alone was 45-55% overall, 16% for VSAA, 47% for SAA and 85% for non-severe aplastic anaemia[9]. In our study, the rate of response is higher in comparison to cyclosporine alone, because, a fair number of non-severe cases were included and addition of prednisolone with cyclosporine might have a cumulative effect to immunosuppression.

Follow up parameter in complete response patients: The mean (±SD) haemoglobin concentration in patients with complete response were 8±1.6, 9.5±1.8 and 10.5±2.4 gm/dl at 3rd, 5th and 6th month respectively after initiation of therapy. The study showed that the concentration of haemoglobin raised gradually in course of time. At the same time, platelet number increased strikingly with immunosuppressive therapy. The mean difference of platelet count at three interval was statistically significant (P=0.01). All the patients were transfused blood and blood product at the initial phase. Most of the patients (21 patients) were prescribed empirical antibiotic at the recruitment phase of the therapy. 5 (14.71%) patients were admitted to hospital for internal bleeding and 6 (17.64%) patients for infection (3 with pneumonia, 2 with enteric fever and 1 with UTI).

Conclusion:

During the last decades, insight in the pathogenesis of aplastic anaemia has increased. In parallel to this, the prognosis of this potentially fatal disorder has improved radically. It can be expected that response rate to immunosuppressive therapy will probably continue to rise partly due to new drugs tested for their efficacy in aplastic anaemia such as mycofenolate mofetil . Outcome after IST has improved over the years. Changes in types of immunosuppressive therapy and patient selection partially explain the cause of improvement and combined treatment was shown to be more effective than single therapy with any drug. In our study, a total of 64.71% patients responded to cyclosporine and prednisolone as complete (20.59%) and partial (44.21%) response. So, the combination of cyclosporine and prednisolone therapy merits further study as primary treatment for aplastic anaemia in patients who are not able to undergo BMT and this small study provides strong justification for performing a well designed, multicenter, randomized control trial of cyclosporine and low dose prednisolone.

References:

1. Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE, eds. Hematology: basic principles and practice. 2nd ed. Vol.1. London: Churchill Livingstone; 1995. p.299-385.

2. Marsh JCW, Ball SE, Darbyshire P, Gordon-Smith EC, Keidan AJ, Martin A, et al. Guidelines for the diagnosis and management of acquired aplastic anaemia. Br J Haematol. 2003; 123: 782-801.

3. Frickhofen N, Kaltwasser JP, Schrezenmeier H, Raghavachar A, Vogt HG, Herrmann F, et al. Treatment of aplastic anemia with antilymphocyte globulin and methylprednisolone with or without cyclosporine. The German aplastic anemia study group. N Engl J Med. 1991; 324(19): 1297-304.

4. Greer JP, Foerster J, Rodgers GM, Paraskevas F, Glader B, Arber DA, et al. eds. Wintrobe's clinical hematology. 12th ed. Vol.1. Philadelphia: Lippincott Williams & Wilkins; 2008. p.1397-413.

5. Gordon-Smith EC, Marsh JCW. Acquired aplastic anaemia, other acquired bone marrow failure disorders and dyserythropoiesis. In: Hoffbrand AV, Catovsky D, Tuddenham EDG. eds. Postgraduate haematology. 5th ed. Victoria: Blackwell Publishing Asia; 2005. p.190-206.

6. Sleijfer S, Lugtenburg PJ. Aplastic anemia: a review. Netherlands J Med. 2003; 61(5): 157-63.

7. Maciejewski JP, Risitano AM. Aplastic anemia: management of adult patients. Am Soc Hematol Educ Program. 2005; 110-7.

8.   Kasper DL, Braunwald E, Fauci AS, Amson JL, Hausu SL. eds. Harrison's principle of internal medicine. ed. McGraw-Hill; 2005. p.617-25.

9.   Maschan A, Bogatcheva N, Kryjanovskii O, Shneider M, Litvinov D, Mitiushkina T, et al. Results at a single center of immunosuppression with cyclosporine A in 66 children with aplastic anemia. Br J Haematol. 1999; 106(4): 967-70.

10. Gluckman E, Esperou-Bourdeau H, Baruchel A, Boogaerts M, Briere J, Donadio D, et al. Multicenter randomized study comparing cyclosporine-A alone and antithymocyte globulin with prednisone for treatment of severe aplastic anemia. Blood. 1992; 79(10): 2540-6.

11. Bacigalupo A, Brand R, Oneto R, Bruno B, Socié G, Passweg J, et al. Treatment of acquired severe aplastic anemia: bone marrow transplantation compared with immunosuppressive therapy - The European group for blood and marrow transplantation experience. Semin Hematol. 2000; 37(1): 69-80.

12. Marsh J, Schrezenmeier H, Marin P, Ilhan O, Ljungman P, McCann S, et al. Prospective randomized multicenter study comparing cyclosporin alone versus the combination of antithymocyte globulin and cyclosporin for treatment of patients with nonsevere aplastic anaemia: a report from the European blood and marrow transplant (EBMT) severe aplastic anaemia working party. Blood. 1999; 93(7): 2191-5.

13. Doney K, Pepe M, Storb R, Bryant E, Anasetti C, Appelbaum FR, et al. Immunosupressive therapy of aplastic anemia: results of a prospective, randomized trial of antithymocyte globulin (ATG), methyl prednisolone, and oxymetholone to ATG, very high-dose methyl prednisolone, and oxymetholone. Blood. 1992; 79(10): 2566-71.

14. Ahn MJ, Choi JH, Lee YY, Choi IY, Kim IS, Yoon SS, et al. Outcome of adult severe or very severe aplastic anemia treated with immunosuppressive therapy compared with bone marrow transplantation: multicenter trial. Int J Hematol. 2003; 78(2): 133-8.