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Diagnostic Utility of Bone Marrow Sampling and Profile of Hematological Abnormalities in Indian HIV-infected Individuals

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ABSTRACT

Background: Hematological abnormalities are a common complication of HIV infection and occur in all stages of the infection. These abnormalities increase as the disease advances. We aimed to evaluate the diagnostic utility of bone marrow sampling in HIV-positive patients.
Methods: 40 HIV-infected individuals were screened for hematological abnormalities. Investigations such as iron studies, hematological work-up, bone marrow evaluation and coagulation profile were performed.
Results: The most common single hematological abnormality was anemia, seen in 8 (20%) patients. However, anemia was seen as a subset of pancytopenia in 21 (52.5%) patients. Microcytic hypochromic anemia was present in 12 (30%) cases while anemia of chronic disease (normocytic normochromic anemia) occurred in 4 (10%) cases. Macrocytic anemia was observed in 32.5% (n=13) cases. Leucopenia and thrombocytopenia was seen in 21 (52.5%) patients as a subset of pancytopenia. However, they were not present as a single hematological abnormality. 45% of the patients showed hypercellular marrow whereas normocellular marrow was seen in 35% (n=14) and hypocellular marrow in 15% (n=6) of the patients. Myelodysplasia was found in 10 (25%) patients. It was commonest in erythroid (12.5%) followed by myeloid series (10%) and megakaryocytic series (2.5%). Tuberculosis was seen in 10% of the cases and gelatinous transformation was seen in 1 (2.5%) case.
Conclusion: Bone marrow sampling has diagnostic utility in HIV-infected patients. Morphological examination in HIV-positive patients plays a distinctive role in ruling out the presence of opportunistic infections.

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Introduction

Hematological abnormalities of all lineages of blood cells are among the most common complications of HIV.[1] These abnormalities are found in all stages of HIV disease and increase in frequency as the disease progresses.[2] Increased severity is seen in late-stage AIDS patients with high viremia. Thus they seem to be dependent on the level of virus replication.[3] Diverse factors responsible for impaired hematopoiesis in HIV infection include suppression of bone marrow by the

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virus or by viral proteins, immune dysregulation, actual infection of the bone marrow progenitor cells by HIV, and alteration of stromal cell element.[4] Irrespective of the cause, the patients usually present with either anemia, thrombocytopenia, leukopenia or with pancytopenia.[5]

The most common hematological abnormality is anemia which is associated with the progression of the disease. Neutropenia is common in the advanced stages of AIDS and often caused or exacerbated by concomitant myelosuppressive drugs. Adverse drug reactions and their complications can cause neutropenia in patients with HIV/AIDS.[3] Thrombocytopenia is correlated with low CD4 cell count and older age.[6-8] A number of characteristic but nonspecific, morphological abnormalities of the bone marrow of AIDS patients have been reported.[9] Bone marrow examination may be useful for definitive assessment of iron stores which can assist in the differentiation of iron-deficiency anemia from anemia of chronic disease.[3]

We systematically assessed the morphology of 40 bone marrow aspirations from patients with serologically established HIV infection. We present our experience with these 40 cases along with their salient hematological features.

Materials and Methods

The present study was done in a large tertiary care hospital of Mumbai, India, from June 2015 to June 2016. The study population included 40 HIV-positive, symptomatic patients with the aim of recognizing the morphological findings sufficiently characteristic of HIV infection. 40 bone marrow smears and trephine biopsies were examined.

Anemia was defined as hemoglobin <13 g/dl (in men) and <12 g/dl (in women). Leukopenia was defined as WBC count less than 4000 cells/µl. Thrombocytopenia was defined as total platelet count $<150\times10^3$/µl.[3] Pancytopenia was a decrease in all cell lines showing anemia, leukopenia and thrombocytopenia.

Bone marrow examination was performed for investigation of anemia, leukopenia, thrombocytopenia or pyrexia of unknown origin. Posterior superior iliac crest was used as the site for bone marrow aspiration and biopsy. All marrow aspirates were stained routinely with Leishman's stain and trephine samples with hematoxylin and eosin, reticulin, Giemsa, Ziehl-Neelsen and other special stains as appropriate. Bone marrow samples were carefully evaluated for cellularity, differential counts, dysplastic changes, fibrosis, granulomas and organisms.

Inclusion criteria were HIV positive patients who had anemia, leukopenia, thrombocytopenia or pyrexia of unknown origin. Exclusion criteria included patients who had chronic liver or renal disease other than explained by HIV or primary hematological abnormalities. Statistical analysis for this study was done using GraphPad statistical software.

Results

We evaluated 40 HIV-positive patients for hematological abnormalities. Age group of the patients ranged from 31 to 40 years. Male to female ratio was 2.3:1 and 12 (30%) patients were women and 28 (70%) were men.

