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Original Research Article

Utility of bone marrow aspiration and trephine biopsy in various haematological malignancies

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ABSTRACT

Background: The bone marrow examination is a useful and cost-effective diagnostic procedure in haematological practice for the diagnosis of haematological disorders. It may either confirm the clinically suspected disease or may provide the previously unsuspected diagnosis. The bone marrow examination can be done by bone marrow aspiration as well as by performing bone marrow biopsy. The aim of the study is to compare the diagnostic accuracy and rate of concordance between two modalities of bone marrow examination in diagnosis of haematological malignancies.
Methods: The study was conducted at haematology section of the Department of Pathology, Government Medical College, Jammu spanned over a period of 2.5 years from June 2020 to December 2022. The clinical data along with physical examination, relevant haematological, biochemical and radiological investigations were also reviewed. After taking the informed consent bone marrow aspiration and bone marrow biopsy were done under aseptic precautions.
Results: A total of 250 cases of haematological malignancies were studied over a 2.5 year period. The most common haematological malignancy was found to be leukemia 194 (77.6%) cases, followed by plasma cell dyscrasia with 30 (12%) cases, lymphomas and myeloproliferative disorders each with 11 (4.4%) cases and metastatic deposits 4 (1.6%) cases. In the present study, 64/250 (25.6%) patients underwent BMA and trephine biopsy simultaneously. A positive concordance was seen in 46 (71.8%) of the cases between the two methods and diagnostic discordance was observed in 10 (16.3%) of the cases.
Conclusions: Bone marrow aspiration and biopsy both complement each other and should be evaluated simultaneously. This study emphasizes the need for greater vigilance in the early diagnosis and an interdisciplinary approach for the effective management of patients as well as inclusion of trephine biopsy as regular procedure for complete evaluation of patients with haematological malignancies.

Keywords: Aspiration, Biopsy, Bone marrow, Concordance

INTRODUCTION

Haematological diseases encompass spectrum of various non neoplastic and neoplastic disorders of blood and blood forming organs. These disorders are common among all age groups and usually range from anemia to malignancies. As a large organ mass, bone marrow is not only the source of many haematological diseases but also a site of involvement of some infections, solid organ metastases and metabolic diseases.[1] In majority of cases, diagnosis is usually made by obtaining complete history, physical and some basic haematological investigations.

Hematological malignancies are broadly divided into myeloid neoplasms, lymphoid neoplasms (leukemia and lymphoma), lymphoproliferative disorders and histiocytic/dendritic cell neoplasms.

The bone marrow examination is a useful and cost effective diagnostic procedure in haematological practice

for the diagnosis of haematological disorders. It may either confirm the clinically suspected disease or may provide the previously unsuspected diagnosis. The bone marrow examination can be done by bone marrow aspiration as well as by performing bone marrow biopsy.[2]

Bone marrow aspiration is a quick technique for marrow evaluation in acute leukaemia and myelodysplastic syndrome, where an accurate enumeration of the blasts is essential for diagnosis. However, in cases of diluted blood smears, dry tap or unsatisfactory marrow aspirate. Marrow trephine biopsy plays an important role for reaching the diagnosis. However, bone marrow aspiration smears and biopsy sections even though perform simultaneously are often assessed at different point of time.[3] Bone marrow biopsy is a lengthy process and bone marrow aspiration is a rapid technique for malignancy evaluation. Most of the time, the results of bone marrow aspiration and bone marrow biopsy are concordant but the disparity is also seen in certain cases.

We at the haematology section of GMC Jammu, attempted the diagnostic accuracy and rate of concordance between these two procedures to ascertain whether simultaneously assessment of bone marrow aspiration smears and biopsy sections serve as a better approach to solve the diagnostic dilemma and improves diagnostic accuracy or not.

METHODS

The study was conducted at haematology section of the Department of Pathology, Government Medical College, Jammu spanned over a period of 2 ½ years from June 2020 to December 2022. All those patients visiting government medical college, Jammu with history and clinical features like pallor, fatigue, bleeding in the form of bruise or petechiae, persistent fever, bone or joint pains with or without organomegaly and/or lymphadenopathy and pancytopenia were taken up for detailed haematological evaluation.

Inclusion criteria

All patients who underwent simultaneous bone marrow aspiration and trephine biopsy were included in study.

