Original Research Paper

Volume - 13 | Issue - 03 | March - 2023 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Pathology

COMPARATIVE STUDY OF CELLULAR MORPHOLOGY OF BONE MARROW ASPIRATION, IMPRINT SMEAR CYTOLOGY AND TREPHINE BIOPSY IN EVALUATION OF HEMATOLOGICAL DISORDERS

Priti Toppo	Postgraduate Student, Department of Pathology, Gandhi Medical College Hamidia Hospital, Bhopal.
Maneesh Sulya*	Associate Professor, Department of Pathology, Gandhi Medical College Hamidia Hospital, Bhopal.*Corresponding Author
Upasana Uniya	Assistant Professor Department of Pathology, Gandhi Medical College Hamidia Hospital, Bhopal.
R. K. Nigam	Professor, Department of Pathology, Gandhi Medical College Hamidia Hospital, Bhopal.
Reeni Malik	Professor and HOD, Department of Pathology, Gandhi Medical College Hamidia Hospital, Bhopal.
Rita Saxena	Associate Professor, Department of Medicine, Gandhi Medical College Hamidia Hospital Bhopal.

ABSTRACT **BACKGROUND**Hematological disorders encompass a broad spectrum of disorders affecting blood & bone marrow. Among a myriad of diagnostic tests that can be applied to the analysis of hematological diseases, bone marrow examination is one of the most valuable diagnostic tools. The Bone marrow aspirate and imprint & trephine biopsy specimens are complementary and when both are obtained, provide a comprehensive evaluation of the bone marrow. **AIMS/PURPOSE** To compare the cellular morphology of Bone marrow aspiration and imprint smears for diagnosis of hematological disorders and compare with biopsy which is taken as gold standard. **METHODS** A total number of 60 cases in whom bone marrow examination was indicated were evaluated by Bone marrow aspiration (BMA), bone marrow imprint (BMI) and bone marrow biopsy (BMBx). **RESULTS** The diagnosis of BMI correlated with the diagnosis of BMBx in 92% cases, which was higher than the value observed with BMA smears (75%). The spreading quality was better and cytological details were better appreciated in BMI as compared to BMA. All of these findings were reflected in the higher diagnostic accuracy of BMI than BMA. **CONCLUSION** Bone marrow aspiration and biopsy are important tools for evaluating haematological disorders. Bone marrow imprint should be a standard practice in bone marrow sampling procedure.

KEYWORDS : Bone marrow, aspiration, imprint, biopsy, pancytopenia

INTRODUCTION

Haematological disorders constitute a broad spectrum of disorders, in which patients can present with various clinical presentations, primarily or secondarily affecting blood or bone marrow may manifest with peripheral pancytopenia, bicytopenia, thrombocytopenia, thrombocytosis, leukopenia, leucocytosis or any morphological alteration.[1]

Pancytopenia includes diseases which are easily treatable to life threatening conditions.[2]

A multi-parameter stepwise approach is essential to reach the final diagnosis. Among a myriad of diagnostic tests that can be applied to the analysis of hematological diseases, Bone Marrow Examination is one of the MOST VALUABLE diagnostic tools.[3-4]

MATERIAL AND METHODS

The present study was a prospective observational study undertaken for a period of 18 months from January 2021 to June 2022 at Department of Pathology, Gandhi medical college, Bhopal. Patients of all age and sex were included in the study. All patients with indications of bone marrow examination in which all three i.e., aspiration, imprint & biopsy was done, were included in the study.

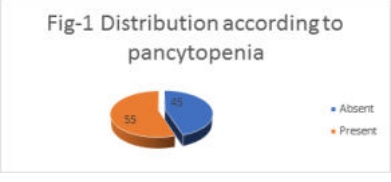
Bone marrow aspiration and biopsy was performed from posterior superior iliac spine using Salah's needle and Jamshidi's needle respectively. The biopsy imprints were made by gently touching and rolling the core biopsy in between two slides. Smears were fixed and stained by Leishman stain. Trephine biopsy specimens were fixed in formalin fixative then decalcified for 6 hrs. Biopsy sections after histological processing were stained by Haematoxylin and Eosin and reticulin stain. Aspiration smears were stain with Perl's stain for assessment of iron stores. The present study's aim was to compare the cellular morphology in aspiration, imprint and biopsy to diagnose various haematological disorders.

