

# Diagnosis of Non-Neoplastic Haematological Disorders using Bone Marrow Aspiration and Trephine Biopsy

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## ABSTRACT

**Introduction:** Proper diagnosis of various hematological and non-hematological disorders is important in the field of haematology. Bone marrow examination is considered as valuable diagnostic tool for evaluation and diagnosis of various hematological and non-hematological disorders. Therefore present study aimed to diagnose non-neoplastic haematological disease through bone marrow aspiration or biopsy and also to explain the prognosis of different diseases.

**Material and method:** Present study was conducted in department of pathology, GSVM Medical College, Kanpur, on 30 patients suffering from chronic haematological disease. The relevant history of patients was recorded and informed consent was taken. Patients were investigated for complete blood count, coagulation profile, reticulocyte count and peripheral blood film (PBF) examination. Bone marrow aspiration and Bone marrow biopsy were done under aseptic precautions.

**Results:** Out of 30 cases, 11 (36.66%) are having non-neoplastic haematological diseases and most common disease is iron deficiency anaemia. In iron deficiency and megaloblastic anaemia, bone marrow aspiration findings are more useful and diagnostic. In aplastic anaemia, final conclusive diagnosis was made by bone marrow biopsy.

**Conclusion:** In some haematological disease, Bone marrow aspiration study alone fails to demonstrate disease processes, therefore trephine biopsy is essential. Hypocellular marrow, unexplained pancytopenias and marrow fibrosis are also strong indications for Bone marrow biopsy.

**Keywords:** Bone Marrow Aspiration, Trephine Biopsy, Non-Neoplastic, Anaemia.

and non-Hodgkin's lymphoma, hairy cell leukemia, myeloproliferative disorders and plasma cell dyscrasias like multiple myeloma respectively.<sup>5</sup> Definitive diagnosis of several hematological diseases, such as unexplained pancytopenias, leukemias and other bone marrow disorders require bone marrow aspiration/biopsy.<sup>6</sup> Bone marrow morphological examination includes peripheral blood film (PBF), buffy coat, direct particle, bone marrow aspiration smears and trephine biopsy imprints for sections and marrow volumetric data respectively. In various non-hematological conditions like fever of unknown origin, tuberculosis, leishmaniasis etc., bone marrow examination is done.

Nutritional anaemia is most common haematological disorder found on bone marrow examination, of which commonest being megaloblastic anaemia.<sup>8</sup> Bone marrow examination is diagnostic of iron deficiency anaemia.<sup>9</sup> On bone marrow examination parasitic infestations like visceral leishmaniasis and malaria can be detected.

There are three ways in which marrow can be obtained-needle aspiration, microtrephine biopsy and surgical biopsy, microtrephine biopsy is a simple procedure. It is now widely used and almost replaced surgical biopsy. It is relatively painless and can be performed on outdoor patients. The disadvantage of aspiration technique is that there is problem of dry tap in certain haematological conditions and the relationship of one cell to the other cell are more or less destroyed by the process of aspiration which is not so in microtrephine biopsy. For example in hypocellular marrow/or fibrosis, an aspirate will be inadequate. Therefore trephine biopsy may show marrow architecture and elaborates abnormal distribution of cells, focal lymphoid infiltrates, unexplained pancytopenias etc., are strong indications for

## INTRODUCTION

There have been major advances in the understanding of blood disorders due to increased recognition of the interrelations between the cellular and tissue elements of the marrow. Improvements in biopsy technique have provided additional impetus to bone marrow study in their natural spatial context.<sup>1-2</sup> Improved needles permit the simultaneous performance of aspiration and biopsy. In haematology, bone marrow examination (BME) is needed for differential diagnosis of various haematological disorders and their prognostic classification.<sup>1-4</sup>

Indications for BME include proper diagnosis, staging and therapeutic monitoring of different hematological/non-hematological disorders like refractory hypochromic anaemia, megaloblastic anaemia, in microcytic anaemias evaluation of iron and sideroblasts, lymphoproliferative disorders, such as Chronic lymphocytic leukemia, Hodgkin's

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trephine biopsy.<sup>7</sup> The great value of microtrephine biopsy is that it can provide a perfect view of the structure of relatively large pieces of marrow that is, if the material obtained by biopsy, has been skilfully processed. Studies on large number of cases have been demonstrated that, whereas microtrephine biopsy specimens are superior to films of aspirated material in some circumstances, e.g. for diagnosing marrow involvement in lymphoma or non-haematological neoplastic disease. A disadvantage of most marrow trephine is that not infrequently, the specimen is crushed and its architecture altered. The Jamshidi needle which has tapering end was designed to overcome this problem. Present study was done for the diagnosis of non-neoplastic haematological disease through bone marrow aspiration or biopsy and also to explain the prognosis of different diseases.

