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Original Research Article

Utility of Bone Marrow Examination in Hematological Disorders

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ABSTRACT

Background: Bone Marrow Aspiration (BMA) and Bone Marrow Trephine Biopsy (BMB) are used to diagnose various hematological disorders. BMA is useful for study of cell cytology; material can also be used for ancillary tests like cytogenetics, flow cytometry, which improves the diagnostic utility. BMB allows complete assessment of marrow architecture along with pattern of distribution of abnormal infiltrates. Thus, BMA & BMB serve as complimentary procedures to diagnose various hematological disorders if performed simultaneously.

Material and methods: Hospital based prospective study done within the duration of 2 years starting from August 2020 to August 2022. All the patients who underwent bone marrow examination at Department of Pathology, Tertiary Care Hospital of Ahmedabad, Gujarat, India were included. BMA was done using Salah needle under aseptic precautions from posterior superior iliac spine. 8-10 aspirate smears were prepared, stained by Romanowsky stains. BMB was also done in all cases using Jamshidi needle from same site. The biopsy was processed and slides were stained by Hematoxylin and Eosin (H&E). Evaluation was done by two pathologists.

Results: Total 106 patients were included. Maximum number of patients belonged to 50-59 years age group followed by 60-69 years of age with M:F ratio of 1.6:1. Leading clinical feature was weakness/fatigue followed by pallor/anemia & fever. Megaloblastic Anemia (38%) was the most common hematological disorder in study population followed by Acute Leukemia (11%). 80% positive correlation between BMA & BMB was identified to diagnose various disorders.

Conclusion: Megaloblastic anemia is the most common hematological disease in study population & can present with variable cytopenia. Accurate diagnosis depends on BMA & BMB evaluation along with peripheral smear & biochemical immunoassays. The role of BMA & BMB remains untouched in the diagnosis of various hematological disorders in-spite of newer methods like immunohistochemistry, flow cytometry, cytogenetics and molecular assays.

Keywords: Trephine Biopsy, Hematological Disorders, Bone Marrow Aspiration

INTRODUCTION

Bone marrow examination is used for the diagnosis of various hematological disorders. The two techniques used for the bone marrow examination are: Bone Marrow Aspiration (BMA) and Bone Marrow Trephine Biopsy (BMB).[1]

The Salah and Klimah are bone marrow aspiration needles used to aspirate the bone marrow from either the sternum or posterior superior iliac crest.[2] The

technique for aspiration is simpler and less risky than bone marrow biopsy. The BMA can be used for routine morphological study, as an adjunct to peripheral smear examination for evaluating both hematologic and non-hematologic disorders as well as for cytogenetic, immuno-histochemical and flow cytometric studies. This improves its diagnostic value.[3] BMA is done for diagnostic, therapeutic or for follow-up purposes. It helps in diagnosing various types of anemias, multiple myeloma, leukemias with their sub-typing, staging of lymphomas, checking response to therapy etc. Diagnosis can be made on bone marrow aspiration except in case of dry tap.

BMB allows complete assessment of marrow architecture along with pattern of distribution of abnormal infiltrates.[3] Use of Local anesthesia reduced the pain in patients undergoing biopsy.[2] BMB is essential in cases of inadequate marrow aspirate, myeloproliferative disorders, myelodysplastic syndromes, bone marrow fibrosis, plasma cell dyscrasias, granulomatous lesions, aplastic anemia and metastatic tumor. It serves as the most reliable method to assess marrow cellularity following chemotherapy and to assess status of engraftment after bone marrow transplantation.[3] A touch preparation is important when aspirate is not obtained as it allows cytological details to be studied. It may also demonstrate bone marrow infiltration when it is not detected in an aspiration like in hairy cell leukemia, lymphoma or multiple myeloma.[4,5] BMB is not a substitute, but a complimentary procedure for BMA to diagnose various hematological disorders.

MATERIAL AND METHODS

Study Type: Hospital based observational prospective study.

Study site: Department of Pathology, tertiary care hospital, Ahmedabad, Gujarat, India.

Study duration: 2 years starting from August 2020 to August 2022.

Sample size: 106 patients underwent bone marrow examination.

Ethical clearance: The study was conducted after approval from Institutional Ethical Committee.