RESULTS

A total of 60 patients were studied who fulfilled the inclusion criteria. 31.7% were less than 20 years of age, 23.3% were more than 40 years of age, 25% cases were between 31-40 years of age and 20% were between 21-30 years of age. 55% cases presented with pancytopenia. There was a slight male preponderance.

Table 1 Distribution of patients according to pancytopenia

Pancytopenia	Frequency (n=60)	Percentage
Absent	27	45.0
Present	33	55.0



COMPARISON OF BONE MARROW CELLULARITY:

Bone marrow biopsy was taken as gold standard investigation for cellularity of marrow. Bone marrow biopsy and imprint revealed hypocellular marrow in 15 (25%) cases, whereas hypocellular on bone marrow aspirate was found in 11(18%) cases.

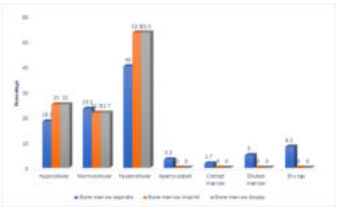
Cellularity of marrow was correctly identified by bone marrow imprint in all cases. Clotted marrow, diluted marrow and dry tap in bone marrow aspirate limited its utility in assessing the cellularity.

Table-2 Comparison of cellularity in Bone marrow aspirate, imprint and biopsy.

Cellularity	Bone marrow aspirate		Bone marrow imprint		Bone marrow biopsy	
	n	%	n	%	n	%

Hypocellular	11	18.3	15	25.0	15	25.0
Normocellular	14	23.3	13	21.7	13	21.7
Hypercellular	24	40.0	32	53.3	32	53.3
Aparticulated	2	3.3	0	0	0	0
Clotted marrow	1	1.7	0	0	0	0
Diluted marrow	3	5.0	0	0	0	0
Dry tap	5	8.3	0	0	0	0
$\chi 2$	81.33		120.0		Standard	
P value	0.0001		0.0001		Standard	

Fig-2 Comparison of cellularity between bone marrow aspirate, bone marrow imprint and bone marrow biopsy



COMPARISON OF ERYTHROID AND MYELOID SERIES IN BONE MARROW ASPIRATE, IMPRINT AND BIOPSY:

Erythroid series in imprint showed 66.7% megaloblastic, 21.7% dimorphic and 11.6% normoblastic, whereas 60% megaloblastic 10% normoblastic and 20% dimorphic in aspiration. 3 cases of dyserythropoiesis missed in aspiration. Myeloid series was seen almost similar in aspiration and imprint except in 4 cases which showed granuloma and higher plasma cells number in imprint. These findings were missed in aspiration. A statistically significant correlation of bone marrow biopsy findings with aspirate and imprint findings in the erythroid and myeloid series was reported. Imprint findings were slightly more similar to biopsy than aspiration.

Table-3 Comparison of cellular reaction in bone marrow aspirate, imprint and biopsy

Reaction	Bone marrow aspirate		Bone marrow imprint		Bone marrow biopsy	
	n	%	n	%	n	%
Normoblastic	6	10.0	7	11.7	7	11.7
Megaloblastic	36	60.0	40	66.7	40	66.7
Dimorphic	12	20.0	13	21.7	13	21.7
Clotted marrow	1	1.7	0	0	0	0
Dry tap	5	8.3	0	0	0	0
$\chi 2$	92.6		120.0		Standard	
P value	0.0001		0.0001		Standard	

Fig-3 Comparison of cellular reaction between bone marrow aspirate, bone marrow imprint and bone marrow biopsy

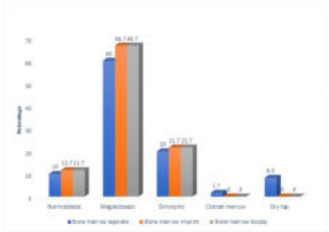


Table-4 Comparison of erythroid series in bone marrow aspiration, imprint and biopsy

