



Estimation of CD34+ in Patients with Hypocellular Marrow and Treatment Response in Aplastic Anemia

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Abstract

Introduction: Hypoplastic myelodysplastic syndrome (hMDS) is morphologically difficult to be distinguished from acquired aplastic anaemia (AA). Response to treatment of AA varies based on the treatment used and duration of follow up. we conducted a prospective study to estimate bone marrow CD 34 cells on hypoplastic marrows (AA and hMDS) and evaluated the haematologic response to treatment in aplastic anaemia.

Methods: We conducted a prospective study in patients with hypocellular BM due to either acquired AA or hMDS. The CD34+cells in the bone marrow were estimated by immunohistochemistry (IHC) prior to treatment. We assessed treatment response to immunosuppressive therapy in patients with AA.

Results: We followed up 53 patients with AA for a minimum of 3 months. Out of 53 patients with AA, 34 (64%) had low CD34+ level, 17 (32%) had normal and 2 (4%) had high CD34+ levels. 34 (64%) patients were on cyclosporine, 14 (26%) patients were on androgens, 3(5.6%) patients received ATG regime and 2(4.4%) patients received blood and blood products alone. At the end of 3 months, 20 (39%) patients showed partial response while the remaining 31 (61%) patients were nonresponders, irrespective of the treatment category.

Conclusion: In conclusion, majority of the patients with AA had low CD34 cells at baseline in our study. The response to treatment varies widely based on the type of treatment. There was no difference in treatment response, irrespective of treatment category based on the CD34 cells at baseline among patients with low and normal CD 34 cells.

Keywords: Aplastic anemia, hypocellular myelodysplastic syndrome (hMDS), CD 34+, immunosuppressive therapy, treatment response.

Introduction

Many medical disorders present with pancytopenia ranging from acute febrile illnesses,

nutritional deficiencies to bone marrow failure syndromes. Bone marrow (BM) study is indicated in many of the above conditions which may reveal

a hypocellular marrow. The causes of pancytopenia with hypocellular marrow include acquired aplastic anaemia (AA), constitutional aplastic anemia, hypoplastic myelodysplastic syndrome (hMDS), aleukemic leukemia, acute lymphoid leukemia, lymphomas of bone marrow, paroxysmal nocturnal hemoglobinuria, hypocellular myelogenous leukemia, tuberculosis (TB), histoplasmosis, HIVinfection, Epstein Barr virus infection etc.¹

Many of the above conditions can be easily distinguished by detailed clinical and specific laboratory tests. But, hMDS is morphologically difficult to be distinguished from acquired AA. The presence of significant bilineage or trilineage dysplasia or the presence of karyotypic abnormalities may indicate a diagnosis of MDS. The often low cellular yield of BM aspirates (BMA) can negatively affect the detection of dysplastic changes and the quality of marrow cytogenetic analyses.² In addition, it takes several days to obtain and report the findings of a chromosomal study.³ Marrow cellularity in AA can be quite patchy, making it an unreliable discriminator.⁴ Even clonal cytogenetic abnormalities, usually considered an exclusion criterion for AA, have not precluded this diagnosis at some centers.^{5,6}

MDS also evolves out of AA in about 10–20% of patients.^{7,8}

A stem-cell CD34 phenotype is presently considered to be one of the best markers of progenitor cells, including B and T lymphoid progenitor cells, and myeloid precursors. CD34 expression is normally confined to 0.1-0.5% of nucleated cells in the peripheral blood (PB) and to 0.8-5% of mononuclear cells in adult BM.⁹

CD34 is expressed in hematopoietic progenitor cells and is fundamental to the pathophysiological mechanisms of both hMDS and AA. MDS clonal expansion emanates from a CD34+ stem cell, and in AA the CD34+ stem cells are the target of an autoimmune attack. Accordingly, the percentage of CD34+ cells is usually <0.3% in AA but is either normal or elevated in hMDS.¹⁰

