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An observational study of the clinicopathological presentation of aplastic anemia in a tertiary hospital

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Abstract: *Introduction:* Pancytopenia in the peripheral smear and hypocellularity in the bone marrow characterise aplastic anaemia (AA). It is far less common in Western countries. The Asian continent has a greater prevalence rate. Aplastic anemia carries a high mortality rate if it is not treated. Hence early diagnosis and treatment is important to reduce the mortality rate. Only few Indian studies are conducted to assess the epidemiology of AA. *Aims and Objectives:* To evaluate clinical and pathological profile of patients with aplastic anemia. *Materials and Methods:* This cross sectional observational study was done over a period of 1.5years in department of medicine, St johns medical college, Bangalore. A detailed clinical history, physical examination and available previous investigations were recorded. *Results:* In our study aplastic anemia had bimodal peak in age distribution noted in groups of 21-30 & 51-60yrs .Male preponderance of 1.33:1 .Most common presenting complaint was Fatigue. Severe (37.6%) and Very severe aplastic anemia (37.6%) were more common.Most common 1st line agent was Cyclosporine and 2nd line agent was Danazol. Out the 35 only 4 (11.4%) showed Complete Response, 19 (54.3%) showed Partial Remission, 10 were transfusion dependent (28.6%) and 2 (5.8%) expired. *Conclusion:* ATG and Cyclosporine showed significant improvement in most parameters. Many patients in the study were unwilling for ATG and bone marrow transplantation due to the cost factor. Many are still requiring frequent transfusions, thus probing us to search for newer novel forms of treatment to reduce the cost of treatment and mortality in aplastic anemia patients.

Keywords: Pancytopenia, Severity, Clinical profile, Aplastic anemia.

Introduction

Pancytopenia in the peripheral smear and hypocellularity in the bone marrow characterise aplastic anaemia (AA). It is far less common in western countries. The asian continent has a greater prevalence rate [1]. Aplastic anemia may be acquired and immune mediated. Most of the cases are idiopathic but environmental factors play significant role including exposure to drugs, toxins and viral infections [2]. Infections and bleeding are the most common causes of death in people who are not treated [3].

The age distribution is bimodal, with peaks in (15-25 yrs) and elderly (>60 yrs) [4]. The beginning of disease happens at a younger age in the Asian Subcontinent, certain HLA alleles and genetic predispositions have been linked to AA aetiology [5]. Allogenic hematopoietic stem cell transplantation (HSCT) is standard of treatment in younger patients. Due to limited provision of the facilities, financial reservations and unavailability of matched donors, HSCT is done for limited number of AA patients. Immune suppression treatment (IST) is one of alternative treatment to HSCT i.e. Antithymocyte globulin (ATG) and cyclosporine treatment. Unfortunately, it is also an expensive treatment option.

Aplastic anemia carries a high mortality rate if it is not treated. Hence early diagnosis and treatment is important to reduce the mortality

rate. Only few Indian studies are conducted to assess the epidemiology of AA [6-7]. Hence this cross sectional study was done in St Johns medical college hospital, Bangalore, Karnataka, with an objective to study the clinicopathological profile of aplastic anemia patients.

Material and Methods

This cross sectional observational study was done over a period of 1.5 years in department of medicine, St Johns Medical College, Bangalore.

Sample size: The sample size was calculated considering the prevalence of infection to be (p) as 39% (by Shukla et al [8] absolute precision (d) of 17% with a 95% confidence interval. Sample size was calculated using the formula n= 4× p × q d2 Where, n is the sample size p is the prevalence q is 100-p d is the absolute precision n = 4pq d2 n= 32 Dropout rate: considering dropout rate as 5% of 32=1.7 Total sample size, n =34~35
Thus, total sample size = 35

Inclusion criteria: All the patients aged above 18 years who fullfilled the definition of aplastic anemia as laid down by International Agranulocytosis and Aplastic anemia Study group, 1987 [9], which is defined as:

1. Peripheral blood showing at least two out of three of the followings:
   a. Hemoglobin less than 10 g/dl or hematocrit less than 30%.
   b. Total leukocyte count less than 3.5 × 109 /l or granulocyte count less than 1.5 × 109 /l.
   c. Platelet count less than 50 × 109 /l.
2. Bone marrow biopsy showing the following:
   a. Decrease in cellularity with absence or depletion of all hematopoietic cells < 25%.
   b. Absence of significant fibrosis or neoplastic infiltration.

