

Differential Diagnosis of Anemia from Bone Marrow Aspiration Cytology: A Prospective Study of 100 Consecutive Cases

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ABSTRACT

Introduction: Anemia is a common clinical problem requiring the precise diagnosis of underlying etiology in order to provide effective therapy. Measurement of red cell indices along with peripheral smear examination is done in most of the cases of anemia to establish the type and possible reasons, but these tests are inconclusive for some cases and they may need bone marrow examination. So this study has been done to emphasize the role of bone marrow aspiration (BMA) cytology in establishing the definite diagnosis of anemia.

Materials and Methods: This is a prospective study that has been conducted for bone marrow examination on 100 cases with anemia. BMA was done and the smears were stained using leishman's stain and some special stains such as myeloperoxidase stain, pearls stain, Periodic acid-Schiffs in selected cases. Trepine biopsy was done in 14 cases for further evaluation.

Results: We have included 100 consecutive cases of anemia, which had undergone BMA for various indications. Most common symptom was prolonged fever. Haemoglobin level was estimated for each patient. Pancytopenia was the commonest indication for marrow examination. Hypoplastic anemia was found in 21% of cases, which was the most common bone marrow examination finding, followed by

haematological malignancies, that was found in 18% of cases. **Conclusion:** BMA was an important tool that helps to reach the underlying etiology of anemia and also to know the response to treatment without using any costly instrument in a lesser time. So Bone marrow examination should be performed in anemic patient for whom the first line investigations are inconclusive.

Key Words: Anemia, Bone Marrow, Pancytopenia, Megaloblastic.

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INTRODUCTION

Anemia is a common clinical problem requiring the precise diagnosis of underlying etiology in order to provide effective therapy. It can be the only manifestation of a underlying disease. Prevalence of anemia is an important health indicator of the population. It is a widespread health problem, mainly in children and pregnant woman.¹ According to World Health Organization (WHO), there are two billions of peoples with anemia in the world and half of the causes of anemia are due to iron deficiency.² In developing countries like India, the estimated prevalence is 39% in children <5 years, 48% in children 5-14 years, 42% in 15-59 years old women, 30% in men 15-59 years, and 45% in adults >60 years.² Though many red cell indices are there, usually anemia is diagnosed basing upon haemoglobin concentration. When the level of haemoglobin in blood is less than the lower limit of the normal range for that age and sex, it is

designated as anemia. With mild anemia, the patients are usually asymptomatic or may have easy fatigability or listlessness. As anemia worsens, dyspnoea, dizziness, faintness, diminished concentration may occur. Though detection of anemia is easy, to reach the underlying etiology is not that simple always, and hence it is difficult to treat also. Classification systems of anemia emphasize on mechanism that reduces red cells number and/or erythrocytes size that is mean corpuscular volume (MCV). Morphological classification based on MCV, divides anemia into three groups, (1) normocytic, (2) microcytic, (3) macrocytic. A valuable aspect of this classification is that, measurement of red cell size is immediately available from automated blood counts. Though the differentials of macrocytic and microcytic anemias are not much complex, but the disease causing normocytic, normochromic anemias are numerous and complicated.

Measurement of red cell indices along with peripheral smear examination is done in most of the cases of anemia to establish the type and possible reasons. Some cases need bone marrow study to know the definite underlying etiology of anemia. Also in assessing anemia, it is useful to know whether the bone marrow has responded with a robust increase in red cell production. So this study has been done to emphasize the role of bone marrow aspiration cytology in establishing the definite diagnosis of anemia.