Erythroid series	Bone marrow aspirate		Bone marrow imprint		Bone marrow biopsy	
	n	%	n	%	n	%
Clotted marrow	1	1.7	0	0	0	0
Dry tap	4	6.7	0	0	0	0
Decreased in number	0	0	1	1.7	1	1.7
Hyperplasia	3	5.0	3	5.0	3	5.0
Hyperplasia Dyserythropoiesis	15	25.0	15	25.0	15	25.0
Hyperplasia Megaloblasts Dyserythrpoiesis	1	1.7	1	1.7	1	1.7
Hypoplasia	19	31.7	22	36.7	22	36.7
Hypoplasia Dyserythropoiesis	6	10.0	7	11.7	7	11.7
Hypoplasia, Few megaloblasts	1	1.7	1	1.7	1	1.7
Normal in number and maturation	9	15.0	7	11.7	7	11.7
Normocellular. Dyserythropoiesis	1	1.7	3	5.0	3	5.0
$\chi 2$	388.5		480		Standard	
P value	0.0001		0.0001		Standard	

Table-5 Comparison of Myeloid series between bone marrow aspirate, bone marrow imprint and bone marrow biopsy

Myeloid series	Bone marrow aspirate		Bone marrow imprint		Bone marrow biopsy	
	n	%	n	%	n	%
Clotted marrow	1	1.7	0	0	0	0
Dry tap	4	6.7	0	0	0	0
Hyperplasia	3	5.0	3	5.0	3	5.0
Hyperplasia Plasma cells increased	1	1.7	2	3.3	2	3.3
Hyperplasia, Basophilia Eosinophilia	2	3.3	1	1.7	1	1.7
Hyperplasia Blasts increased	5	8.3	5	8.3	5	8.3
Hyperplasia Blasts increased Basophilia	1	1.7	1	1.7	1	1.7
Hyperplasia Eosinophils increased	2	3.3	1	1.7	1	1.7
Hyperplasia Promyelocytes markedly increased	0	0	1	1.7	1	1.7
Hyperplasia Giant myeloid precursors	9	15.0	11	18.3	11	18.3
Hyperplasia Lymphocytes markedly increased	1	1.7	1	1.7	1	1.7
Hyperplasia Normal in maturation	1	1.7	1	1.7	1	1.7
Hyperplasia Shift to left	4	6.7	2	3.3	2	3.3
Hyperplasia Toxic granulation	1	1.7	0	0	0	0
Hyperplasia Toxic granulation. Shift to left	1	1.7	1	1.7	1	1.7
Hyperplasia Granuloma	1	1.7	3	5.0	3	5.0
Hyperplasia Basophilia Eosinophilia Blasts increased	0	0	1	1.7	1	1.7
Hyperplasia Hairy cells.	0	0	1	1.7	1	1.7
Hypoplasia	11	18.3	11	18.3	11	18.3
Hypoplasia Dysmyelopoiesis Shift to left	3	5.0	3	5.0	3	5.0
Hypoplasia Lymphocytosis	0	0	1	1.7	1	1.7
Hypoplasia Occassional myeloid cells seen	0	0	1	1.7	1	1.7
Normal in number and maturation	6	10.0	6	10.0	6	10.0
Normocellular Eosinophilia	1	1.7	0	0	0	0
Normocellular Giant myeloid precursors	0	0	2	3.3	2	3.3
Normocellular Plasma cells increased	1	1.7	1	1.7	1	1.7
Normocellular Toxic granulation. Increased plasma cells.	1	1.7	0	0	0	0
$\chi 2$	938.3		1260		Standard	
P value	0.0001		0.0001		Standard	

Table-5 Comparison of Megakaryocyte series between bone marrow aspirate, bone marrow imprint and bone marrow biopsy