Quantification of marrow CD34+ cells may serve as an important tool for distinguishing between aplastic anemia and hypoplastic myelodysplastic syndrome as shown in few studies.^{11,12} In a study by Matsui et al, patients with a normal or increased percentage of CD34+ cells were ultimately diagnosed with hypoplastic MDS based on the detection of clonal cytogenetic abnormalities or progression to refractory anemia with excess blasts/acute myeloid leukemia. Patients with low marrow CD34+ cell numbers met standard clinical criteria for aplastic anemia and did not demonstrate neoplastic transformation with follow up.¹⁰

There is paucity of data on estimation of CD34+ cells among patients with bone marrow hypoplasia in India. Hence, we conducted a prospective study to estimate bone marrow CD 34 cells on hypoplastic marrows and evaluated the haematologic response to treatment in aplastic anaemia.

Objectives

1. To qualitatively estimate CD34+ cells in patients with bone marrow hypoplasia (aplastic anemia and hypoplastic myelodysplastic syndrome)
2. To assess hematological response in patients with aplastic anemia.

Materials and Methods

We conducted a prospective study in patients with hypocellular BM. We included adult in-patients from medical wards of a tertiary care hospital, who had hypocellular bone marrow due to either acquired AA or hMDS in the study. The study was approved by the institutional ethics committee.

The diagnosis of AA was based on the presence of pancytopenia with a neutrophil count fewer than 1500/ μ L (1.5×10^9 /L), a platelet count fewer than 50,000/ μ L (50×10^9 /L), a hemoglobin concentration less than 10 g/dL (100 g/L), and an absolute reticulocyte count fewer than 40,000/ μ L (40×10^9 /L), accompanied by a hypocellular

marrow without abnormal or malignant cells or fibrosis.¹³ MDS is commonly characterized by progressive bone marrow failure, with several of the subtypes often progressing to AML. Not unexpectedly, the more “high-grade” MDS categories that demonstrate extensive bone marrow failure, such as refractory cytopenia with multilineage dysplasia and refractory anemia with excess blasts, more commonly present with pancytopenia.¹⁴

Further, patients with aplastic anaemia were classified into non severe, severe and very severe aplastic anemia based on hypocellular BM, and 2/3 of the following: hemoglobin, neutrophil count, reticulocyte concentration and platelet count.¹⁵ We excluded patients from further follow up and analysis if they had an alternative diagnosis for hypocellular marrow. All patients diagnosed with either hMDS or AA underwent baseline BM CD 34+ estimation. We followed patients with AA for a minimum of 3 months and assessed the treatment response to immunosuppressive therapy. We assessed treatment response by the measurement of blood counts and transfusion requirements and classified patients as non-responders, partial and complete responders as per Camitta 2000 criteria.¹⁶

The CD34+cells in the bone marrow were quantified by immunohistochemistry (IHC) using reagent: Dako FLEXTM Monoclonal Mouse Anti-Human CD34 Class II, which is a ready-to-use monoclonal mouse antibody provided in liquid form in a buffer containing stabilizing protein and 0.015 mol/L NaN₃. Clone: QBEnd 10. Isotype: IgG1, kappa. The normal levels of CD 34 + in the bone marrow are 0.8-5%.⁹ CD 34 + values below 0.8% were considered low and more the 5% high. The CD 34 % were qualitatively reported as low, normal or high by the pathologist based on the discretion used, depending on the percentage marker on IHC found in bone marrows.

The immunosuppressants included either stanazolol (20 mg/day) or cyclosporine (300 mg/day) or anti-thymocyte globulin (ATG) regime which included (ATG [40mg/kg/day for 4 days] +

cyclosporine [6mg/kg/day for 90 days] + Danazolol [5mg/kg/day for 180 days]+ intravenous methylprednisolone [2mg/kg/day for 7 days, then tapererd and stopped over 30 days]).