MATERIALS AND METHODS

This is a prospective study conducted in a tertiary care hospital and medical college of eastern Odisha. The present study has been conducted for bone marrow examination on 100 consecutive cases with anemia with or without other signs and symptoms though mild anemia alone is not an indication of bone marrow

examination, these patients either have severe anemia or anemia refractory to treatment or anemia associated with any other primary disease where bone marrow examination was necessary. All the patients were subjected to peripheral blood smear examination. Bone marrow aspiration (BMA) was done after getting informed consent from the patient or his guardian, using Salah's bone marrow puncture needle. Site of aspiration was posterior superior iliac spine from most of the cases, only in two cases it was anterior superior iliac spine and for one it was tibial tuberosity. BMA smears were stained using leishman's stain and some special stains such as myeloperoxidase stain, pearls stain, Periodic acid-Schiffs were performed in selected cases. Trephine biopsy was done in 14 cases for further evaluation and the tissue sections were stained with H&E stain. Data were analysed using Microsoft Excel. Patients taking chemotherapy, with anemia are excluded from this study.

Table 1: Presenting symptoms of our cases

Clinical presentation	Degree of anemia			Total
	Mild	Moderate	Severe	
Fever/ PUO	3	15	6	24
Pallor	0	3	5	8
Lymphadenopathy	1	4	3	8
Purpura/echymosis/epistaxis	2	6	1	9
Weakness	4	8	3	15
Arthritis	0	2	0	2
Low back pain	1	3	0	4
Mixed	12	15	9	36

Table-2: Age and sex distribution among the cases

Age (yr)	No. of patient	Male	Female
<1	4	2	2
1-10	20	12	8
11-20	13	7	6
21-30	8	5	3
31-40	12	5	7
41-50	14	7	7
51-60	10	7	3
61-70	15	8	7
71-80	4	1	3
Total	100	54	46

Table 3: Indication for bone marrow aspiration

Indication of marrow aspiration	No. of cases
Anemia	14
Leukemia	13
Pancytopenia	26
Lymphoma staging	15
Metastatic deposits	12
Bicytopenia	7
Platelet disorder	3
PUO	6
Others	4

Table 4: Differentials diagnosis of anemia from BMA cytology

Diagnosis from bone marrow cytology		No of cases	
Hypoplastic anemia		12	
Megaloblastic anemia		18	
Haematological malignancy	ALL	10	21
	AML	6	
	Multiple myeloma	5	
Metastasis to bone marrow		5	
Erythroid hyperplasia		18	
Infectious etiology		2	
Myelodysplastic syndrome		3	
Hyper-splenism		2	
Others		4	
Normal marrow		15	