Study population: All the patients with suspected haematological disorders of any age & sex fulfilling below criteria:

Inclusion Criteria: All age groups, any sex, Patients with anemia or erythrocytosis, thrombocytopenia or thrombocytosis, leukocytopenia or leukocytosis, pancytopenia or bicytopenia, panmyelosis, hepato-splenomegaly, plasma cell dyscrasia, suspected for lymphoma, suspected for infectious diseases or metastasis, post transplantation.

Exclusion Criteria: History of radiotherapy/ chemotherapy, Unwilling patients, Patients with bleeding/local infection, recent blood transfusion.

Study method: Informed written consent was taken for BMA and BMB from the patient or guardian in cases of minor. Complete blood count, reticulocyte count, prothrombin time, HIV, HBsAg tests were done before procedure.

BMA was done using Salah needle no.16 for adults and no.18 for children under aseptic precautions. Aspirate was withdrawn with a 10 ml disposable plastic syringe from posterior superior iliac spine. 8-10 aspirate smears were prepared, air dried, fixed and later stained by Leishman, Giemsa or Field stain. BMB was also done in all cases at nearby site by using Jamshidi needle with trocar and cannula. After taking impression, biopsy material (at least 1.5-2cm long) was sent to histopathology lab in 10% neutral buffered formalin, decalcified in 5% nitric acid, processed in routine paraffin embedding, 4-5µm sections were cut by Leica RM 2125 microtome. The slides were stained by Hematoxylin and Eosin (H&E) and then reported by two pathologists with consensus. Reticulin stain was performed to assess marrow fibrosis.

Data collection: All data including age, sex, hemoglobin, reticulocyte count, total white cell and platelet counts, clinical signs and symptoms were collected from clinical case records of patients. BMA, BMB diagnosis were collected from laboratory records.

Statistical analysis: Data was entered in Microsoft excel sheets in computer. The statistical calculations were done using a Statistical Package for the Social Sciences Version 21 (SPSS, IBM company, Chicago, USA). The results were analyzed using frequency distribution. The cases were also distributed according their age, sex and presenting clinical signs and symptoms. BMA and BMB findings were also compared and analyzed.

RESULTS

The present study was carried out in Pathology department of tertiary care hospital in Ahmedabad, Gujarat, India. It lasted for 2 years starting from August 2020 to August 2022. Total 106 patients diagnosed or suspected of hematological disorders were included.

Figure 1 shows maximum (24) number of patients belonged to 50-59 years age group followed by 19 patients of 60-69 years of age. Figure 2 indicates male patients were more in number compared to females and M:F ratio was 1.6:1.

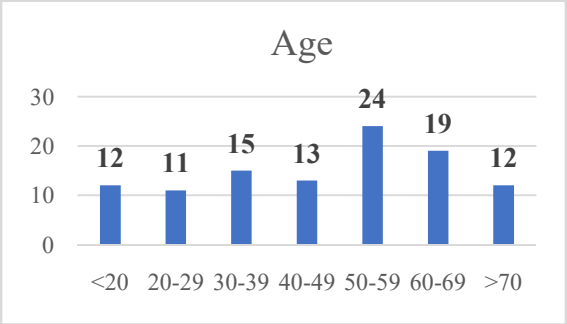

Figure-1: Frequency of Age Distribution

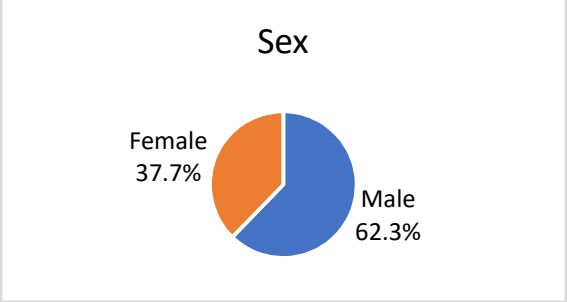

Figure-2: Frequency of Sex Distribution

Table 1 shows weakness/fatigue as leading clinical variable followed by pallor/anemia & fever. The analysis of hematological parameters of 106 patients showed 50 patients had severe anemia (Hb<7g/dl) & 45 patients had moderate anemia (Hb 7-10g/dl) while rest 11 patients had mild anemia (Hb 10-12g/dl). 56 patients had leucopenia (Total leukocyte count [TLC]<4000/cumm), 27 had normal count (TLC-4000-11000/cumm) & 23 patients had leukocytosis (TLC>11000/cumm). 77 patients had platelet count <1 lac/cumm and 29 patients had platelet count >1 lac/cumm.