Statistical analysis

Statistical analysis was performed using SPSS 18.0 for Windows. Descriptive statistics were analyzed using percentage, frequencies, mean with standard deviation. Student t test was used to find out the difference in means between continuous variables. Chi-square test was applied to find out the significance between response (non-responders and partial responders) and baseline CD34. A p-value of 0.05 was considered significant.

Results

We analysed 61 patients with aplastic anaemia and hMDS who underwent BM CD34 estimation at diagnosis. The mean age of the patients was 47.30 (SD 16.88) years. Of the 61 patients studied, 33 (54.1%) patients were males and 28 (45.9%) patients were females. The presenting symptoms in our study included bleeding in 22 patients (42%), symptoms of anemia in 17 patients (32%) and fever in 14 patients (26%).

We followed up 53 patients with AA for a minimum of three months (Fig 1). Among the 53 patients, 25 (47%) patients were females and 28 (53%) were males, the ratio of male: female was 1.1:1. The mean age of the patients was 39.8 (SD 16.1) years. No significant difference in age was noted between male and females ($p = 0.445$).

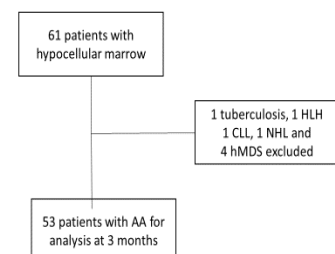


Fig 1: Study flow chart

Out of 53 patients with AA, 34 (64%) had low CD34+ level, 17 (32%) had normal and 2 (4%) had high CD34+ levels. Out of 4 patients with hMDS, 2 (50%) had low and 2 (50%) had high CD34+ levels (table 1).

Table 1: CD 34+ levels in AA and hMDS

CD 34+	Normal	Low	High	Total
AA patients	17	34	2	53
hMDS patients	0	2	2	4

AA Aplastic anemia, hMDS hypoplastic myelodysplastic syndrome

All the 53(100%) patients were followed up for a minimum of 3 months. Out of these, 13 (24%) patients had 6 months of follow up, 4 (7.5%) patients had 1 year and 1(1.8%) patient had 2 yrs of follow up. Out of the 53 patients, presence of a small clone of CD55/59 deficient population (AA + PNH) was detected in 3(6%) patients at diagnosis. Thirty three (63%) patients had non severe AA, 18 (34%) had severe AA, thus comprising 97% of acquired idiopathic AA. Only 2 (3%) patients fell into the very severe AA category.

Treatment Modalities and severity of AA

Out of the 53 patients with AA, 34 (64%) patients were on cyclosporine, 14 (26%) patients were on androgens, 3(5.6%) patients received ATG regime and 2(4.4%) patients received blood and blood products alone (table 2).

Table 2: Treatment modality and severity of disease

Treatment	Severity of disease		
	Non severe AA	Severe AA	Very severe AA
Transfusion	1	0	1
Androgen	8	4	2
Cyclosporine	20	10	4
ATG regime	1	2	0

At the end of 3 months, 20 (39%) patients showed partial response while the remaining 31 (61%) patients were nonresponders, irrespective of the treatment category. Based on the treatment category, out of the 34 patients who received cyclosporine, 13 (46%) patients showed partial response and 21(54%) showed no response to treatment. Out of the 14 patients who received

androgen, 4(29%) patients showed partial response and 10 (71%) showed no response to treatment. Out of the 3 patients who received ATG regime, all 3(100%) patients showed partial response to treatment (Table 3). There was no patient with complete response. 2 Patients who were treated with only blood transfusion were excluded from assessing the response to treatment.

Table 3: Treatment category and response

Treatment category	Partial response	No response
Cyclosporine	13(46%)	21(54%)
Androgen	4(29%)	10(71%)
ATG regime	3(100%)	0

We evaluated the treatment outcome by individual treatment category and baseline CD34 percentage. 34 patients received cyclosporine monotherapy. Out of the 21 patients who were non responders to cyclosporine, 14 had low CD34% and 5 had normal CD34%. Among 13 patients who were partial responders to cyclosporine, 9 had lowCD34% and 4 had normal CD34%.