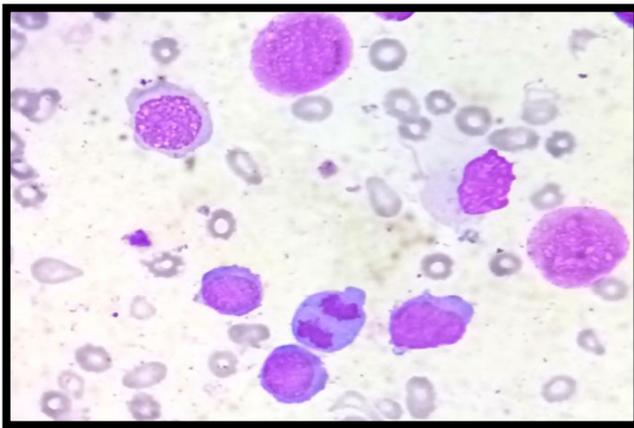


Figure 1: Leishman stain (1000X): Megaloblastic anemia

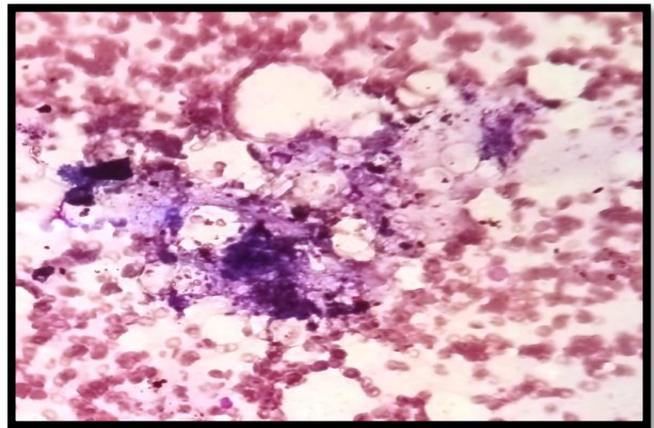


Fig 2: Leishman stain(100X): Hypoplastic Anemia

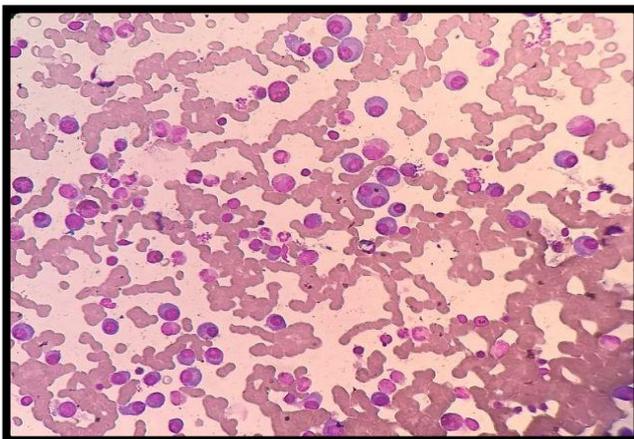


Fig 3: Leishman stain (400X): Multiple Myeloma

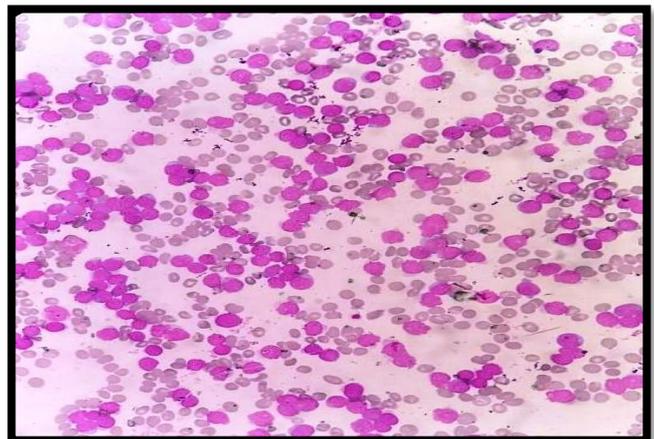


Fig4A: Leishman stain (400X): ALL-L1

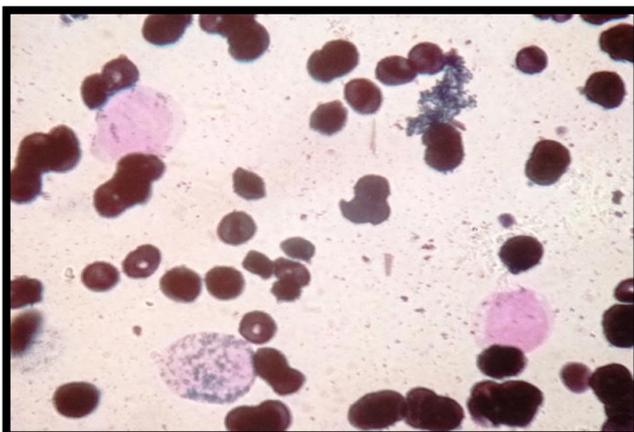


Fig 4B: Myeloperoxidase stain (1000X):
Blast are negative for MPO stain

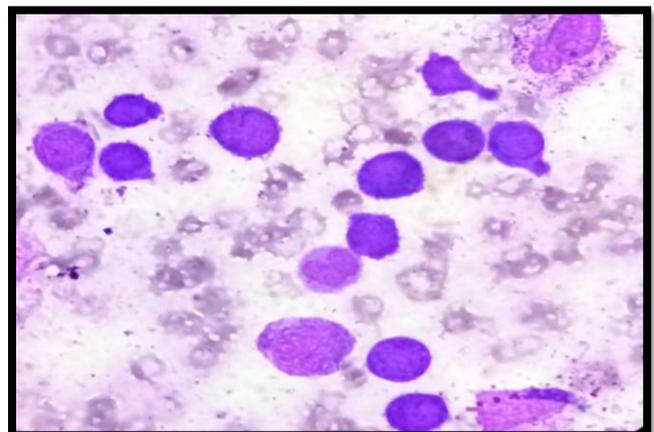


Fig 5A: Leishman stain (1000X): AML-M3

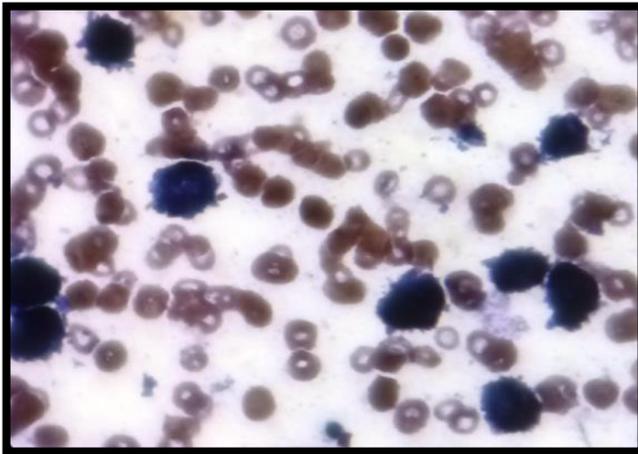


Fig 5B: Myeloperoxidase stain (1000X): Blast is strongly positive for MPO

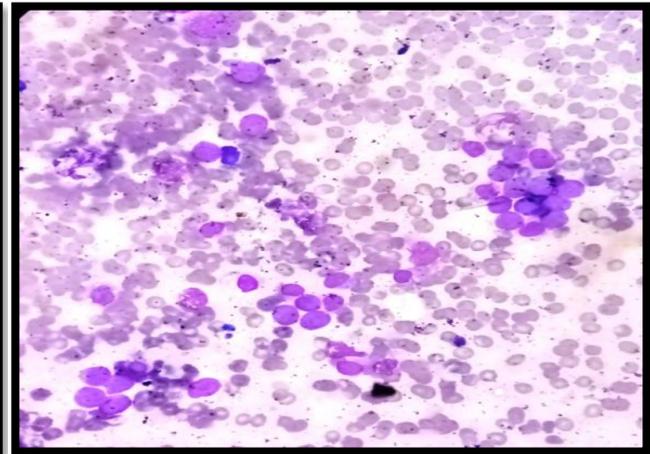


Fig 5C: Leishman stain (400X): AML-M2

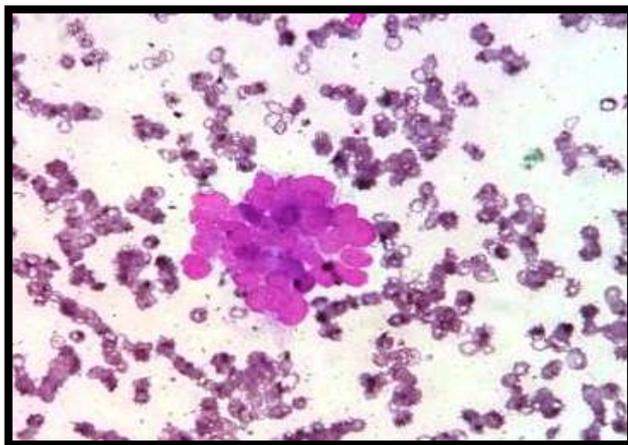


Fig 6: Leishman stain (400X): Metastasis to bone marrow

RESULTS

There were 100 consecutive cases of anemia, who were underwent bone marrow aspiration for various indications. Clinically the patients presented with different symptoms and signs, majority have more than one complain (miscellaneous group- 36%). 24% patients presented with prolonged fever only, among which 5% patients had pyrexia of unknown origin.(table-1) 15% patients came to the outpatient department with complain of severe weakness only. Bleeding manifestations were complained by a significant number of patients.8% patients had a complain of lymphadenopathy. Only paleness of skin and mucosa was seen in 8% cases. Haemoglobin levels were first measured in all these patients by colorimetry, using darbkins reagent. Comparing patients haemoglobin concentration with the WHO standards for that age and sex, they were classified into three groups like, mild, moderate and severe. (Table-1)

