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Study of Hematological Disorders on Basis of Bone Marrow Examination with Special Reference to Ferro Kinetics

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Abstract

Bone marrow examination is considered as valuable tool to assess the various hematological and non-hematological disorders. It helps in the diagnosis, staging, and therapeutic monitoring of leukemia's and lymphomas along with evaluation of cytopenias, thrombocytosis, leukocytosis, anaemia, and iron status.

Keywords: Bone marrow examination; Hematological disorders; Anemia; Iron stores

Introduction

Bone marrow examination is useful in the diagnosis of both hematological and non-hematological disorders. Indications have included the diagnosis, staging, and therapeutic monitoring of lympho-proliferative disorders such as chronic lymphocytic leukemia, Hodgkin's and non-Hodgkin's lymphoma, hairy cell leukemia, and multiple myelomas. Furthermore, evaluation of cytopenias, thrombocytosis, leukocytosis, anaemia, and iron status can also be done.

Bone marrow examination is also an important tool in the diagnosis of non-hematological disorders like storage diseases, granulomatous lesions, metastatic carcinoma, PUO, and disseminated infection in immune compromised hosts. The two most important techniques used for the diagnosis are bone marrow aspiration and bone marrow trephine biopsy which are complementary to each other. Aspiration of the marrow is primarily utilized for cytological assessment with analysis directed towards morphology and obtaining a differential cell count. Biopsy is essential for diagnosis in cases where a dry/blood tap is obtained on aspiration, which occurs when the marrow is fibrotic or densely cellular. Only a biopsy allows a complete assessment of marrow architecture and pattern of distribution of any abnormal infiltrates.

The aim of this study was to evaluate the morphology of various hematological disorders by bone marrow aspiration with special emphasis on evaluation of bone marrow iron status [1].

The microscopic examination of stainable iron in the bone marrow aspirate smear is generally considered the reference standard for determining the body iron stores [2,3].

Material and Methods

In present study 100 cases were studied by bone marrow aspiration. Adequate material was obtained in 98 cases and 2 cases showed bloody tap. These 2 cases of bloody tap were further followed up by bone marrow trephine biopsy.

All the procedures were done under aseptic precaution. Both bone marrow aspiration and bone marrow trephine biopsy were performed through same incision with two different needles. The aspiration was done from posterior superior iliac crest and sternum in adults patients and from anterior tibia in infants and children. The slide were fixed in alcohol Giemsa staining was done for aspiration smears and H&E stain was used for biopsy. PAS, MPO, NSE, ZN stain and Sudan black was done whenever indicated. Iron stain was done in all the cases.

Observations and Results

In present study 110 cases with various haematological disorders underwent bone marrow aspiration during the period January 2015 to December 2016. Out of these 10 cases were excluded from present study due to inadequate material.

Out of 100 cases, 02(02%) cases showed bloody tap which were followed up and confirmed by bone marrow trephine biopsy.

Table 1: Distribution of patients according to the site of bone marrow aspiration and trephine biopsy (N=100).

S. NO	Site of procedure	No of cases	Percentage
1.	Posterior iliac crest	77 (03 cases of BMT)	77.0%

2.	Sternum	13	13.0%
3.	Medial surface of proximal part of tibia	10	10.0%
Total		100	100%

Posterior iliac crest was the most common site of aspiration (77%). In 13 cases aspiration was done from sternum and in 10 cases from the medial surface of proximal part of tibia. Bone marrow trephine biopsy was performed from posterior iliac crest in 3 cases.

Table 2: Mode of clinical presentation of cases.

Clinical presentation	No of cases	Percentage %
Anemia	79	79.0%
Organomegaly	35	35.0%
Pancytopenia	32	32.0%
Fever	32	32.0%
History of blood transfusion	20	20.0%
Bone pain	15	15.0%
Abdominal pain	07	7.0%
Lymphadenopathy	06	6.0%
Lytic bony lesion	05	4.0%
Jaundice	03	3.0%

The most common clinical presentation observed was anemia (79.0%), followed by organomegaly (35.0%), pancytopenia and fever.

Table 3: Distribution of patients presented with pancytopenia in peripheral smear (N=32).

Hematological disorders	BMA	%
Megaloblastic anemia	15	46.88%
Dimorphic anemia	08	25.00%
Hypoplastic marrow	03	09.38%
MDS	02	06.25%
Micronormoblastic marrow	02	06.25%
Aplastic anemia	01	03.12%
DLBCL	01	03.12%

It was found that Megaloblastic anemia was the most common cause of pancytopenia (Tables 1-8) followed by dimorphic anemia (25%). 03 cases had hypoplastic marrow.