Patients who do not give consent for the procedure, patients having pancytopenia and a cellular marrow on bone marrow aspiration or biopsy and patients having pancytopenia and hypocellular marrow, but with neoplastic infiltration were excluded from the study.

Methodology: After obtaining approval and clearance from the institutional ethics committee, 35 patients fulfilling the inclusion criteria were enrolled for the study after obtaining informed consent. A detailed clinical history, physical examination and available previous investigations were recorded. Complete blood count, including reticulocyte count, peripheral blood film examination, bone marrow aspiration and biopsy was done in all the patients. Data collected including demographics, laboratory and clinical measurement including age, gender, blood counts, cytogenetic, viral profile, treatment offered. Status of alive and death of patients during the study period was also recorded.

Camitta classification was used to classify severity of disease:

Classification	Criteria
Severe	BM Cellularity < 25% (or< 50% if < 30% of BM is hematopoietic cells) AND $\geq$ 2 of the following : • Peripheral blood neutrophil count < 0.5 x $10^{9/L}$ • Peripheral blood platelet count < 20 x $10^{9/L}$ • Peripheral blood reticulocyte count < 20 x 19 $^{9/}$ L
Very Severe	As above, but peripheral blood neutrophil count must be < 0.2 x $10^{9/}$L
Nons evere	Hypocellullar BM with peripheral blood values not meeting criteria for severe aplastic anemia.

Sstatistical analysis: Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. *Chi-square test* was used as test of significance for qualitative data.

Continuous data was represented as mean and SD. *Paired t test* was the test of significance for paired data such as before and after treatment for quantitative data. *ANOVA (Analysis of Variance)* was the test of significance to identify the mean difference between more than two groups for quantitative data.

Graphical representation of data: MS Excel and MS word were used to obtain various types of graphs such as bar diagram, Pie diagram. *p value* (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Statistical Software: MS Excel**,** SPSS version 22 **(**IBM SPSS Statistics, Somers NY, USA) was used to analyze data.

Results

During the study period 35 patients were included. Out of 35 patients 20 were male and 15 were female Most of the patients belonged to age group of 51 - 60 years (31.4%). Aplastic anemia was more common in patients with blood group of B +ve. Most common co morbid Condition which was associated was hypertension (14.5%) followed by diabetes (2.9%) and hypothyroidism (2.9%).

Commonest symptom was fatigue or generalized weakness (62.9%) followed by bleeding manifestation (54.3%) and fever (48.6%). Among 35 patients 15 patients had bleeding manifestation and commonest bleeding site was menorrhagia in females (33.3%) followed by GI tract bleeding (26.7%). In majority of our patients the organism causing infection cannot be detected (60%) while among the organism which were detected staphylococcus aureus (8.6%) was the commonest organism isolated.

The commonest finding in bone marrow aspirate was hypocellular marrow (51.4%) followed by hypocellular marrow with increased iron stores (28.6%) where as commonest finding in bone marrow biopsy was also hypocellular marrow (68.6%).

Baseline laboratory parameters with there mean value is shown in table 1.

Table-1: Baseline Laboratory parameters distribution

	Mean	SD	Median
Hb	6.21	1.68	6.20
Reticulocyte	.98	.81	.63
Platelet count	22.06	23.70	14.00
WBC Count	1.97	.88	2.02
Neutrophil	25.25	21.27	21.00
Lymphocyte	62.23	25.63	67.30
Monocyte	3.35	2.36	3.00
ANC	.52	.51	.44
ARC	.41	.39	.26
LDH	199.94	194.04	160.00
Creatinine	.85	.31	.80
Total Bilirubin	1.22	1.77	.88
Direct Bilirubin	.54	.74	.30
Total Protein	6.38	.58	6.40
Albumin	3.46	.57	3.60
SGOT	45.89	101.38	21.00
SGPT	48.17	100.89	26.00
ALTP	89.26	51.08	84.00

Our study population was divided into 3 groups as per severity: Non severe, Severe and Very Severe. The numbers being 9 (25.7%) for Non Severe, 13 (37.6%) each for Severe and Very Severe (Figure-1). HAMS test and PNH clone were positive in 3 patients. Cytogenetics study among 35 patients showed 1 (2.9%) to be having CD20+, 2(5.8%) type 2 PNH clone and 1 (2.9%) having type 3 PNH clone.