The most common single hematological abnormality was anemia seen in 20% of the patients. However, anemia was seen as a subset of pancytopenia in 52.5% of the patients. Microcytic hypochromic anemia was present in 30% of the cases while anemia of chronic disease (normocytic normochromic anemia) occurred in 10%. Macrocytic anemia was observed in 32.5% of cases. Leukopenia and thrombocytopenia were seen in 52.5% of patients as a subset of pancytopenia. However, they were not seen as a single hematological abnormality (table 1).

Table 1: Cytopenias on peripheral smear

A. Anemia	
Single abnormality	20% (n=8)
Subset of pancytopenia	52.5% (n=21)
B. Type of anemia	
Microcytic hypochromic anemia	30% (n=12)
Macrocytic anemia	32.5% (n=13)
Normocytic normochromic anemia	10% (n=4)
Total cases with anemia	72.5 % (n=29)
C. Leukopenia	52.5 % (n=21)
D. Thrombocytopenia	52.5 % (n=21)

Bone Marrow Changes

The following parameters were evaluated on bone marrow: 1) cellularity; 2) myeloid-erythroid (M:E) ratio; 3) morphology of hematopoietic lineages; 4) plasma cells; 5) lymphocytes; 6) others (table 2).

Table 2: Bone marrow changes

A. Cellularity	
Hypercellular	45% (n=18)
Hypocellular	15% (n=6)
Normocellular	35% (n=14)
B. Hyperplasia	
Myeloid	10% (n=4)
Erythroid	45.5% (n=17)
C. Erythroid maturation	
Normoblastic	50 % (n=20)
Micronormoblastic	20 % (n=8)
Megaloblastic	25% (n=10)
D. Megakaryocytes	
Increased	-
Decreased	2.5 % (n=1)
E. Dysplastic changes	
Myeloid	10% (n=4)
Erythroid	12.5% (n=5)
Megakaryocytic	2.5% (n=1)
F. Plasma cells	37.5% (n=15)
G. Lymphocytes	30% (n=12)
H. Granulomas	10% (n=4)

Dysplasia was found in 25% of patients in all series. It was the most common abnormality in erythroid series (12.5%), followed by myeloid (10%) and megakaryocytic dysplasia in 2.5% of the patients. The most significant dysplastic changes in myeloid series were cytoplasmic vacuolation, nuclear dysmorphism and giant

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metamyelocytes. Erythroid series showed basophilic stippling and megaloblastoid changes and megakaryocytic series showed some degrees of hypolobulation. Out of 10 patients showing dysplasia, 6 (15%) had anemia, 3 (7.5%) had leukopenia and 1 (2.5%) had thrombocytopenia.

Plasmacytosis and decrease in number of lymphocytes was seen in 15 (37.5%) and 12 (30%) cases, respectively.

Tuberculosis is known to be associated with HIV and was the only co-infection we encountered. Amongst the 40 cases studied, 10 patients were clinically diagnosed with pulmonary tuberculosis and 4 with abdominal tuberculosis who were taking anti-tuberculosis medication. Epithelioid granulomas as an indicative of tuberculous infection of the bone marrow were observed in 4 of the 10 cases studied.

Gelatinous transformation was seen in one patient. Out of the 6 cases showing hypocellular marrow, 2 cases showed fibrosis.

As shown in table 3, out of 40 patients with HIV, there were 29 patients with anemia in which 21 patients were showing pancytopenia on peripheral smear while 8 patients were found to have anemia as a single hematological abnormality. It was found that there were 17 patients showing erythroid hyperplasia and 12 were without erythroid hyperplasia. In the 17 patients showing erythroid hyperplasia, 12 had pancytopenia while 5 had only anemia. These findings when compared by two by two contingency table were statistically insignificant (P>0.05). But this result is mainly because of small sample size and less patients of anemia as a single hematological abnormality in the study population (in which one of the text box is having value less than 5).

Discussion

HIV/AIDS have been implicated in causing a spectrum of hematological abnormalities and morphological changes in the bone marrow.[2,10] These are possibly due to either direct effect of HIV, nutritional deficiencies, opportunistic infections or bone marrow suppression by antiretroviral therapy and other drugs used in the treatment of HIV infection.[11] 90% of patients are reported to have bone marrow abnormalities during the course of disease in the form of increased cellularity, dysplasia or granulomatous involvement.[12]

Low blood counts are common in patients with HIV,[10] especially in advanced stage[l] with anemia, leukopenia and thrombocytopenia each developing in more than half of infected patients,[10] causing symptoms and contributing to complications such as severe infections and bleeding.[2]