## MATERIAL AND METHODS

Present study was conducted in department of pathology, GSVM Medical College, Kanpur, on 30 patients suffering from chronic haematological disease. These patients were admitted in LLR and associated hospitals of GSVM Medical College, Kanpur. Patients suspected of having their bone marrow involvement by any hematological or non-hematological disorders were included in study. The relevant history of patients was recorded and informed consent was taken. Patients were investigated for complete blood count, coagulation profile, reticulocyte count and peripheral blood film (PBF) examination. Equipments included were Sahali's apparatus, Salah's Bone marrow aspiration needle and Jamshidi's trephine biopsy needle, 5 ml and 20 ml disposable syringes, xylocaine (1%), glass slides, fixative-isopropyl alcohol, leishman's stain, Prussian blue stain

Disease	No. of Cases	Percentage
Non-neoplastic	11	36.66
Neoplastic	19	63.34

**Table-1:** Distribution of cases according to disease

Disease	No. of Cases	Percentage
Iron deficiency anaemia (IDA)	3	27.26
Megaloblastic anaemia (MA)	2	18.18
Aplastic anaemia (AA)	3	27.26
Hypersplenism (HS)	2	18.18
Parasitic (Microbilaria)	1	9.09

**Table-2:** Distribution of cases according to disease

Age group (Yrs)	IDA		MA		AA		Hypersplenism		Parasitic	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
01-10	-	1	-	-	1	-	-	-	-	-
11-20	-	-	-	-	-	-	-	-	-	-
21-30	-	2	-	2	-	-	1	1	1	-
31-40	-	-	-	-	-	-	-	-	-	-
41-50	-	-	-	-	2	-	-	-	-	-
51-60	-	-	-	-	-	-	-	-	-	-
61-70	-	-	-	-	-	-	-	-	-	-

**Table-3:** Distribution of cases according to age & sex

and other antiseptic materials respectively. Bone marrow aspiration and Bone marrow biopsy were done under aseptic precautions. Bone marrow biopsy was taken by Zamshidi needle from the posterior iliac crest and specimen obtained is then gently removed with long probe on a slide. Obtained biopsy tissue is preserved in FAA solution, 5 ml ethyl alcohol, 10 drops acetic acid and 2 drops formalin after 6 hours fixation. The tissue is decalcified in E.D.T.A. solution, refixed in formalin and processed with paraffin-wax embedding sections, 1µm-thick, were cut and were stained by Hemotoxylin and Eosin (H&E) stain. The staining for reticulin fibers with Gomori's Silver impregnation method was done. Bone marrow biopsy and aspiration findings were analysed in context of clinical signs, symptoms and other laboratory investigations.

## RESULT

Table 1 shows that out of 30 cases, 11 (36.66%) were having non-neoplastic haematological diseases while 19 (63.34%) were suffering from neoplastic haematological diseases.

Table 2 shows that most common diseases were Iron deficiency and Aplastic anaemia each having 3 (27.26%) cases.

Table 3 shows age and sex incidence in non-neoplastic cases. All 3 cases of Iron deficiency anaemia were female, 1 was in 1<sup>st</sup> decade and other 2 in 3<sup>rd</sup> decade. Both cases of Megaloblastic anaemia were female and from 3<sup>rd</sup> decade. One male and one female patient was from 3<sup>rd</sup> decade and they were suffering from hypersplenism. One male case of parasite was from 3<sup>rd</sup> decade.