Exclusion criteria

Patients with severe thrombocytopenia with platelet count <20,000/cumm or prolonged PT, INR or severe bleeding were excluded.

After taking ethical approval and written informed consent of the patients, bone marrow aspiration was performed on posterior superior iliac spine under all aseptic precautions by using Salah's aspiration needle. After a test dose for hypersensitivity reaction, skin, subcutaneous tissue and periosteum overlying the selected site was infiltrated with 2% xylocaine (2-5 ml). With boring movement bone marrow aspiration needle was passed perpendicularly into the cavity of the ilium at the centre of the oval posterior iliac spine. Decreased resistance indicated penetration of cortex and entry into the marrow cavity. Stillette was removed when the bone was penetrated. The 10 ml syringe was attached and 1ml marrow contents were sucked into the syringe. The 3-5 cm long marrow smears were prepared by using 2 cm wide smooth edged glass spreader. The marrow fragments were dragged behind the spreader and trail of cells was left behind. After drying, the smears were stained with May Grunwald Giemsa stain. Special stains like myeloperoxidase, PAS, NSE etc were used, wherever required.[4]

Bone marrow trephine biopsy was done in selected cases that had dry tap, blood tap and aspiration showing inadequate material and also in cases of myelodysplastic syndrome, myeloproliferative disorders, lymphomas and some cases of leukemia. Bone marrow trephine biopsy was performed by using the Jamshidi needle from the same site. It was obtained by inserting the biopsy needle into the bone and using to and fro rotation to obtain a core of tissue. Imprint smears were taken before the specimen was transferred into the fixative. The bony core was gently dabbed or rolled across the slide, which was then fixed and stained with MGG stain to prepare imprint smears. Specimen obtained was fixed in Bouins fluid for 12-48 hours prior to dehydrating and embedding in paraffin. Bony tissue was decalcified using 5% nitric acid and kept for 1-4 days. Formalin fixed tissue was dehydrated with ascending grades of alcohol, cleared in xylene and finally embedded in paraffin, 3-5u thick paraffin sections were cut on a rotary microtome, dewaxed and stained with H and E.[5]

RESULTS

The present study was carried out in the postgraduate Department of Pathology, Government Medical College, Jammu, over a period of 2.5 years from calendar year June 2020 to December 2022. A total of 250 patients were diagnosed as haematological malignancies during this period. The pattern of haematological malignancies was analysed by calculating the specific percentages out of the presented data.

In our study, total of 250 cases were seen over a 2.5-year period. The most common haematological malignancy was found to be leukemia 194 (77.6%) cases, followed by plasma cell dyscrasia with 30 (12%) cases, lymphomas and myeloproliferative disorders each with 11 (4.4%) cases and metastatic deposits 4 (1.6%) cases. Acute leukemias were found to be more common with 141 (56.4%) cases than chronic leukemias with 66 (26.4%) cases. AML were slightly more common than ALL. On acute leukemia cases, cytochemical stains like MPO, NSE and PAS were also done and diagnosis was based on morphology and cytochemistry that showed AML cases to be 65 and ALL cases were found to be 64. Also there

were 8 (3.2%) cases of acute leukemia which were not further categorised into lymphoid or myeloid malignancies on morphology and cytochemistry for which flowcytometry and immunophenotyping was advised. CML 40 (16%) cases were found to be more common than CLL 17 (6.8%) cases (Figure 1). Other than CML, chronic myeloproliferative disorders included in the study were essential thrombocythemia 6 (2%) cases, polycythemia 2 (0.8%) cases, chronic neutrophilic leukemia 2 (0.8%) cases and single case of primary myelofibrosis. Among lymphomas, NHL 9 (3.6%) cases were found to be more than Hodgkin's lymphoma with 2 (0.8%) cases. Among plasma cell dyscrasia, majority of the cases 29 (11.6%) were of multiple myeloma with only one case of MGUS noted. Metastatic malignancy with 4 (1.6%) cases was the least common haematological malignancy in our study (Table 1).