In the present study, the most common single hematological abnormality was anemia, seen in 8 patients. However, anemia was seen as a subset of pancytopenia in 52.5% of the patients. This was iconsistent with a previous study,[3] where anemia was the most common presentation. Microcytic hypochromic anemia was seen in 12 cases in present study which correlated with another study,[10] where microcytic anemia was seen in 20% of their patients, commonly in women. Tripathi and colleagues reported microcytic anemia in 6.56% of the cases,[2] whereas Dikshit and colleagues reported iron deficiency anemia in 49.2% of cases.[3] Different studies reported anemia of chronic disease ranging from 50.8% to 88.52%,[2,3,10] which was in agreement with other reports in the literature.[13-15] In our study, anemia of chronic disease was seen in 10% of the cases. This might possibly be due to small sample sizes. Probable etiologies of anemia of chronic disease could be chronic disorders such as recurrent pneumonia, tuberculosis and opportunistic infections involving the bone marrow[10] or myelodysplastic changes.[16] In present study, macrocytic anemia was seen in 13 patients. It was reported in 4.9% of patients who were all on anti-retroviral therapy.[2]

Leukopenia and thrombocytopenia were seen in 52.5% of patients as a subset of pancytopenia in the present study. Various studies have reported leukopenia in 12%[10] and 7.27 %[2] of AIDS patients and in 20%[10] and 5.26%[2] of patients without AIDS. This difference can be attributed to the difference in the size of the study groups. Thrombocytopenia is known to be a frequent complication of HIV infection.[1,6] Prevalence of thrombocytopenia in patients with HIV/AIDS has been variably reported in 13-61% of cases.[15,17]

Pancytopenia was observed in 23%[14] of HIV-positive patients. Mir and co-workers,[18] in a cohort of 60 HIV-infected individuals reported anemia, thrombocytopenia, leukopenia and various combinations of these in most individuals. In our study, pancytopenia was seen in 52.5% of cases, which was in concordance with the above studies. The most common cause of pancytopenia is reported to be megaloblastic anemia (44%).[5] This increased figure of megaloblastic anemia might be correlated with the high prevalence of nutritional anemia in our country. This group of patients responded very well to appropriate therapy. The second major cause of pancytopenia was aplastic/hypoplastic anemia. However, we did not encounter any case of aplastic/hypoplastic anemia in our study.

Hypercellularity, increase in number and morphological alterations of megakaryocytes, raised reticulin content, mild plasmacytosis and frequent presence of reactive lymphoid aggregates are features suggestive, though not diagnostic, for HIV infection on bone marrow biopsy.[19]

Marrow from HIV-infected patients is sometimes difficult to aspirate and the trails are of decreased

Table 3: Relation between presence and absence of erythroid hyperplasia on bone marrow aspiration with anemia on peripheral smear in the study population

Erythroid hyperplasia	Pancytopenia	Anemia only	Total	P value
Present	12	5	17	1.00 (NS)
Absent	9	3	12	
Total	21	8	29	

NS: Not significant; P<0.05 considered to be significant; Fisher's exact test applied

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cellularity. True marrow cellularity is better appreciated on trephine biopsy which is reported to be hypercellular in the majority of the patients.[20] Previous reports indicate that most of the cases with HIV have hypercellular bone marrow.[12] However, Tripathi et al.[2] revealed bone marrow to be normocellular in 75.68%, hypocellular in 6.75% and hypercellular in 17.57% patients. The difference is difficult to explain but it is likely to be due to different cohort of patients included in various studies. Majority (n=72.9%) of patients observed by Tripathi et al[2] had full blown AIDS where bone marrow could likely be normocellular or hypocellular rather than hypercellular which is predominant in early stages of the disease.[21] In a study by Rudresh et al[10], the majority of HIV infected patients had a normocellular bone marrow. In the present study, majority of the patients had a hypercellular marrow (45%), whereas 35 % showed normocellular and 15% showed hypocellular marrow. It should be claimed that mixed reports are available on this finding, as some studies concur while others disagree with our data.[13,21-23] A hypercellular marrow may be seen in early stages of the disease (HIV infection), but it is more likely to be normocellular or hypocellular in advanced disease (AIDS).