In 3 cases of iron deficiency anaemia, 2 had normal reticulin network, in one case increased reticulin network. In both cases of megaloblastic anaemia, reticulin network was normal in quantity. In all 3 cases of aplastic anaemia, reticulin network was less than normal. In case of hypersplenism, one had slightly increased reticulin network and other has normal. (Table 4)

Table 5 shows that in cases of iron deficiency anaemia, 2 cases had got decreased iron and in one case iron was increased. In both cases of megaloblastic anaemia iron was increased. In 3 cases of aplastic anaemia, 2 had got decreased iron and one normal iron staining.

All the 3 cases of iron deficiency anaemia aspiration findings were more useful and diagnostic. Same was the case in megaloblastic anaemia and hypersplenism. In all 3 cases of aplastic anaemia last conclusive diagnosis was made by

Disease	Total cases no.	Normal pattern	Increased	Decreased
Iron deficiency anaemia	3	2	1	-
Megaloblastic anaemia	2	2	-	-
Aplastic anaemia	3	-	-	3
Hypersplenism	2	1	1	-
Parasitic (Microbilaria)	1	1	-	-

**Table-4:** Reticulin pattern on bone marrow biopsy

Disease	Total cases no.	Normal pattern	Increased	Decreased
Iron deficiency anaemia	3	-	1	2
Megaloblastic anaemia	2	-	2	-
Aplastic anaemia	3	1	-	2

**Table-5:** Iron staining in cases in anaemia

Disease	Total No. Cases.	Bone marrow aspiration	Bone marrow biopsy
Iron deficiency anaemia	3	3	-
Megaloblastic anaemia	2	2	-
Aplastic anaemia	3	-	3
Hypersplenism	2	2	-
Parasitic (Microbilaria)	1	1	-

**Table-6:** Comparison of bone marrow aspiration with bone marrow biopsy (Diagnostic)

bone marrow biopsy. In one case of parasite aspiration was diagnostic. (Table 6)

## DISCUSSION

Present study was conducted on 30 patients suffering from chronic haematological disorders. The trephine biopsy was done either to diagnose the basic cause of disease or to confirm the diagnosis of peripheral blood smear and aspiration or for explaining the prognosis of the disease. These patients were admitted in L.L.R and associated hospitals of G.S.V.M. Medical College, Kanpur.

In present study bone marrow biopsy was performed on 30 cases. In 20 (66.66%) cases, biopsy tissue was sufficient, long enough to report. But in 10 cases, we have to report on the aspiration findings. Problem in 10 cases was that either the tissue get fractured or sometimes crushed during biopsy procedure.

Similar problems have been reported by Inwood et al<sup>10</sup> who has used the same needle. The needle was widely used for core biopsy of the posterior iliac crest. But in about half of the cases, the sample fractures while it is being extracted and only the outer portion is retrieved. Furthermore in a small number of cases the core is not served at its base and the whole specimen is left in situ as the needle is withdrawn, thus required a second or third attempt to secure adequate sample.

In the cases in which biopsy was failure it was not possible to do repeat biopsy because most of the patients refused for this procedure and it is also advisable not to do repeat biopsy from the same site within 4-6 months<sup>10</sup>.

In the present study the iron deficiency anaemia and aplastic anaemia has same incidence. There are 3 cases of each (27.3%), out of 11 cases although iron deficiency anaemia is quite common. The same incidence may be because of

random selection of cases in present work.

The highest incidence of iron deficiency anaemia is in women during reproductive age group is a common cause of chronic fatigue and ill health. In a study in Sweden, Raney RB et al.<sup>11</sup> found that marrow iron stores were absent in 32 patients of 28 year old females. IDA is especially common in women with persistently heavy blood loss, and in women who have many pregnancies and in rapid succession. A mild degree of anaemia is not uncommon in young girls after onset of menstruation. Significant blood loss may occur as a result of miscarriages, especially when these are repeated. Inadequate iron intake due to poor diet, anorexia (during pregnancy), diminished bioavailability or impaired absorption may act as a contributing factor. Thus iron deficiency anaemia is more common in women of low socio-economic status, probability due to inadequate intake of food rich in iron such as meat, eggs and green vegetables. Foulds et al<sup>12</sup> and Goldberg et al<sup>13</sup> said that megaloblastic anaemia is due to deficiency of folic acid and most often in the third trimester or shortly after delivery. Folate requirement increases during pregnancy and the diets of many pregnant patients are insufficient to meet the increased need although the folate deficiency occurs most often in economically deprived patients.