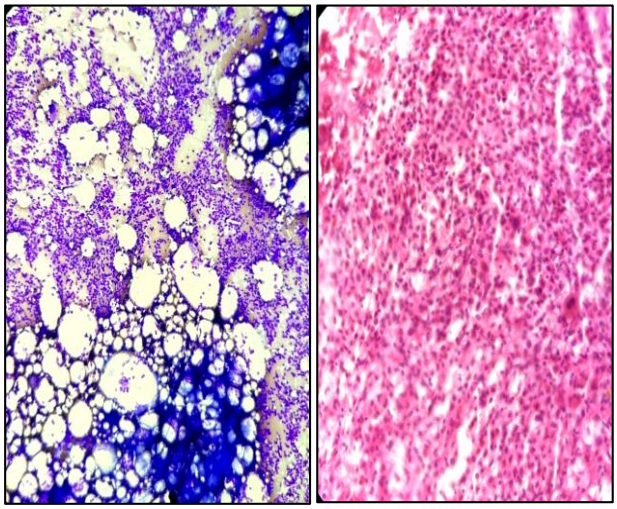

Figure 1: CLL; bone marrow aspiration and biopsy showing predominantly mature lymphocytes replacing hematopoietic elements, (MGG and H&E, 100X).

The age of the patients ranged from 6 months to 85 years. Acute leukemias were more common among younger age groups with maximum of the ALL cases between 1st and 2nd decade of life and AML among mid age groups, while chronic leukemias, lymphomas, multiple myeloma and metastatic malignancies were common among elderly age groups more than 50 years of age group (Table 2).

In the present study, majority of the cases were males accounting for 127 (50.8%) cases as compared to females with 123 (49.2%) cases. It was observed that in overall, the cases were more in males as compared to females except for the cases of AML and CML where females were found to be more common with 40 (16%) cases and 27 (10.8%) cases respectively (Table 3).

Among 194 cases of leukemia, trephine biopsy was done in 29 cases. BMA alone was found to be sufficient for making diagnosis in 165 cases. Among myeloproliferative neoplasm excluding CML cases, BMB was done along with BMA in 3 cases of essential thrombocythemia (ET) and in 2 cases of polycythemia vera as 4 of these patients were non cooperative. Among ET, all cases (3/6) that underwent trephine biopsy showed increased megakaryocytes with large forms in intertrabecular spaces. Among polycythemia vera (PV) cases, BMB found to be useful to assess overall cellularity, morphology of megakaryocytes and presence of fibrosis. Of the 2 PV, one case was diagnosed as myelofibrosis on BMB (Figure 4).

Among lymphomas with 10 cases, there were 9 cases of NHL and 2 cases of Hodgkin's lymphoma (HL). BMB was done in 8 cases of NHL. Out of 8 cases, 6 cases of NHL showed marrow involvement on both BMA and BMB. One case was diagnosed ALL on aspiration but on BMB it showed high grade NHL with diffuse interstitial pattern of infiltration with retained hematopoietic elements (Figure 2). Another case showed normoblastic hematopoiesis on BMA whereas it was diagnosed as NHL on BMB. In case of Hodgkin's lymphoma, both marrow aspiration and biopsy results were same.

Among plasma cell dyscrasia, 16/29 patients underwent BMA as well as BMB. Among these 10 cases were reported as multiple myeloma on both aspiration and biopsy (Figure 3). However, 6 cases on biopsy showed normoblastic marrow with occasional plasma cells only and diagnosis was given on BMA only.

Among metastatic malignancies, 3 cases showed similar deposit on BMA and BMB (Figure 5), however in one case marrow aspiration showed metastatic deposits whereas BMB was normal with trilineage hematopoiesis (Table 4).

Table 1: Distribution of haematological malignancies.

Hematological malignancies	N	Percentage (%)
Leukemias		
Acute lymphoblastic leukemia	64	25.6
Acute myeloid leukemia	65	26
Chronic lymphocytic leukemia	17	6.8
Chronic myeloblastic leukemia	40	16
Acute leukemias	8	3.2
Myeloproliferative disorders except CML		
Essential thrombocythemia	6	2.4
Polycythemia	2	0.8
Chronic neutrophilic leukemia	2	0.8
Primary myelofibrosis	1	0.4
Lymphomas		
Non Hodgkin's lymphoma	9	3.6
Hodgkin's lymphoma	2	0.8
Plasma cell dyscrasia		
Multiple myeloma	29	11.6
MGUS	1	0.4
Metastatic deposits	4	1.6
Total	250	100

In the present study, 64/250 (25.6%) patients underwent BMA and BMB simultaneously. A positive concordance was seen in 46 (76.6%) of the cases between the two methods and diagnostic discordance was observed in 10 (16.3%) of the cases. The high concordance between the methods clearly indicates that the methods are complementary to each other. Six cases were diagnosed on biopsy only due to hypocellular marrow/diluted marrow aspirate smears. Similarly, inadequate biopsy was observed in 2 cases due to lesser inter trabecular spaces and fragmented tissue (Table 5).