Table 4: Distribution of various hematological disorders in bone marrow aspiration.

Hematological Disorders	Sub Types	No. of cases	%
Normal Marrow		1	1%
Anemia	Megaloblastic anemia	18	18%
	Dimorphic anemia	15	15%
	IDA	7	7%
	MDS	5	5%
Primary BM Malignancies	ALL	9	9%
	AML	5	5%
	CML	11	11%
	CLL	2	2%
	Multiple myeloma	5	5%
	CEL	1	1%
BM Infiltration	NHL	1	1%
	Mantle cell lymphoma	1	1%
	Metastatic Adenocarcinoma	1	1%
	Metastatic Malignant Melanoma	1	1%
ITP		7	7%
Parasite	Leishmania	1	1%
	Histoplasmosis	1	1%
Bloody Tap		2	2%

Others	Hypoplastic marrow	3	3%
	Erythroid hyperplasia	1	1%
	HES	1	1%
	Granulomatous	1	1%111111
Total		100	100%

In the present study, diagnosis or diagnostic clue to the diseases process was ascertained in 97 cases. One case revealed a normal marrow study. The most common diagnosis rendered on aspiration was anemia 45 cases followed by 33 cases of primary bone marrow malignancies.

Megaloblastic anemia was found in 18 cases (40%), followed by 15(33.33%) cases of combined deficiency anemia or dimorphic anemia.

Among primary bone marrow malignancies, 11 cases were diagnosed as chronic myeloid leukemia and 09 (27.27%) cases of acute lymphoblastic leukemia. Three cases of hypoplastic marrow, one case of erythroid hyperplasia, one case of HES and one case showed granulomas in the marrow were also noted.

Table 5: Distribution of Lymphoproliferative diseases on Bone marrow aspiration (N=4).

LPD	No of cases
CLL	2(50%)
DLBCL	1(25%)
Mantle cell lymphoma	1(25%)
TOTAL	04(100%)

CLL was found in 2 cases, while DLBCL and Mantle cell lymphoma was observed in 1 case.

Table 6: Distribution of metastasis in Bone marrow aspiration (N=4).

Disorders	No. of cases
NHL	02
Adenocarcinoma	01
Malignant melanoma	01
Total	02

Bone marrow metastasis was observed in 2 cases of NHL, 1 case of adeno-carcinoma from stomach and in 1 case of malignant melanoma of eye origin.

Bone Marrow Trephine Biopsy

Bone marrow trephine biopsy was done in 3 cases.

Table 7: Distribution of cases undergoing Bone marrow trephine biopsy (N=03).

Disorder	No. of cases	Total
APLASTIC ANEMIA	02	02(66.67%)
MANTLE CELL LYMPHOMA	01	01(33.33%)
Total	03	03(100%)

Out of 03 cases, 02 cases were diagnosed as Aplastic anemia and one case was of Mantle cell lymphoma.

Table 8A: Predominant marrow findings and marrow iron storage in various hematological conditions.

Condition	Number of cases	Iron grade
Megaloblastic anemia	18	1-4
Dimorphic anemia	15	1-4
Chronic myeloid leukemia	11	1-4
Acute lymphoblastic leukemia	09	1-3
Iron deficiency anemia	07	0-4
Idiopathic thrombocytopenic purpura	07	1-2
Acute myeloid leukemia	05	2-4
Multiple myeloma	05	2-5
Myelodysplastic syndrome	05	3-5
Bone marrow infiltration	04	2-3
Hypoplastic marrow	03	1-2
Aplastic anemia	02	0-1
Chronic lymphocytic leukemia	02	2-3
Parasite	02	2-3
Chronic eosinophilic leukemia	01	4
Hyper eosinophilic syndrome	01	3
Erythroid hyperplasia	01	4
Granulomatous disease	01	5
Normal marrow	01	1

Total	100	

Table 8B: Gale's grading of iron in various hematological disorders.