Table-1: Frequency of Clinical Variables (n=106)

Clinical Variables	Frequency	Percentage
Weakness/Fatigue	51	48.11
Pallor/anemia	40	37.74
Fever	38	35.85
Hepatomegaly + Splenomegaly	19	17.92
Abdominal pain	17	16.04
Splenomegaly	12	11.32
Bone pain	09	8.49
Bleeding/hematemesis	08	7.55
Weight loss	07	6.60
Vomiting	06	5.66
Headache	05	4.72
Petechiae	04	3.77
Lymphadenopathy	02	1.89
Deep vein thrombosis	02	1.89

Table 2 indicate megaloblastic anemia as most common hematological disorder in present study population accounting 40 cases with 38% frequency. The second common disorder was acute leukemia including Acute Myeloid Leukemia & Acute Lymphoid Leukemia accounting 11% frequency.

Table-2: Hematological Diagnosis (n = 106 Cases)

Sr. No.	Diagnosis	No. of cases	BMA	BMB	Concordance of BMA & BMB (%)
1	Megaloblastic anemia (37.74%)	40	34	40	85.0%
2	Acute leukemia (11.32%)	12	10	12	83.3%
3	CML(8.49%)	09	09	09	100%
4	Multiple myeloma (8.49%)	09	09	09	100%
5	MDS (6.60%)	07	07	05	71.4%
6	ITP (5.66%)	06	06	06	100%
7	Aplastic anemia (3.77%)	04	-	04	
8	CLL (3.77%)	04	03	04	75.0%
9	Lymphoma (1.89%)	02	01	02	50.0%
10	Essential Thrombocythemia(1.89%)	02	02	02	100%
11	Marrow Hypoplasia (1.89%)	02	-	02	
12	Polycythemia vera (0.94%)	01	01	01	100%
13	Normocellular (3.77%)	04	04	04	
14	Inconclusive (3.77%)	11	11	06	
15	Dry tap	09	09	-	
	Total		106	106	79.25%

(Abbreviations- CML- Chronic Myeloid Leukemia, MDS- Myelodysplastic Syndrome, ITP- Immune Thrombocytopenic Purpura, CLL- Chronic Lymphocytic Leukemia)

Dry tap was seen in 09 cases on BMA. Causes of dry tap were packed marrow due to hypercellularity, hypocellularity, fatty degeneration of marrow or due to increased fibrosis. They were later on diagnosed on BMB as aplastic anemia (04 cases), acute leukemia (02 cases), lymphoma (01 case) and hypoplastic marrow (02 cases). Such findings indicate complimentary role of BMA and BMB if performed together. It also shows role of BMB in assessing cellularity, detection of focal lesion, marrow fibrosis and fat.

Total 11 BMA were inconclusive due to dilution by peripheral blood and/or attributed to faulty technique/inadequate material. Six cases were also not reaching concluding interpretation by BMB due to very small biopsy length as sometimes tissue gets fractured or crushed during procedure. In these cases, repeat BMB was not possible due to denial of

consent by patient & it is also not advisable to repeat at the same site within 4-6 months. 80% positive correlation between BMA & BMB was identified in present study.

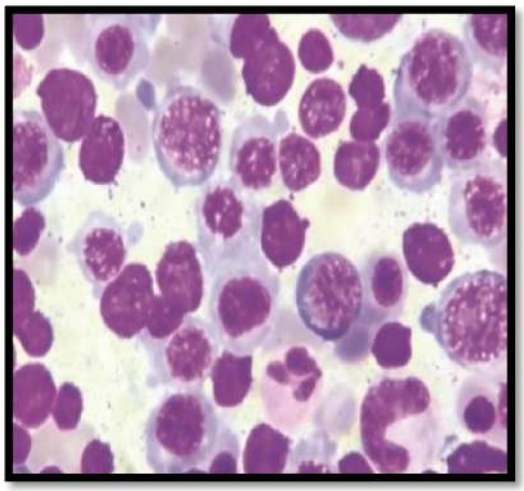

Figure 3: Megaloblastic anemia BMA: Megaloblast with sieve like chromatin, Leishman stain, 100x.