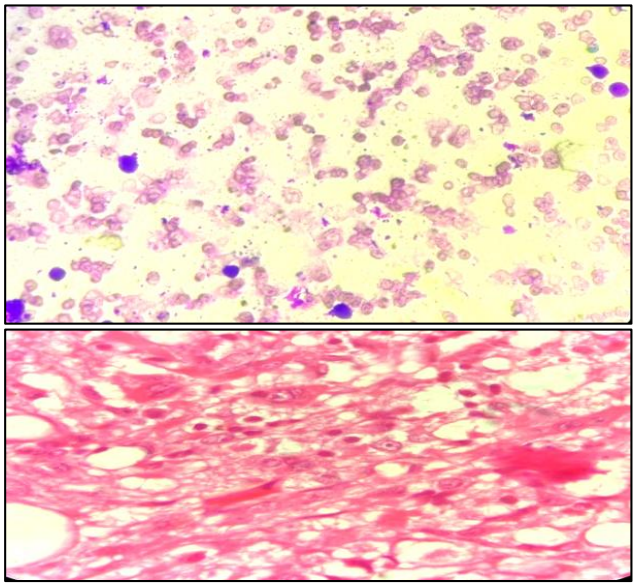

Figure 2: NHL; marrow aspiration of diluted smear with small lymphoid cells (MGG, 400X). Trephine section shows lymphoid cells infiltrating the marrow (H and E, 400X).

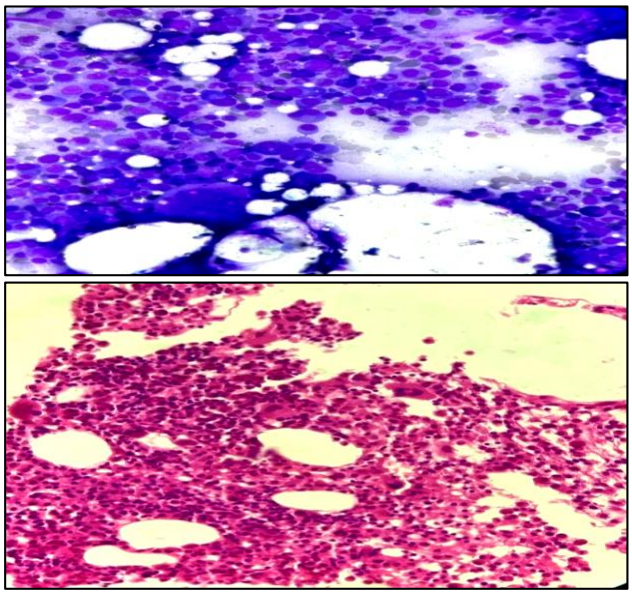

Figure 3: Multiple myeloma: aspiration smear and biopsy section of sheets of plasma cells with few binucleated forms (MGG, H and E, 400X).

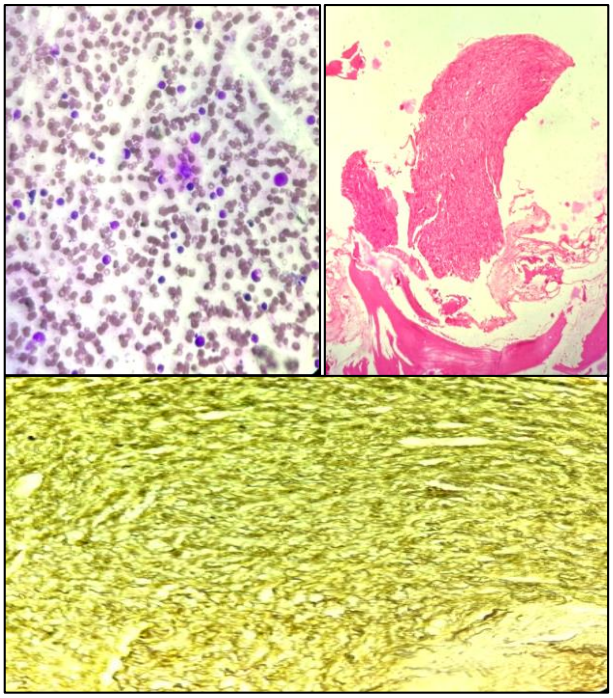

Figure 4: Primary myelofibrosis: marrow aspiration shows diluted hypocellular smear (MGG, 400X). Biopsy showing myelofibrosis (H and E, 100X). Reticulin stain shows grade IV fibrosis (400X).

