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Original Research

A COMPARATIVE ASSESSMENT OF HEMATOLOGICAL DISORDERS USING BONE MARROW ASPIRATE AND TREPHINE BIOPSY, ALONG WITH DETERMINATION OF OPTIMAL LENGTH IN THE ASSESSMENT OF BONE MARROW INVOLVEMENT WITH SPECIAL REFERENCE TO LYMPHOMA AND LEUKEMIA

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Abstract

Introduction: The diagnostic procedures of bone marrow aspiration and biopsy form the backbone of evaluation of patients admitted in haematology, medical oncology and a large proportion of patients in general medicine as well.

Aims: To conduct a comparative analysis between bone marrow aspirate and bone marrow trephine biopsy, as well as to ascertain the most favorable length of trephine biopsy for detecting lymphoma infiltration.

Method: A total of 2006 patients were evaluated over a period of four years. All of these patients underwent bone marrow aspirate and trephine biopsy examination. Biopsies were fixed in formalin, and then decalcified in 5 % nitric acid , processed and stained with H & E, and special stains, wherever required. Results of Aspirate and Biopsy examination were compared, correlation noted and validity parameters for aspirate were calculated. Relationship between marrow infiltration by lymphoma cells and trephine length was studied and correlation was assessed.

Results: Aspirate was found to have a high sensitivity for acute leukemia (100%) and multiple myeloma (80.48%), moderate for myeloproliferative neoplasm's (74.19%) and metastasis (58.33%) and low for Non-Hodgkin lymphoma (38.46%). Lymphoma positivity increased with trephine length, with maximum positivity (85.71%) seen in 25-28 mm group and no further gain beyond 30 mm.

Conclusion: Aspirate exhibits a notable level of specificity, while its sensitivity varies depending on the specific disease being considered. With the exception of a few cases where aspirate alone is satisfactory, biopsy is necessary in the majority of situations. When evaluating lymphoma positivity, it was discovered that preprocessing trephine length of 25-28 mm, examined at multiple deeper levels, yielded optimal results.

Keywords: H&E, biopsy, leukaemia, multiple myeloma, Non-Hodgkin lymphoma

INTRODUCTION

Bone marrow examination is an indispensable diagnostic tool in the evaluation of various haematological disorders, non haematological malignancies, pyrexia of unknown origin and infective diseases.[1] It is also valuable for follow up of patients undergoing chemotherapy and bone marrow transplantation.[1,2] Involvement of marrow by metastases has a significant impact on patient management and prognosis.[3] Bone marrow examinations serves to establish or confirm a primary

diagnosis of lymphoma or to determine the extent of disease dissemination for staging purposes.[4] Rarely, bone marrow examination has been useful in detecting non-hematopoietic malignancy in clinically unsuspected cases.[5] At times, marrow metastases may have normal serum chemistry and hematologic parameters and may even be missed by bone scans and advanced imaging modalities.[6] Bone marrow aspiration (BMA) is a simple, reliable and rapid method of marrow evaluation. Trephine biopsy provides more comprehensive information regarding the marrow cellularity, architectural patterns and overall hematopoiesis. But biopsy is a painful procedure and its processing takes at least 48–72 hours. Few studies have analyzed the diagnostic accuracy of bone marrow aspirate in comparison with trephine biopsy.[7–10] Literature on correlation of lymphoma positivity with trephine biopsy length is even sparse.[11,12]

To obtain and decide an optimum length for diagnostic purpose of bone marrow biopsy has been a matter of debate among hematopathologists, hence we aim to compare the positivity rates of lymphomas in marrow with the lengths of respective biopsies.

MATERIAL AND METHODS

From May 2019 to December 2022, 2006 patients were received in the Department of pathology and underwent both bone marrow aspirate and biopsy simultaneously. Of these, 72 (3.58%) biopsies were inadequate for assessment and were excluded from analysis. Patient information, comprising of clinical history including physical examination findings, chemo/radiotherapy, complete blood count with peripheral smear examination and indication of bone marrow were collected. Aspirate findings were compared to that of trephine biopsy.Of these, 228 patients were diagnosed or follow up cases of hematolymphoid malignancy. 1033 patients presented with Pancytopenia/Bicytopenia.