Fig-1: Severity of Aplastic Anemia distribution

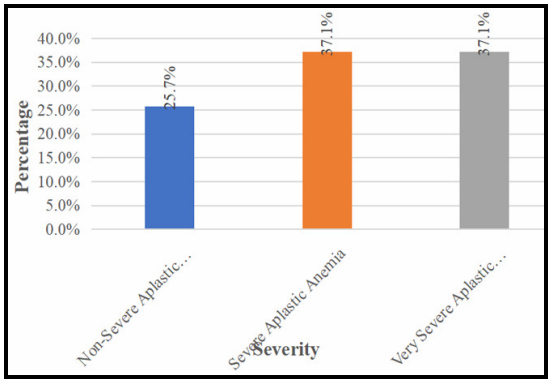

Table 2 shows laboratory parameters comparison with respect to severity.

Table-2: Laboratory parameters comparison with respect to severity										
	Severity									P value
	Non-Severe AA			Severe AA			Very Severe AA			
	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	
Hb	7.59	1.68	7.70	5.41	1.59	6.00	6.06	1.19	6.20	0.007*
Reticulocyte	1.03	.87	.94	1.23	.87	.90	.68	.67	.35	0.225
Platelet count	46.78	34.36	46.00	11.46	8.31	11.00	15.54	10.45	13.00	<0.001*
WBC Count	2.29	.95	2.25	2.25	.87	2.41	1.47	.62	1.41	0.028*
Neutrophil	41.59	20.78	46.00	27.06	16.50	24.00	12.12	18.21	5.00	0.003*
Lymphocyte	52.54	22.83	50.00	67.55	17.82	67.80	63.63	33.06	84.00	0.402
Monocyte	4.29	3.02	4.00	3.66	1.59	4.00	2.39	2.32	1.70	0.150
ANC	.90	.61	.75	.63	.41	.52	.14	.19	.07	0.001*
ARC	.55	.52	.44	.46	.38	.27	.25	.22	.15	0.175
LDH	179.89	75.50	160.00	162.15	23.77	156.00	251.62	312.36	170.00	0.483
Creatinine	.92	.48	.85	.87	.21	.80	.77	.25	.66	0.540
Total Bilirubin	.61	.38	.52	1.64	2.56	1.09	1.23	1.32	.88	0.415
Direct Bilirubin	.23	.13	.17	.52	.58	.36	.78	1.04	.44	0.229
Total Protein	6.18	.55	6.30	6.53	.66	6.60	6.36	.50	6.40	0.388
Albumin	3.32	.55	3.40	3.58	.72	3.80	3.45	.42	3.60	0.568
SGOT	18.56	5.05	17.00	41.31	69.95	23.00	69.38	151.76	27.00	0.516
SGPT	26.00	12.51	22.00	31.69	34.13	24.00	80.00	160.66	32.00	0.365
ALTP	81.56	20.78	84.00	83.00	33.09	84.00	100.85	76.02	79.00	0.600

Of the 35 patients, 32 (91.5%) were started on Cyclosporine. Of the 32, 10 (28.6%) were in combination with ATG. The remaining 3 patients were on Danazol, Stanazol and Wysolone-Romiplostim combination each (2.9%) as first line of treatment. 12 patients received 2nd line of treatment. Of which, 8 (66.7%) received Danazol and 2 (16.7%) received Revolade and 2(16.7%) received Stanazol. Table 3 and 4 shows response, side effects and cause of death after first and second line of treatment.

Table-3: Response, Side effects and Cause of Death after Dose 1			
		Count	%
Response	Expired	2	5.7%
	Complete Response	4	11.4%
	Partial Response	19	54.3%
	Transfusion Dependent	10	28.6%
Side effects	Nil	27	77.1%
	Fever	1	2.9%
	Hypertension	1	2.9%
	Nausea	3	8.6%
	Nephrotoxicity	1	2.9%
Cause of Death	Nil	33	94.3%
	Hemorrhagic Shock	1	2.9%
	Urosepsis	1	2.9%

Table-4: Response, Side effects and Cause of Death; after Dose 2			
		Count	%
Response	Transfusion dependent	3	25.0%
	Partial remission	9	75.0%
Side effect of Rx	Nil	7	58.3%
	Acne	2	16.7%
	Sweating, Flushes	1	8.3%
	Transaminitis	1	8.3%
	Vulval dryness and itch	1	8.3%
Death	Nil	0	0%

Complete responses were mostly seen in NSAA patients with only 1 SAA patient attaining complete response after 1st Line All four of them took ATG and cyclosporine treatment. Partial responses were attained almost in equal proportions (regardless of severity). Both the patients who expired were Severe and Very Severe respectively.