Various studies have reported myelodysplastic changes to be a common feature in AIDS patients.[22] These changes in bone marrow were found in 32.43% of cases[2] as compared to 50-90 % reported in literature.[12] Cells most commonly showing dysplasia in the study of Tripathi et al[2] were granulocytes (27.03%) followed by erythroid in 4.05% and megakaryocytes in 1.35% of cases. Some authors have previously reported erythroid dysplasia to be the commonest type of dysplasia; whereas others reported granulocytic series the most affected.[12] These changes can explain normocytic or macrocytic anemia which is common in patients with AIDS.[2] Myelodysplasia in early stages of HIV infection may not be reflected in peripheral blood smear and thus may remain undiagnosed. This also suggests that prevalence of myelodysplasia in HIV infection may be much higher than what is reported. This is evident from one study[2] where 7.69% of patients showing erythroid dysplasia did not have anemia. Dysplasia seems to be an important factor in HIV-induced leukopenia, while it may not have a major impact in causing anemia and thrombocytopenia. Higher incidence of dysplasia in advanced stages of the disease is probably due to increased HIV-RNA load, cytokine mediated effects, drug related changes and also indirect effects of infections.[2]

In the present study, myelodysplasia was found in 25% of patients. It was commonest in erythroid series [12.5% (n=5)], followed by myeloid [10% (n=5)] and megakaryocytic series [2.5% (n=1)]. Out of 10 patients showing dysplasia, 6 had anemia, 3 had leukopenia and one had thrombocytopenia. Myelodysplasia was observed in 37% of patients[10] predominantly involving the granulocytic series followed by erythroid and megakaryocytic series. Karcher et al.[14] reported myelodysplasia in 69% of HIV positive patients.

Plasmacytosis has also been reported to occur in HIV infection.[24] Increased plasma cells were observed in 57.9% of non-AIDS and 65.45% of patients with AIDS.[2] Plasmacytosis was not only confined to advanced disease with possibility of opportunistic infections but was also seen in patients at an early stage with no concurrent infection.[2] Increased plasma cells could thus be a polyclonal B cell response to HIV infection and can occur at any stage of HIV disease. Increased plasma cells were seen in 27% of HIV infected patients.[10] Other studies have reported plasmacytosis in 25% and 22% of patients, respectively.[22,25] In the present study, 15 patients showed increase in plasma cells.

Reduced bone marrow lymphoid cells were seen in 37% of HIV-infected patients[10] and in 36.84% of non AIDS and 60% of AIDS patients.[2] Lymphopenia is probably a result of direct attack of lymphocytes by HIV through CD4 binding sites.[26] Decrease in marrow lymphoid cells could possibly explain lymphopenia in HIV patients.[2] In the present study, 12 (n=30%) patients showed decrease in lymphoid cells.

Granulomas are an infrequent finding in bone marrow biopsies and may be associated with a broad spectrum of infectious and non-infectious disorders.[22] Tuberculosis is well known to be associated with HIV and was the only co-infection we encountered. Amongst the 40 cases studied, 10 patients were clinically diagnosed with tuberculosis; pulmonary in 6 and abdominal in 4. However, epithelioid granulomas indicative of tuberculous infection were seen in 4 of the 10 cases. Granulomas were seen in 9% of patients in the AIDS group.[10] Other studies have reported granulomas in 16% and 12% of cases, respectively.[23,25]

Out of the 6 cases showing hypocellular marrow, 2 cases showed fibrosis; while, 2 cases were inadequate for opinion in the present study. Inadequate aspiration of the bone marrow was seen in 11% cases[10], a consequence of focal fibrosis, which was seen in 14% of cases. Two other studies[2,13] observed marrow fibrosis in 54% of HIV-infected cases, whereas one study documented marrow fibrosis in 20% cases.[14]

Along with increase in prevalence of HIV infection worldwide, it is important for the hematopathologist to recognize the hematological abnormalities and morphological changes in the bone marrow associated with HIV infection.[10]

Treatment of hematological abnormalities aims primarily at reducing replication of HIV, thereby diminishing suppression of hematopoiesis by the virus, and at controlling opportunistic infections during the course of the disease. Hematologic complications of HIV infection will definitely decrease as infectious complications are improved. Further research on hematological complications of HIV disease will lead to effective management of the patients and reduce morbidity and mortality from these complications. There were limitations in our study like small sample size, mental trauma due to HIV and reluctance for further investigations. Even if the patients were ready for the investigations, the procedure was invasive and painful and thus, voluntariness was affected to a certain degree. Also, we did not culture the aspirates for possible opportunistic pathogens.

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Conclusion

In conclusion, peripheral blood and bone marrow abnormalities are common in HIV- infected individuals and patients with AIDS. These abnormalities become more frequent as the disease progresses. Bone marrow study is an important investigation in HIV- infected patients with peripheral hematological abnormalities and is recommended routinely when other studies are not conclusive. Early diagnosis and effective management of anemia in patients with HIV infection is of tremendous importance as anemia in such patients may be an indicator of poor prognosis and progression to advanced disease. Bone marrow morphologic examination plays a distinctive role in ruling out the presence of opportunistic infections or associated neoplasms. The yield of bone marrow examination was considerable in our setting.

Conflict of Interest: None declared.

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