On taking brief history of our patients we found that drug idiosyncrasy was the main cause of aplastic anaemia. In two cases, there was history of chloramphenicol ingestion for very long duration and other one was due to phenytoin toxicity. As there is no bar for drug intake in any age so it may occur in any age group. Similar picture has been reported by Wallerstein et al<sup>14</sup> in regard to chloramphenicol, the risk of developing fatal aplastic anaemia may be in the range of 1 in 20000 cases i.e. about 13 times the frequency of aplastic anaemia in the population not so exposed. In the analysis

of 408 cases of chloramphenicol associated non-neoplastic depression of one or more blood cell types reported, showed persons of all ages to be affected, there being a broad age range from 25-65 years, old for adults and a childhood peak.<sup>15</sup> Similarly more than 48 cases of aplastic anaemia associated with anticonvulsant drug therapy especially phenytoin have been reported the onset of the anaemia occurred as early as 2 weeks or as long as 30 months after starting the treatment, but in most instances after 4 to 30 months. There was no relation to age, sex or drug dosage (Robins, 1962).

As we have found 2 cases of hypersplenism one was male and other was female. In these cases PBS was negative for Malaria Parasite. Bone marrow smears revealed *P.vivax*. The parasite was found in 1 male of 23 years old. It was an accidental finding with case of refractory anaemia.

In present study in iron deficiency anaemia or megaloblastic anaemia the bone marrow reticulin was either increased or normal. The form of network, however, does not differ from the normal though in present cases the reticulin fibers were demonstrated rather more uniformly throughout the marrow.

In conditions like leukemia reticulin network was prominent and continuous throughout the greater part of the marrow. Masugi (1926) was of the opinion that the increase in reticulin observed in benign hyperplasias was due to a thickening after a preliminary thinning of pre-existing reticulin fibers. Haschen (1926) shared this view and extended it to include all cases in which the content of reticulin was increased.

Out of 30 cases, in 7 cases, diagnosis was made only by bone marrow biopsy. In 5 of these cases, it was dry tap on aspiration (3 cases of aplastic anaemia, 2 cases of CML and 1 each case of CLL & CMM).

In 3 cases of IDA aspiration was useful for diagnosis and there was no difference on bone marrow biopsy and aspiration as bone marrow H/E section was showing erythroid hyperplasia. As aspiration for pearl iron staining aspiration films were better than biopsy because they get better stained with iron. Same was the finding in cases of megaloblastic anaemia. Jacobsen et al<sup>16</sup> had reported that the examination of aspirates may be more reliable than the biopsy since iron may be lost during biopsy preparation.

All 3 cases of aplastic anaemia aspiration was dry tap or inconclusive or acellular one case each. So bone marrow biopsy was highly significant. Secondly to see the proportion of cells which is better seen in bone marrow biopsy to explain the prognosis. Vilter et al (1960) reported that when a patient thought to have aplastic anaemia is found to have a normally cellular or hypercellular marrow have a normally cellular or hypercellular marrow. One explanation for such a contradictory finding is that the biopsy needle has entered an area in which bone marrow is regenerating after severe damage. Another not uncommon dilemma a patient thought to have leukemia. In most situations a larger marrow sample obtained by biopsy will solve the problem.

Li et al reported that in high number of cases on bone marrow aspirate there was dry tap or chiefly mature red cells seen in aplastic anaemia. Such finding makes it advisable to obtain a larger specimen, by biopsy to be sure that one has obtained

true bone marrow and not blood, and also to see how fatty the marrow is. However, the most important bone marrow finding is the proportion of cells that are nonmyeloid and not erythroblasts since the proportion of such cells has been found to be directly to motrality.

## CONCLUSION

From above results we conclude that iron deficiency anaemia is the most common disease in non-neoplastic haematological disease. Commonest age/sex in iron deficiency anaemia and megaloblastic anaemia is reproductive age group females. In most of the cases of aplastic anaemia there was dry tap. In case of aplastic anaemia, bone marrow biopsy is almost always necessary as we get larger true representative bone marrow tissue piece. So we can see the proportion of cells in better way. By that prognosis can be explained. Results of aspiration and bone marrow and bone marrow biopsy are almost same, but in aspirated marrow, individual cells are perfectly preserved in well made films.

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