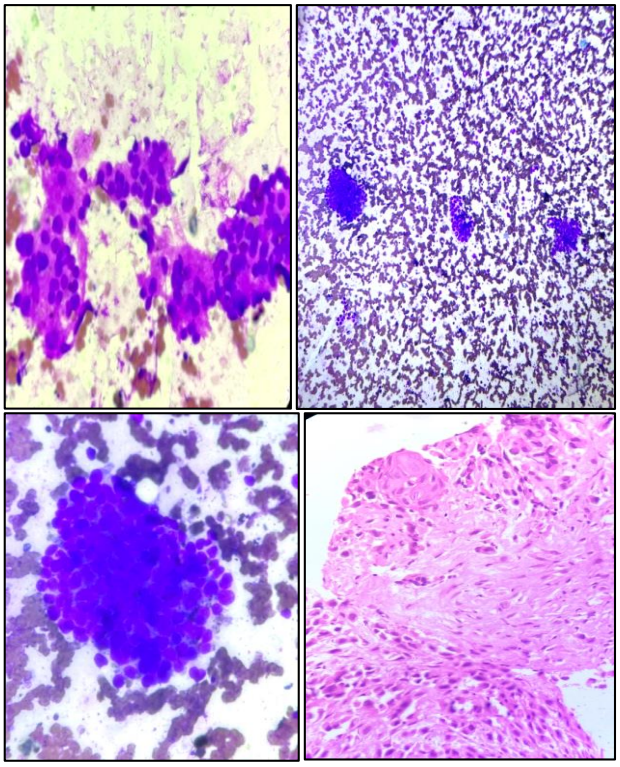

Figure 5: Metastatic deposits: bone marrow aspiration of metastatic deposits of round cell tumor (MGG, 400X) and adenocarcinoma (MGG, 100X and 400X). Trephine biopsy section shows metastatic deposits of squamous cell carcinoma (H and E, 400X).

Table 2: Distribution of haematological malignancies among different age groups.

Age (Years)	ALL	AML	CML	CLL	MPN except CML	Plasma cell dyscrasia	Lymphomas	Metastatic	Acute leukemia
0-10	34	1	0	0	0	0	0	0	2
11-20	17	8	0	0	1	0	0	0	3
21-30	5	5	10	0	0	0	0	1	1
31-40	4	9	8	0	3	0	1	0	1
41-50	3	17	13	1	2	6	2	0	1
51-60	1	11	5	3	0	13	5	0	0
60-70	0	8	4	8	5	6	2	1	0
>70	0	6	0	5	0	5	1	2	0
Total	64	65	40	17	11	30	11	4	8

Table 3: Sex distribution of various types of haematological malignancies.

Gender	ALL	AML	CML	CLL	MPN except CML	Plasma cell dyscrasia	Lymphomas	Metastatic	Acute leukemia
Male	40	25	13	12	4	17	8	3	5
Female	24	40	27	5	7	13	3	1	3
Total	64	65	40	17	11	30	11	4	8

Table 4: Cases diagnosed on bone marrow aspirate and trephine biopsy.

Hematological malignancies	N	BM aspiration	BM trephine biopsy
Leukemia			
Acute lymphoblastic leukemia	64	64	2
Acute myeloid leukemia	65	65	4
Chronic lymphocytic leukemia	17	17	9
Chronic myeloblastic leukemia	40	40	10
Acute leukemias	8	8	4
Myeloproliferative disorders			
Essential thrombocythemia	6	6	3
Polycythemia	3	3	2
Chronic neutrophilic leukemia	2	2	0
Lymphomas			
Non Hodgkin's lymphoma	9	9	8
Hodgkin's lymphoma	2	2	2
Plasma cell dyscrasia			
Multiple myeloma	29	29	16
MGUS	1	1	0
Metastatic deposits	4	4	4
Total	250	250	64

Table 5: Concordance between BMA and BMB, (n=64).