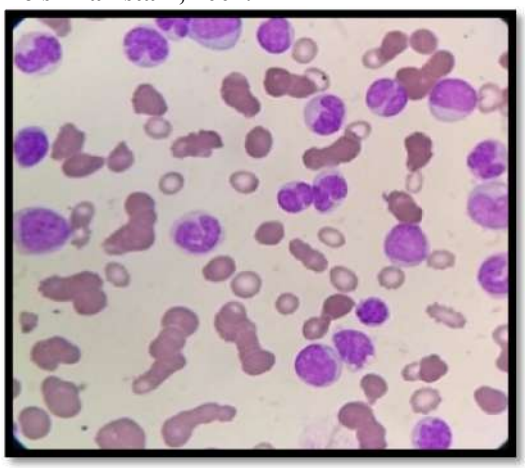

Figure 4: Acute Myeloid Leukemia BMA: Myeloblast, Field stain, 100x

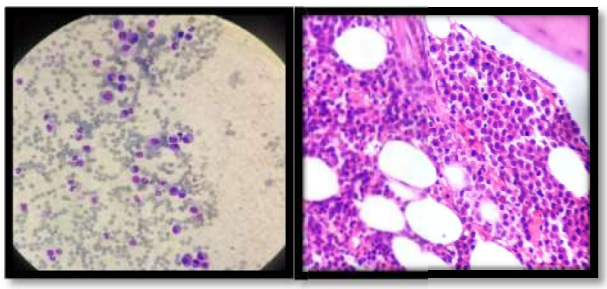

Figure-5: Plasma cell dyscrasia BMA and BMB: plasma cells infiltration, Field stain and H & E stain, 40x

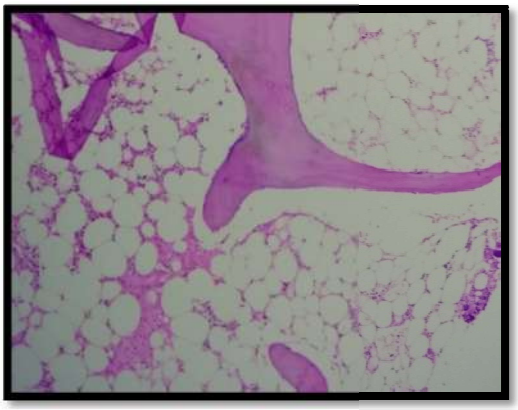

Figure-6: Aplastic anemia BMB: Hypoplastic marrow with increased fat spaces, H & E stain, 10x

DISCUSSION

Bone Marrow Examination is very important to diagnose various hematological as well as non-hematological diseases. The aspiration is mainly used for cytological details while biopsy is useful for cellularity, fibrosis, architectural pattern, metastatic deposits and suitable for immunohistochemistry. These can also be used in follow up of patients taking chemotherapy to check the response to therapy. In case of dry tap due to some pathology in marrow, bone marrow biopsy becomes absolute indication.[3]

In this study, 106 cases of hematological disorders were studied and analyzed for age, sex, clinical variables, BMA & BMB findings. The results were compared with other similar studies.

In this study, maximum number of cases was seen in the age group of 50-59 years (25.44%) and the least in age group of 20-29 years. They were very well comparable with findings in studies of Gilotra M et al[1] and Shah R et al.[6] Male to female ratio was1.6:1 which was in concordance with male preponderance seen in other studies.[1,6,7]

The most common presenting complaint in this study was generalized weakness in 48% of the cases, followed by fever in 36% cases. Pallor was the most common physical sign seen in 38% of cases, followed by hepatosplenomegaly in 18% cases. These findings were close to findings obtained by studies of Shah R et al[6] and Vaidya S et al.[7]