Hematological disorders	G-0	G-1	G-2	G-3	G-4	G-5	G-6
Megaloblastic anemia	-	3	2	6	7	-	-
Dimorphic anemia	-	7	2	4	2	-	-
Chronic myeloid leukemia	-	3	1	5	2	-	-
Acute lymphoblastic leukemia	-	3	4	2	-	-	-
Iron deficiency anemia	3	2	1	-	1	-	-
Idiopathic thrombocytopenic purpura	-	3	4	-	-	-	-
Acute myeloid leukemia	-	-	1	2	2	-	-
Myelodysplastic syndrome	-	-	-	1	1	3	-
Multiple myeloma	-		1	2	1	1	-
Bone marrow infiltration	-	-	2	2	-	-	-
Hypoplastic marrow	-	1	2	-	-	-	-
Chronic lymphocytic leukemia	-	-	1	1	-	-	-
Parasitic	-	-	1	1	-	-	-
Aplastic anemia	1	1	-	-	-	-	-
Chronic eosinophilic leukemia	-	-	-	-	1	-	-
Hyper eosinophilic syndrome	-	-	-	1	-	-	-
Erythroid hyperplasia	-	-	-	-	1	-	-
Granulomatous disease		-	-	-	-	1	-
Normal marrow	-	1	-	-	-	-	-
Total	4	24	22	27	18	9	100

Iron store was evaluated in all the 100 cases. It was observed that range of 1-4 was evident in megaloblastic anemia, dimorphic anemia and Chronic myeloid leukemia whereas range of 2-3 was in Bone marrow infiltration, Chronic lymphocytic leukemia and in Parasitic infestation. Myelodysplastic syndrome showed iron grade in range of 3-5 and granulomatous disease showed Grade 5 iron. Minimum grade was seen in Aplastic anemia (grade 0-1) and in Iron deficiency anemia (grade 0-4).

Discussion

Bone Marrow Aspiration is an important diagnostic procedure for diagnosis of hematological and non-hematological disorders and malignancies. Posterior superior iliac crest is the most common safe site for bone marrow evaluation [4]. Both biopsy and aspiration can be done from the same site. The most common clinical indication for aspiration was anemia followed by pancytopenia. The other indications includes pyrexia of unknown origin, history of multiple blood transfusion, organomegaly etc. The nutritional anemia was found in 40% of the patients and was the most prevalent anemia in our studies. The sensitivity of aspiration in diagnosing anemia was 100%. Megaloblastic anemia was the most common anemia and also the cause of pancytopenia. Mahajan et al. [5] also found the similar findings in his studies.

Pancytopenia is described as the deficiency of all the three cellular elements. It is one of the common hematological entities that a pathologist comes across in routine practice.

Khodke et al. [6], Metikurke et al. [7], Thakkar et al. [8] and Sweta et al. [9] have documented similar findings; while Kumar et al. [10] and Pathak et al. [11] reported hypoplastic marrow as the commonest cause.

The various studies done throughout the world have reported aplastic anemia as the commonest cause of pancytopenia. However these findings do not match with results obtained in the current study. This seems to reflect the higher prevalence of nutritional anemia especially vitamin B12 and folic acid deficiency in Haroti region. Of the two micronutrients, possibly vitamin B12 deficiency is more common due to vegetarian life style of people. It is a correctable disorder and should be treated immediately as it can present acutely in critically ill patients.

Iron deficiency anemia was less frequent. This could be due to the fact that, bone marrow examination is not routinely done to diagnose iron deficiency anemia and is treated as outpatient

basis. However from public health view point, deficiency of B12 /Folate has been regarded to contribute little to nutritional anemia. Moreover B12/Folate deficiency more commonly present with severe form of anemia and pancytopenia, and hence frequent submission to Bone marrow studies. At present, Anemia control or prophylaxis program in India give only Iron and Folic acid.

Kibira et al. [12] observed AML (27.7%) and Parajuli [13] observed erythroid hyperplasia (13.3%) as common hematological disorder in patients undergoing bone marrow aspiration. This could be explained by the fact that cases belonged to rural, sub-urban region and agricultural backgrounds with lower socio-economic status and lack of appropriate education, therefore, they had exposure to various risk factors co-incidentally and finally faced to hematological abnormalities.

Primary hematological malignancies represented 33% cases. In diagnosing acute leukemia, the sensitivity and specificity of aspiration was 100% in our study, which is in concordance with other studies. Anjum et al. (2014) [14] found 23 cases of acute leukemia out of 39 cases. However flow cytometry and cytogenetics evaluation were done for further subtyping of leukemia.