Age range of the patients varied from 8months-79years. anemia can be seen in any age group , so lowest age of our patients was 8months and highest was 79 years. Maximum number of patients (20%) were children, belongs to 1-10yr age group. 15% of patients were belonged to 61-70yrs, 14% cases were in 40-50yrs. Out of 100 cases 54 were male and 46 were female with a male to female ratio of 1.17:1. (Table-2)

Pancytopenia is the most common indication for marrow aspration, seen in 26% of cases, (Table-3). In 15% cases, BMA was done to know marrow involvement in lymphoma patients.

12% cases were known to had malignancy and BMA was done to rule out metastatic deposits in marrow.14% of patients had symptoms due to anemia only. Haematological malignancies (21%) were the commonest diagnosis from bone marrow aspiration cytology i.e. 21%, followed by megaloblastic anemia (18%), erythroid hyperplasia (11%), metastasis to marrow (5%), hypersplenism, myelodysplastic syndrome and infectious etiology.12% cases have hypoplastic marrow picture. In 15% of cases the bone marrow aspiration study revealed no pathology.

DISCUSSION

Anemia is a common health problem found in all age group, from paediatric to geriatric, known to be associated with increased morbidity and mortality. Many haematological and non-haematological disorders can result in anemia. Although these disorders have various clinical symptoms and also involve the blood, but peripheral blood picture alone does not reflect the disease process. Depending upon the suspected diagnosis from the clinical symptoms and peripheral blood picture, indication