Diagnosis	N	Features
Cases diagnosed positive with BMA and BMB, (n=46)		
Non-Hodgkin's lymphoma	06	
Multiple myeloma	09	
Chronic myeloid leukemia	10	
Chronic lymphoid leukemia	09	
Acute myeloid leukemia	04	
Acute lymphoblastic leukemia	01	
Myeloproliferative neoplasms	03	
Metastatic malignancy	02	
Hodgkin's lymphoma	02	

Continued.

Diagnosis	N	Features
Dry tap cases diagnosed on BMB, (n=6)		
Primary myelofibrosis	1	Hypocellular marrow with marked fibrosis with dysplastic megakaryocytes on biopsy.
Acute myeloid leukemia	2	Imprint smears showed >20% blast cells. MPO +ve.
Acute lymphobastic leukemia	2	BMB showed increased blast with the bloody tap on the BMA
Metastatic deposits	1	Marrow infiltration by neoplastic cells with fibrosis.
Inadequate biopsy and diagnosed on BMA, (n=2)		
Myeloproliferative disorder	2	Inadequate biopsy
Diagnostic discordance, (n=10)		
BMA		**BMB**
Normoblastic hematopoiesis	1	Non Hodgkin's lymphoma
ALL-L2	1	High grade-NHL with grade 3 fibrosis.
Metastatic malignancy	1	Cellular marrow with trilineage hematopoiesis on BMA
Multiple myeloma	6	Normoblastic marrow with few plasma cells on BMB
Polycythemia	1	Marked fibrosis.

Diagnostic correlation: BMA+BMB, concordance: 46, discordance: 10.

DISCUSSION

Examination of the bone marrow is one of the diagnostic pillars of haematological practice. Bone marrow aspiration and trephine biopsy are the two procedures done for the diagnosis of neoplastic and non-neoplastic haematological disorders. The two procedures are complementary to each other. In our study a comparative evaluation of bone marrow aspiration and trephine biopsy was done to determine the diagnostic usefulness of both the procedures.

In the present study, total of 250 cases were seen over a 2.5-year period. The most common haematological malignancy was found to be leukemia with 194 (77.6%) cases whereas metastatic deposits were the least common with 4 (1.6%) cases. Similar to our study, Dogan et al also found leukemia to be the most common haematological malignancy while metastatic deposits being the least common.[1]

Acute leukemias with 137 (54.8%) cases were more common as compared to chronic leukemias with 57 (22.8%) cases. Similar findings were also observed by Khan et al in their study in which majority of cases were acute leukemias.[2] Acute myeloid leukemia with 65 (26%) cases accounted for maximum of the cases followed by acute lymphoblastic leukemia with 64 (25.6%) cases which was in concordance with the study done by Rathod et al in which AML cases accounting maximum of the cases among acute leukemias.[6] Similar findings were also observed by Gupta et al.[7] Among chronic leukemia, we encountered maximum of chronic myeloid leukemia (CML) with 40 (16%) cases followed by chronic lymphocytic leukemia with 17 (6.8%) cases. In the studies conducted by Piplani et al, Khatik et al, Atchyuta et al, Rana et al the most common disorder encountered was CML among chronic leukemia as in the present study.[8-11]

In the present study, we observed majority of the cases were in the age group of 1st and 2nd decade of life with a slight male preponderance which is similar to study done by Manju et al where majority of the cases were in the age group of 11-20 years.[12] Rathod et al also observed that maximum number of haematological disorders were in the age group of 21 to 30 years with male preponderance whereas Vahini et al in their study observed that most of the cases were in the age group of 46-60 years with male preponderance in contrast to our study.[6,13]

Among 250 cases included in the study, 64 patients underwent simultaneous BMA and BMB. Majority of the cases showed positive correlation of 71.8% (46/64) between marrow aspiration and biopsy. Of the 64 cases, we encountered 29 cases of leukemia reported on both aspirate as well as on biopsy. Out of these 29 cases of leukemia, 4 cases showed inadequate aspiration smears so that the diagnosis was made only on trephine biopsy. Among the remaining 25 cases of leukemia, a single case showed discordance which was reported as ALL on aspiration but on biopsy it diagnosed as high grade-NHL with grade III fibrosis. Hence, overall concordance rate of 24/25(96%) was observed among the leukemia between the two procedures. CML and CLL showed 100% concordance between two methods. It is similar to the study done by Puri et al where highest concordance rate was encountered in acute leukemia, CML and CLL.[3] Dogan et al also found similar results in their study.[1]