Megakaryocyte series	Bone marrow aspirate		Bone marrow imprint		Bone marrow biopsy	
	n	%	n	%	n	%
Almost absent	1	1.7	2	3.3	1	1.7
Clotted marrow	1	1.7	0	0	0	0
Dry tap	4	6.7	0	0	0	0
Dysmegakaryopoiesis, Emperipolesis	1	1.7	1	1.7	1	1.7
Hyperplasia	2	3.3	2	3.3	2	3.3
Hyperplasia Dysmegakaryopoesis	13	21.7	13	21.7	13	21.7
Hyperplasia Dysmegakaryopoiesis Micromegakaryocytes	0	0	2	3.3	2	3.3
Hyperplasia Hypolobated megakaryocytes	1	1.7	1	1.7	1	1.7
Hyperplasia Normal in maturation	2	3.3	2	3.3	2	3.3
Hyperplasia Unlobated megakaryocytes	4	6.7	4	6.7	4	6.7
Hypoplasia	10	16.7	13	21.7	14	23.3
Hypoplasia Dysmegakaryopoiesis.	3	5.0	3	5.0	3	5.0
Hypoplasia Emperipolesis	1	1.7	0	0	0	0
Hypoplasia Hemophagocytosis	1	1.7	2	3.3	2	3.3
Hypoplasia Micromegakaryocytes	2	3.3	2	3.3	2	3.3
Normal in number and maturation	10	16.7	10	16.7	10	16.7
Normocellular Dysmegakaryopoesis	4	6.7	3	5.0	3	5.0
$\chi 2$	662.3		747.8		Standard	
P value	0.0001		0.0001		Standard	

COMPARISON OF MEGAKARYOCYTE SERIES IN BONE MARROW ASPIRATE, IMPRINT AND BIOPSY:

A statistically significant correlation of bone marrow biopsy findings with aspirate and imprint findings for identification of features of megakaryocytic series. 4 cases of hyperplasia, dysmegakaryopoiesis and hypoplasia missed in aspiration. 1 case of megakaryocytic hypoplasia missed in imprint.

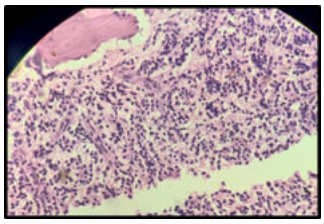
IMPRINT CORRELATING MORE WITH BIOPSY as compared to aspirate.

Bone marrow aspirate could not identify aplastic anemia in any case, 1 out of 5 cases of acute leukemia, 1 out of 2 cases of Lymphoproliferative disorder, 2 out of 3 cases of multiple myeloma, 2 out of 14 cases of megaloblastic anemia; 2 out of 3 cases of granulomatous disease and 1 out of 2 cases of hemophagocytic lymphohistiocytosis. Bone marrow imprint could not identify 6 out of 11 cases of aplastic anemia.

Table-6 Comparison of final impression in bone marrow aspirate, imprint and biopsy

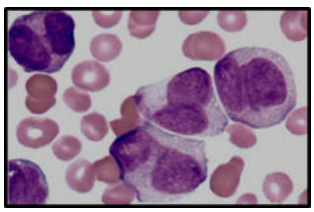
Impression	Bone marrow biopsy		Bone marrow aspirate		Bone marrow imprint	
	n	%	n	%	n	%
Acute leukemia	5	8.3	4	6.7	5	8.3
Chronic myeloid leukemia	5	8.3	5	8.3	5	8.3
Lymphoproliferative disorder	2	3.3	1	1.7	2	3.3
Myelodysplastic Syndrome	2	3.3	2	3.3	2	3.3
Multiple myeloma	3	5.0	1	1.7	3	5.0
Immune thrombocytopenia	4	6.7	4	6.7	4	6.7
Dimorphic anemia	4	6.7	5	8.3	4	6.7
Reactive to infection	5	8.3	10	16.7	5	8.3
Aplastic anemia	11	18.3	0	0	5	8.3
Megaloblastic anemia	14	23.3	12	20.0	14	23.3
Granulomatous disease	3	5.0	1	1.7	3	5.0
Hemophagocytic lymphohistiocytosis	2	3.3	1	1.7	2	3.3
Clotted marrow	0	0	1	1.7	0	0
Dry tap	0	0	2	3.3	0	0
Hypocellular marrow	0	0	11	18.3	6	10.0

IMAGE-1



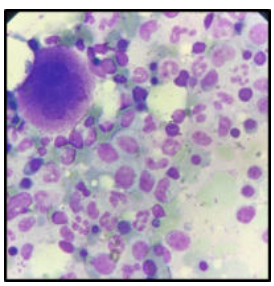
Bone marrow biopsy showing hypercellularity.