In this study, there were 13 cases of chronic leukemias - chronic myeloid leukemia [CML] 11 cases, chronic lymphocytic leukemia [CLL] 2 cases. Aspirate was hypercellular with granulocytic hyperplasia.

Bone marrow aspiration is essential for appropriate evaluation of plasma cell differentiation and thus was 100 % diagnostic in 5 cases found in the current studies.

Four cases of bone marrow metastasis were seen. One was a case of adenocarcinoma (25%) which was further confirmed by endoscopic biopsy of Gastric ulcer which was reported as well differentiated adenocarcinoma. Toi et al studied 160 cases and found 3 cases of adenocarcinoma [15]. The other case was of malignant melanoma which was confirmed on retrospective evaluation, Non-hodgkin's Lymphoma, high grade NHL – DLBCL and Mantle cell Lymphoma. The current study also highlights that bone marrow aspiration if done properly can diagnose metastatic lesion. However bone marrow biopsy provides valuable information regarding spatial distribution, extent of infiltrate, cellularity, and fibrosis in NHL which cannot be determined from aspiration. It is more useful in postchemotherapy patients to assess the residual tumor cell burden and degree of chemotherapy response [16]. The combined procedure of aspiration and biopsy gives a higher yield and is essential in patients with suspected carcinoma and lymphoma.

2 cases of dry tap observed underwent trephine biopsy and were diagnosed as aplastic anemia. Mukiibi et al. studied 2880 cases and exhibited 116 cases as dry tap on aspiration. He documented that the single commonest underlying pathological cause of 'dry tap' was aplastic anaemia which accounted for 56 (48.3%) cases [17].

None of the 'dry tap' cases showed normal bone marrow by trephine biopsy in our case. It can be concluded that the finding of a 'dry tap' should never be dismissed as being due to faulty technique and always be subjected to a subsequent Bone marrow biopsy. An important limitation of bone marrow obtained by aspirate is diluted marrow which may not allow for reliable estimates of marrow cellularity.

Bone marrow aspiration is done in ITP to exclude other hematological malignancy [18]. Only 2 cases of ITP have undergone aspiration in the present study.

The increasing prevalence of multiple co-morbidities among anaemic patients with chronic diseases have made the use of serum ferritin (which is also an acute phase reactant) and transferrin saturation more challenging in diagnosing iron deficiency. Microscopic examination of bone marrow aspirate is the gold standard" for assessing marrow iron store. However, conventional Gale's method assesses iron in marrow fragments alone provides little valuable information about functional iron deficiency seen in many chronic diseases.

In the present study, minimum grade was seen in Aplastic anemia (grade 0-1) and in Iron deficiency anemia (grade 0-4).

Out of 7 cases of iron deficiency anemia, 06 showed low-absent iron store. However one case unusually showed grade 4 iron. This could possibly be explained by a history of prolonged blood transfusion and associated anemia of chronic disorder.

Bableshwar et al. [1] studied iron status category of the 80 patients by both, Gale's method and intensive method. Gale's grading method revealed hypoferremic state in 38.75% cases and normal iron stores in 61.25% cases. This closely matched with the intensive grading system, which demonstrated normal marrow iron store in 37.5% cases, depleted iron stores in 16 % cases, functional iron deficiency in 23% cases, and combined deficiency seen in 22% patients. This study and the study done by Pujara et al. [19] are comparable with the results of our study.

In dimorphic anemia 02 cases out of 15 cases showed grade 4 iron due to haematinic therapy and prior blood transfusions. 13 cases showed grade 1-3 iron this was comparable with other studies.

In present study higher grades, according to Gale's iron grading system, Grade 2-3 marrow iron was observed in Bone marrow infiltration, Chronic lymphocytic leukemia and in Parasitic infestation. Myelodysplastic syndrome showed iron grade in range of 3-5 and granulomatous disease showed Grade 5 iron.

Conclusion

Bone marrow is an important tool in diagnosing various hematological and non-hematological disorders. Its importance is further highlighted in cases where routine investigations fail to reach a conclusive diagnosis. Treatable conditions like nutritional anemia and malaria can be diagnosed and the mortality can be decreased. This procedure is also done for follow up of patients

undergoing chemotherapy, bone marrow transplantation and other forms of medical treatment.

Bone marrow iron in BM aspirates is the gold standard for assessing marrow iron store. Where sophisticated instruments and facilities are not available for measuring Iron in blood by chemical methods, this simple technique is efficient, cost-effective and result oriented.

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