Among myeloproliferative disorders except CML, out of 6 cases of essential thrombocythemia, simultaneous BMA and BMB was done in 3 cases which showed 100% concordance. Of the two cases of polycythemia vera, one case showed myelofibrosis on trephine section and had dry tap marrow aspirate thereby emphasising the role of BMB to assess overall cellularity and degree of fibrosis. Overall, in myeloproliferative neoplasms, a positive correlation of 80% (4/5 cases) was found in

myeloproliferative neoplasm except CML. The biopsy was found to be only diagnostic method for myelofibrosis similar to the study done by Vijayamohanan et al, Gilotra et al.[14,15]

Among lymphomas with 11 cases, there were 9 cases of NHL and 2 cases of Hodgkin's lymphoma (HL). BMB was done in 8 cases of NHL. Out of 8 cases, 6 cases of NHL showed marrow involvement on both BMA and BMB. One case was diagnosed ALL on aspiration but on BMB it showed high grade NHL with diffuse interstitial pattern of infiltration with retained hematopoietic elements. Another case showed normoblastic hematopoiesis on BMA whereas it was diagnosed as NHL on BMB. Hodgkin's lymphoma showed similar findings on both marrow aspiration and biopsy. The concordance between BMA and BMB was 80% (8/10 cases) and disagreement was observed in 2 cases where biopsy was found to be better for the diagnosis of NHL. Overall, the pattern of infiltration was better seen on trephine sections, most common being the diffuse followed by nodular pattern which is a valuable prognostic indicator. Conlan et al in their study found 94% concordance between BMA and BMB.[16] Similar findings were observed in the study done by Piplani et al which also concluded that the biopsy is important for the diagnosis and pattern of involvement.[8]

Among plasma cell dyscrasia, 16/29 patients underwent BMA as well as BMB. Among these 10 cases were reported as multiple myeloma on both aspiration and biopsy. However, 6 cases on biopsy showed normoblastic marrow with occasional plasma cells only and diagnosis was given on BMA only. Thus, indicating that diagnosis of multiple myeloma could be easily possible with peripheral blood smear and aspiration alone along with radiological findings. BMB is indicated only in cases where there is inadequate BMA or focal involvement of the marrow. Similar findings were also observed in the study done by Gupta et al.[7]

Among metastatic malignancies, 3/4 cases showed similar deposit on BMA and BMB, however in one case marrow aspiration showed metastatic deposits whereas BMB was normal with trilineage hematopoiesis. We found only one case of metastatic deposits of prostatic adenocarcinoma, with infiltrating cells in both aspirate and biopsy. Other two were metastatic deposits of squamous cell carcinoma and round cell tumor. Therefore a highest positive correlation of 75% was obtained between aspiration and biopsy sections. Savage et al in their study obtained a correlation of 75% between marrow aspiration and biopsy for detection of metastatic deposits which is similar to our study.[17]

We therefore encountered that among the haematological disorders leukemia has highest rate of diagnosis on marrow aspiration as compared to trephine biopsy. The biopsy sections are required for the diagnosis of myelofibrosis, to assess the pattern of infiltration in case of lymphomas and in case of inadequate aspirate where marrow biopsy can aid in reaching the diagnosis as well as gives information about prognosis of patient.

CONCLUSION

Bone marrow aspiration and biopsy both complement each other and should be evaluated simultaneously. However, significantly more number of trephine sections provided diagnosis in case of dry tap/ scanty material, for assessment of lymphoma tumor infiltration, cellularity, megakaryocyte density and proliferating cell lines in myeloproliferative disorders. Further fibrosis of bone marrow, pattern of bone marrow involvement and topographical alterations were appreciable only on trephine sections. This study emphasizes the need for greater vigilance in the early diagnosis and an interdisciplinary approach for the effective management of patients as well as inclusion of trephine biopsy as regular procedure for complete evaluation of patients with haematological malignancies.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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