Bone marrow aspirate and trephine biopsy: Bone marrow aspirate(BMA) was done using 16G Salah's needle and 0.25 to 0.5 ml of aspirate was withdrawn with a 20 ml plastic syringe from posterior superior iliac spine and smears were prepared immediately. After that, trephine biopsy was performed using Jamshidi's needle , 11G . Imprints from Bone marrow biopsy(BMB) were taken as well. Leishmans staining was performed on Peripheral smear examination(PBF) and BMA. BMB was fixed in 10% neutral buffered formalin,decalcified in 5% nitic acid solution , length ofbiopsy measured, processed in automated tissue processor and embedded in paraffin wax blocks. 2-3µ sections were cut, stained with hematoxylin and eosin and3 to 4 deep sections cut at intervals of 0.1-0.2 mm were examined. BMA and biopsy findings were compared. Wherever indicated, Special stains and immunohistochemistry was performed.

Statistical Analysis: Analysis using SPSS software was done. The results of Aspirate and Biopsy were compared. Proportion of trephine biopsies showing lymphoma infiltration was plotted on y axis and total preprocessing trephine length on x axis, in increments of 4 mm. Lengths of trephine biopsies were compared for positive lymphoma infiltration.

RESULTS

Pancytopenia/bicytopenia

Of the total patients, pancytopenia was seen in 1033 patients. The majority of these cases were found to have megaloblastic anaemia (609) followed by normocellular marrow (257), acute leukemia(75), followed by lymphoma (65), metastases(12) and aplastic anaemia(10). Only one case each of hemophagocytic syndrome(HPS) and Leishmaniasis were received.

Table 1: Distribution of patients of Pancytopenia

Type of Anemia	No. of Patients	Percentage
Megaloblastic Anemia	609	58.95
Normocellular Marrow	257	24.87
Aplastic Anemia	10	0.96
Lymphoma	65	6.29

Leukemia	78	7.55
Metastasis	12	1.16
HPS	1	0.09
Leishmaniasis	1	0.09
Total	**1033**	**100**

Megaloblastic anemia: 58.95% of cases of pancytopenia comprised of megaloblastic anemia Aspiration and biopsy were concordant in 100% of cases of megaloblastic anemia.
Acute leukemia: Acute Leukaemias accounted for 34.21% of haematological malignancies. BMA showed concordance with biopsy in all patients.
Lymphoma: Lymphomas accounted for 28.94% of haematological malignancies. Of these, 98.4% (65/66) patients were of Non Hodgkin Lymphoma (NHL) and Hodgkin lymphoma comprisedonly 1.6%(1/66). Of 66 trephines being evaluated for NHL staging, 25(38.46%) showed marrow infiltration in the form of paratrabecular nodular, interstitial or diffuse pattern. 24 out of these 25 aspirates were reported as positive for lymphoma infiltration, 1 was reported as negative. In 40 cases, both biopsy and aspirate were negative for lymphoma infiltration.
In the single case of Hodgkin lymphoma we received, marrow involvement was absent.
Myeloproliferative neoplasm: Of 31 patients,28 were positive on bone marrow biopsy. 8 aspirates were discordant and did not show positivity whereas 20 (71.42%) were concordant.

Table 2: Spectrum of disorders on bonemarrow examination

Provisional Diagnosis	Number	Positive (%)	Negative (%)
Non-Hodgkin Lymphoma	65	25 (38.46)	40
Hodgkin Lymphoma	1	0 (0)	1
CMPN	31	23 (74.19)	8
Acute Leukemia	78	78 (100)	0
Multiple Myeloma	41	33 (80.48)	8
Metastasis	12	7 (58.33)	5

Metastases: Evidence of marrow infiltration with metastases was seen in 7 out of 12 patients (58.33%),the major primaries in the cases were adenocarcinoma from prostate, breast,gastrointestinal tract and lung. Aspirate was negative in only 2 cases and showed concordance with biopsy in 5 cases (71.42%)
Multiple myeloma:41 patients were received, 33 (80.48%) showed positive biopsy and concordant aspirate was detected in 32 (96.96%) cases. Marrow metastases from neuroblastoma and small cell carcinoma along with secondary myelofibrosis on biopsy presented as pancytopenia, for which marrow was done.

Distribution of 228 patients of Haematological malignancies and proportion of Bone Marrow Positivity.