for bone marrow aspiration was summarised. In the present study male to female ratio is 1.17:1 and the age range is wide i.e. from 8months baby to 79yrs old. Our results are similar with the results of many studies done by Tilak et al in 1999³, Khodke et al in 2001⁴, Atla BL et al in 2015.⁵ But this result differ from results obtained from studies done by Kumar et al. in 2001⁶, and Das et al. in 2011⁷, as they excluded children from their study. All cases had anemia mild, moderate, or severe. In 26% of cases anemia is a component of pancytopenia and pancytopenia was the commonest indication of marrow aspiration. Atla BL et al in 2015⁵, also reported pancytopenia as the most common indication of bone marrow examination. Out of 26 cases of pancytopenia, 16 cases (61.5%) were found to have features of megaloblastic anemia (figure 1) in bone marrow cytology. The second common cause of pancytopenia was aplastic/ hypoplastic anemia (figure 2) (7=26.9%). This result are with similar Atla BL et al⁵ and Gayatri et al.⁸ Rest 3 cases of pancytopenia, diagnosed as hypoplastic acute lymphoblastic leukemia (ALL) from bone marrow cytology. In 15% of cases, indication of bone marow examination was to find out marrow involvement in lymphoma. Both BMA and trephine biopsy was done in these cases. As involvement of bone marrow by lymphoma is a definite evidence of disseminated disease^{9,10}, the assessment of BM status in patients with lymphoma provides

important information for decisions regarding treatment. The occurrence of BM involvement in lymphoma was 26.7%, which is nearly same as data documented by Shi YF et al¹¹, in 2015, who got 16% BM involvement in lymphoma.