14 patients received androgen monotherapy. Out of the 10 patients who were non responders to androgen, 7 had low CD34% and 3 had normal CD34%. Among 4 patients who were partial responders to androgen, 2 had low CD34% and 2 had normal CD34%. 3 patients received ATG, all 3 were partial responders, 2 of them had normal CD34% and 1 had low CD34%. Treatment outcome based on baseline CD34+, irrespective of treatment category is shown in table 4.

Table 4: Baseline CD 34 and treatment response

CD34 level	No Response	Partial Response	Total
Normal	08	08	16
Low	21	12	33
High	02	0	02
Total	32	20	51

There was no significant difference in response to treatment based on the baseline CD34 level, p=0.82, after excluding 2 patients with high CD 34 cells as both of them showed no response to treatment at 3 months.

Discussion

We conducted a single institution cross-sectional study to estimate BM CD34% in 53 patients

diagnosed to have aplastic anemia at baseline and assessed treatment response to immunosuppressants at 3 months with reference to their baseline CD34%. To the best of our knowledge this is the first study to assess response to treatment based on pre-treatment BM cd34 cells at baseline.

In our study, majority of the patients with AA had low BM CD34 cell. This is supported by other studies which report that CD34 cells are significantly decreased in bone marrow of patients with AA.^{17,18}

Normal CD34 levels may be explained by a relatively low cellular area of bone marrow (being evaluated for CD 34) early in the disease among severe AA or a non-severe AA. As MDS cytogenetics were not done on all hypocellular bone marrows, the 2 patients with AA but high CD34 levels probably were hMDS.

Hematopoietic stem-cell transplantation (HSCT) or bone marrow transplantation (BMT) is the treatment of choice for young patients who have a matched sibling donor. Immunosuppression with either anti-thymocyte globulin and cyclosporine or high-dose cyclophosphamide is an effective therapy for patients with SAA who are not suitable BMT candidates owing to age or lack of a suitable donor.¹⁹ Anabolic steroids have also been used to treat AA in those with no access to HSCT or ATG + Cyclosporine.²⁰ with limited data on response for non SAA, but there is no practical and reliable method to predict response to treatment. The immunosuppressants used in our study included standard doses of either stanazolol, cyclosporine or ATG regime. In each treatment category, we had more patients with non severe AA than severe and very severe AA. Our decision on the choice of immunosuppressants was based on the affordability of patients. One third of the patients who received stanazolol were partial-responders. Almost half of the patients who received cyclosporine and all patients who received ATG regime were partial responders. Complete hematological response was not noted in our patient population at 3 months. The

plausible explanation maybe that few patients presented late in their illness and few others may be late responders. The hematopoietic response rate in SAA after ATG/CsA is 60–70% and the probability of survival at 5 years ranges from 60 to 85%.²¹

There is only one prospective randomized trial of CsA alone or the combination of ATG/CsA treatments in patients with NSAA. In this study the end point was the hematologic response at 6 months. A significantly higher overall response rate of 74% was found in the ATG and CsA group, with 57% complete and 17% partial responders ($p=0.02$).²²

Two thirds of our patients (64%) with AA had low CD34 % on BM, and two thirds of these were partial responders at 3 months. One third of our patients had normal CD34 % and half of them were partial responders. There was no significant difference in response to treatment based on the BM CD 34 cells at baseline, irrespective of the treatment category.

The limitations of the study include non-availability of cytogenetic analysis on all hypocellular BM which would have helped in identifying hMDS from the AA population. The minimum follow up of all the patients was for 3 months, which may be short a period to look for late responders.

In conclusion, majority of the patients with AA had low CD34 cells at baseline in our study. The response to treatment varies widely based on the type of treatment. We did not have patients with complete responders at the end of 3 months. There was no difference in treatment response, irrespective of treatment category based on the CD34 cells at baseline among patients with low and normal CD 34 cells.

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