Discussion

Though mostly considered as immune-mediated disease, many host and environmental factors have been found to be associated with AA. Therefore, the epidemiology of AA has been an area of active research. This is a study done in a tertiary care centre in south India. Studies from Western populations show bimodal age distribution among AA patients with peaks at 15–29 years and above 60 years [10] while in Asian populations the disease tends to show an unimodal distribution with the highest incidence between 15 and 24 years [11].

In our cohort of 35 patients, ages ranging from 18-65 years, the mean age of subjects was 42.74 ± 16.008 years. With maximum population in 21-30 and 51-60 age groups, showing a Bimodal peak, i.e. comparable to the meta analysis done in Barcelona [4]. Conflicting data were seen on sex distribution of AA in Asian population. Some showed equal distribution and others showed a rather gross male preponderance [12]. In our population, male preponderance was seen at the ratio of 1.33 : 1.

The most common symptom symptom was fatigue or generalized weakness (62.9%) followed by bleeding manifestation (54.3%) and fever (48.6%) in our study. A similar result was drawn by a study [13] in Texas which showed that patients with MDS, AA, and PNH have severe levels of fatigue with decreased Quality of Life. Our study population had significant past history of Autoimmune diseases in 3 (8.6%), Viral serology positivity in 2 (5.7%) (HIV & HBsAg each) and Drug intake (heavy metals in the form of non allopathic medication) in 1 (2.9%).

Among the patients who had diagnosed infections, most common was Lungs (Pneumonia) in 4 (11.4%), followed by Urinary tract infection in 3 (8.6%) which similar to study done by Shano naseem et al [14] where pneumonia was commonest infection. In Hematological lab parameters, the median of all three cell lineage was low with Hb 6.2g/dl, TLC 2.02 (10^9/l) and platelet counts 14(10^9/l) which is similar to study done by Das et al [15]. Cytogenetic analysis among 35 patients showed normal karotyping in 31 patients (88.6%), 1 (2.9%) showed CD20+ , 2 (5.8%) type 2 PNH clone and 1 (2.9%) having type 3 PNH clonen which is higher as compared to the study of Das et al. and Gupta et al [15-16].

Our study population was divided into 3 groups as per severity: Non severe, Severe and Very Severe. The numbers being 9 (25.7%) for Non Severe, 13 (37.6%) each for Severe and Very Severe which is similar to study done by Jha SC et al [17]. Of the 35 patients 32 (91.5%) were started on Cyclosporine. Of the 32 , 10 (28.6%) were in combination with ATG. The remaining 3 patients were on Danazol, Stanazol and Wysolone-Romiplostim combination each (2.9%).12 patients received 2nd line of treatment. Of which, 8 (66.7%) received Danazol and 2 (16.7%) received Revolade and 2(16.7%) received Stanazol.

In our study none of the patient underwent bone marrow transplantation because of financial constraints. There was statastically significant improvement in mean Hb value, mean total count and mean ANC (p values <0.001) in patients taking both 1st and 2nd line of treatment whereas there was no significant improvement in the mean Platelet count after the 1st line of treatment. But post 2nd line, there was significant improvement of mean Platelet count with p value <0.001. Of the 35 who received 1st Line treatment, only 4 (11.4%) showed Complete Response, 19 (54.3%) showed Partial Remission, 10 were transfusion dependent (28.6%) and 2 (5.8%) expired.

The deaths were due to Hemorrhagic Shock and Urosepsis respectively. Complete responses were mostly seen in NSAA patients with only 1 SAA patient attaining complete response after 1st Line. Partial responses were attained almost in equal proportions (regardless of severity). Both the patients who expired were Severe and Very Severe respectively. All Transfusion dependent patients were of Severe (SAA & VSAA) category.

Conclusion

In view of variability in clinicopathological presentation of AA in different populations and scarcity of studies from Asia, we

conducted this Cross sectional Descriptive Study of 35 AA patients. Their clinicopathological correlates were analysed and inferences were made. There was significant male preponderance, which may also be due to bias in the health seeking behaviour in our population. The pattern of presentation guides us to keep a lower threshold for Bone marrow evaluation in patients presenting with Pancytopenia without Hepatosplenomegaly. There was significant correlation in outcome of treatment and prognosis to the initial severity of AA on initial presentation. ATG and Cyclosporine showed significant improvement in most parameters. Many patients in the study were unwilling for ATG and bone marrow transplantation due to the cost factor. Many are still requiring frequent transfusions, thus probing us to search for newer novel forms of treatment to reduce the cost of treatment and mortality in aplastic anemia patients.

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