Table-3: Comparison of Findings of Various Studies

Sr. No.	Study	Country	Publishing Year	Number of cases	Commonest hematological disease	Second most common disease
1.	Vaidya S et al[7]	Nepal	2015	83	Megaloblastic anemia	Aplastic anemia
2.	Gilotra M et al[1]	India	2017	100	Anemia	Acute leukemia
3.	Shah R et al[6]	India	2019	145	Megaloblastic anemia	Hypoplastic anemia
4.	Shah N et al[8]	India	2021	74	Megaloblastic anemia	Aplastic anemia
5.	Present study	India	2022	106	Megaloblastic anemia	Acute leukemia

Most common hematological disorder in present study was megaloblastic anemia followed by acute leukemia & these were similar to other Indian studies done by Gilotra M et al[1], Shah R et al[6], Vaidya S et al[7] and Shah N et al[8] (Table 3). Thus, indicating that megaloblastic anemia is widely prevalent hematological disease in India. It is due to Vitamin B12 or folate deficiency. Causes can be decreased oral intake, excess vegetarian food intake, malabsorption states or due to chronic inflammatory disorders of the intestine like chronic diarrhea/parasitic infestations.[7]

Megaloblastic anemia comprises 40 cases in present study. Out of which 34 were diagnosed on BMA but rest 6 cases were of diluted smears may be due to faulty technique leading to inconclusive interpretation, later diagnosed on BMB by histopathogical features of hypercellularity & megaloblastoid erythroid hyperplasia suggesting Vitamin B 12 deficiency. They were very well correlated with clinical diagnosis of Vitamin B12 deficiency anemia.

Acute leukemia cases were 12 in present study including Acute Myeloid Leukemia (AML) & Acute Lymphoid Leukemia (ALL). AML was common in older adults while ALL in children.

10 out of 12 cases were showing blasts >20% in BMA leading to diagnose as acute leukemia. Rest 02 cases were of dry tap on BMA due to hypercellularity later diagnosed by BMB with help of Immunohistochemistry. Positive correlation between BMA & BMB in diagnosing acute leukemia was 83.3% and very well compared with studies like Gilotra et al[1], Shah R et al[6] and Thakur S et al[9].

All cases of Chronic Myeloid Leukemia (CML) & Multiple Myeloma showed 100% positive concordance between BMA & BMB establishing complementary role of both of them. Similar results were noted in studies of Gilotra M et al[1] and Shah R et al.[6] BMA is essential in CML to classify phases compared to biopsy while in multiple myeloma it is for the evaluation of plasma cell differentiation. However, BMB provide additional information in both disorders about pattern & extent of involvement by atypical cells.[1]

Suspected aplastic anemia (04) cases had dry tap on marrow aspiration. On BMB they showed hypocellularity or acellularity with increase in fat cells and lymphoplasma cells without fibrosis or abnormal cells infiltration. These results were similar with Gilotra M et al[1] and Shah R et al.[6]

Immune Thrombocytopenic Purpura cases were 6 and shows 100% complementary role of BMA & BMB which was well correlated with other authors results.[1,6] They showed mature & immature megakaryocytic hyperplasia without dysplasia in bone marrow examination while peripheral smear showing thrombocytopenia. It establishes immune etiopathogenesis of disease.

Limitations

In the present study**,** inconclusive cases were more. They could be attributed to procedures performed by trainee doctors. It has small sample size over a defined period of time, so results obtained from this study are not generalizable to society.

Future implications

Vitamin B12 deficiency or Megaloblastic anemia is very common in India. So, supplementation of food for vegetarian population in India is recommended to prevent its occurrence.

CONCLUSION

Bone marrow examination is a rapid outpatient procedure with minimal patient discomfort. Aspiration smears are used for cytological diagnosis and trephine biopsies for histological diagnosis as cellularity, architectural patterns and fibrosis are better visualized. Metastatic deposits and focal lesions have better diagnostic yield on BMB. Both procedures are complementary and cost effective to each other if performed simultaneously. BMB becomes very important for diagnosis in cases where dry tap is obtained or in unsatisfactory smears of aspirate.

Megaloblastic anemia is the most common hematological disease in India and many times, it presents with cytopenia. The causes of which are diverse and diagnosis depends on family, personal, drugs & alcohol history. The accurate diagnosis depends on bone marrow examination in addition to peripheral smear & biochemical immunoassays. The role of BMA & BMB remains untouched in diagnosis of various hematological disorders despite newer methods like immunohistochemistry, flow cytometry, cytogenetic analysis and molecular assays.

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