But our result was much lower than the 40% incidence rate of previous reports from the series of western countries¹². Though bone marrow examination is not a first line investigation in anemia, BMA was done in 14 cases only for anemia. All these cases couldn't be diagnosed from the basic investigations. In BM examination 5 cases were diagnosed as aplastic anemia. Multiple myeloma (figure-3) was also found in 6 cases after marrow examination. 2 cases were of megaloblastic anemia, one case was diagnosed as myelodysplastic syndrome, i.e. refractory anemia with unilineage dysplasia. 13 percent of cases were aspirated as suspected cases of leukemia, as their peripheral smear revealed presence of atypical cells of varying proportion. In these cases BM aspiration smears were stained with myeloperoxidase stain and periodic acid Schiff (PAS) stain along with leishman stain. Out of 13, 7 cases were of acute lymphoblastic leukemia (ALL) (Figure-4A, 4B) and 6 cases were of acute myeloid leukemia (AML), (AML-M1 in 1 case, AML-M2 in 1 Case and AML-M3 in 4 cases) (Figure-5A, 5B, 5C). In our study, we have total 9 cases of ALL, out of which 6 cases had leukocytosis with presence of atypical cells in peripheral blood. But 3 cases had pancytopenia, and diagnosed as hypoplastic ALL from BM aspiration. Many studies reported the incidence of hypoplastic ALL is 2%.^{13,14} But in present study the incidence is very high (33.3%). The major difference may be due to less number of ALL cases in our study. One of the major indications of marrow aspiration in our anemic study group was to know the occurrence of metastasis to BM in patients of certain systemic malignancy. 5 out of 12 (41.7%) had shown marrow involvement. Anemia in these patients may be due to primary tumour or may be due to metastasis to marrow that hamper erythropoiesis. Out of 5 cases 3 were adenocarcinoma (figure-6), with primary focus in prostate and 2 female patients had their primary focus in breast. Bone marrow metastasis can commonly see following development of metastatic breast cancer.¹⁵ Schmid et al. evaluated 1068 patients and found 17.8% of them as metastatic to bone marrow. In their study, they reported, prostate, breast, lung, and gastric cancer were the most common cancers metastasized to bone marrow.¹⁷ The difference of BM metastasis is, perhaps due to the difference in the study population. That means, we have included the patients who have less haemoglobin along with systemic malignancy and not the whole population. 2 cases had BM findings within normal limit, and rest 5 cases revealed erythroid hyperplasia. Pyrexia of unknown origin (PUO) was also one of the major causes of bone marrow aspiration i.e. 6% of cases clinically diagnosed as PUO with anemia were included in our study group. We found infectious etiology in 2 cases (one is mycobacterial infection and other is malarial), haematological malignancy in one case. But BM examination findings were within normal limit for 3 cases. In the present study the diagnostic spectrum for PUO is typical of that seen in a developing country like India, where the infectious etiology is the major contributor of PUO, unlike Western countries.¹⁸⁻²⁰ Among neoplastic causes, haematological malignancies are the commonest neoplastic cause of PUO.^{21,22} Erythroid hyperplasia was a predominant BM aspiration diagnosis in our study. This finding is comparable with

data documented in some recent studies by Atla BL et al⁵ 2015, Parajuli et al²³ and Khan et al²⁴, 2014, Chandra et al²⁵ 2011. Out of 100 cases of BM examination patients with anemia, 15 cases had BM picture which was completely normal. Majority of these patients had undergone marrow examination to find out the occurrence of BM involvement either in lymphoma or systemic malignancy.

CONCLUSION

Usually bone marrow examination is deferred in cases of mild anemia. BMA was an important tool that is required to perform, not only to reach the underlying etiology of anemia but also to know the response of these cases to treatment. The major advantage of this technique is that we don't need any costly instrument and also its needs very less time. We find many differentials from bone marrow examination. Hence bone marrow examination should be performed in anemic patient for whom the first line investigations are inconclusive. Bone marrow aspiration and/or biopsy should be included in investigation panel for anemia

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