IMAGE-2



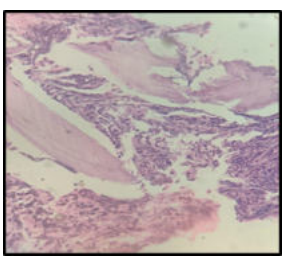
Bone marrow imprint smear shows promyelocytes hypogranular variant of APML

IMAGE-3



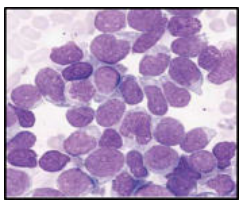
Bone marrow imprint showing hypolobated megakaryocyte.

IMAGE-4



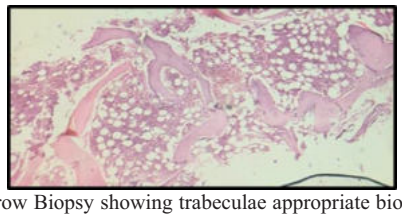
Bone marrow biopsy showing increased fibrosis.

IMAGE-5



Bone marrow aspiration showing blasts.

IMAGE-6



Bone Marrow Biopsy showing trabeculae appropriate biopsy should have minimum 5 bony trabeculae.

DISCUSSION

Aspiration and imprint are invaluable tools for assessing the cytomorphological details of the cellular elements of the marrow. Both these modalities are helpful in diagnosis of many hematological disorders. Bone marrow biopsy helps in assessment of cellularity of marrow, distribution of cellular elements and degree of fibrosis within the marrow. Bone marrow biopsy, imprint and aspiration are complementary to each other. We assessed and compared the three modalities in terms of cellularity, reaction, erythroid series, myeloid series and Megakaryocyte series. We observed bone marrow imprint to be as effective as biopsy in assessing the cellularity, reaction, erythroid series and myeloid series. We observed cellularity as well morphology to be BETTER IN IMPRINT as compared to aspirate, taking biopsy as standard. Pant S et al (2020) included 63 cases with hematological malignancies, and documented the diagnostic accuracy of aspirate as 84.12% and that of imprint was 95.23%5 Taori G et al (2019) documented correlation of biopsy with aspiration in 79.28% and biopsy with imprint in 87.39% cases.6 Chandra S et al (2011), reported correlation of BMA and BMI with BMB in 78% and 84.3% cases respectively.[7]

CONCLUSION

The study concludes that all the three preparations of aspirate, touch imprint & trephine biopsy are complementary to each other for evaluating any bone marrow. Vigilant examination of aspirate smears and meticulously prepared imprint cytology smears are almost equally efficient and rapid method. Bone marrow imprint was found to be more superior than aspiration cytology, in diagnosing the cases. Biopsy specimens can be preserved for IHC and sent to higher centers later. Bone marrow aspirate and imprint smears stained by Leishman or Romanowsky staining can be very much useful for diagnosing hematological disorders quickly as well as they can be performed at peripheral centers easily which will initiate not only treatment but also referral to higher centers, if needed for benefits of patients.

REFERENCES

1. McKenzie Clinical Laboratory Haematology. New Jersey: Pearson Education publishers; 2004. pp. 43- 46.
2. Kishore Khodke, S Marwah, G Buxi, RB Yadav, NK Chaturvedi. Bone Marrow Examination in Cases of Pancytopenia. Journal of Indian Academy of Clinical Medicine 2001;1: 55-59.
3. Bain BJ. The bone marrow aspirate of healthy subjects. Br J Haematol 1996; 94:206–9.
4. Candido A, Rossi P, Menichella G, et al. Indicative morphological myelodysplastic alterations of bone marrow in overt AIDS. Haematologica 1990;75:327–33.
5. Pant S, Dhingra H, Gupta A, Misra RK. Comparative Analysis of Bone Marrow Aspiration Smears, Touch Imprints and Trephine Biopsy in Haematological Malignancies. International Journal of Contemporary Medical Research 2020;7(6):F12-6.
6. Taori G, Ukey A, Bajaj P. Comparison of Bone Marrow Aspiration Cytology, Touch Imprint Cytology and Bone Marrow Biopsy for Bone Marrow Evaluation at a Tertiary Health Care Institute. MVP Journal of Medical Sciences. 2019 Dec 1:152-7
7. Chandra S, Chandra H. Comparison of bone marrow aspirate cytology, touch imprint cytology and trephine biopsy for bone marrow evaluation. Hematology reports. 2011 Oct;3(3):e22.