Table 3: Validity parameters for bone marrow aspirate

Type of Tumours		Biopsy		Total	Sensitivity	PPV	NPV
		Positive	Negative				
Lymphoma	Positive	24	0	24	96.00	100.00	100.00
	Negative	1	0	1			
	Total	25	0	25			
CMPN	Positive	15	0	15	65.22	100.00	100.00
	Negative	8	0	8			

	Total	23	0	23			
Acute Leukemia	Positive	78	0	78	100.00	100.00	100.00
	Negative	0	0	0			
	Total	78	0	78			
Multiple Myeloma	Positive	32	0	32	96.97	100.00	100.00
	Negative	1	0	1			
	Total	33	0	34			
Metastatic Solid Tumors	Positive	7	0	7	58.33	100.00	100.00
	Negative	5	0	5			
	Total	12	0	12			
Megaloblastic Anemia	Positive	609	0	609	100.00	100.00	100.00
	Negative	0	0	0			
	Total	609	0	609			
Aplastic Anemia	Positive	9	0	9	90.00	100.00	100.00
	Negative	1	0	1			
	Total	10	0	10			
Leishmaniasis	Positive	1	0	1	100.00	100.00	100.00
	Negative	0	0	0			
	Total	1	0	1			

Comparison of bone marrow aspirate with trephine biopsy: Concordance rates were calculated between BMA and trephine biopsy. In larger subgroups we also calculated validity parameters taking trephine biopsy as gold standard.

Table 4: Concordance rate of BMA and BMB in various hematological disorders

	Positive Biopsy	**BMA Inconsistent**	**BMA Consistent**	**Concordance Rate**
Lymphoma	25	1	24	96%
CMPN	23	8	15	65.21%
Acute Leukemia	78	0	78	100%
Multiple Myeloma	33	1	32	96.96%
Metastatic Solid Tumors	7	2	5	71.42%
MegaloblasticAnemia	609	0	609	100%
Aplastic Anemia	10	1	9	90%
Leishmaniasis	1	0	1	100%
HPS	1	0	1	100%
Total	**787**	**13**	**774**	**98.34%**

Correlation of trephine length with lymphoma positivity: We had 67 adequate biopsies for lymphoma staging in our study. Of these 38.46% (25) were positive for lymphoma infiltration after examining three sections at deeper levels. The mean length of trephine core was 14 mm, ranging from 1-36 mm. We found that lymphoma positivity showed a rising trend with length of trephine core, with maximum positivity (85.71%) seen in 25 - 28 mm group, but no further improvement beyond 30 mm Based on this, two groups were made, taking 18mm as cut-off. Fischer's exact test was applied and the difference in both the groups was found to be statistically significant (lymphoma positivity ≤16mm=40.3% and ≥17mm=66.1%, p=0.0011).

Table 5: Lengthof T biopsy(mm)				
Length of T biopsy (mm)		Lymphoma		Total
		Positive	Negative	
05 to 08	No. of Patients	0	3	3
	Percentage	0.0%	7.7%	5.2%
09 to 12	No. of Patients	1	12	13
	Percentage	5.3%	30.8%	22.4%
13-16	No. of Patients	5	9	14
	Percentage	26.3%	23.1%	24.1%
17-20	No. of Patients	5	10	15
	Percentage	26.3%	25.6%	25.9%
21-24	No. of Patients	1	3	4
	Percentage	5.3%	7.7%	6.9%
25-28	No. of Patients	6	1	7
	Percentage	31.6%	2.6%	12.1%
33-36	No. of Patients	1	1	2
	Percentage	5.3%	2.6%	3.4%
Total	No. of Patients	19	39	58
	Percentage	100.0%	100.0%	100.0%
Chi-square		14.51		
p-value		0.024		

DISCUSSION

In this tertiary care analysis, a total of 2006 patients with various hematological disorders were included. Both BMA and BMB were performed on each patient, and the diagnostic results were compared. The findings of this tertiary care analysis contribute to the understanding of the diagnostic utility of BMA and BMB in hematological disorders. The high diagnostic concordance between these techniques suggests that they should be used in combination to obtain a comprehensive assessment of the bone marrow. Literature on correlation of lymphoma positivity with trephine biopsy length is even sparse.[11,12]

Of the total patients, pancytopenia was the chief complaint in 1033 patients. The majority of these cases were found to have megaloblastic anaemia (609) followed by normocellular marrow (257), acute leukemia(75), followed by lymphoma (65), metastases(12) and aplastic anaemia(10). Only one case each of HPS and Leishmaniasis were received.58.95% of cases of Pancytopenia comprised of megaloblastic anemia Aspiration and biopsy were concordant in 100% of cases of megaloblastic anemia. Acute Leukaemias accounted for 34.21% of haematological malignancies.BMA showed concordance with biopsy in all patients.Lymphomas accounted for 28.94% of haematological malignancies. Of these, 98.4% (65/66) patients were of Non-Hodgkin Lymphoma (NHL) and Hodgkin lymphoma comprisedonly 1.6%(1/66). Of 66 trephines being evaluated for NHL staging, 25(38.46%) showed marrow infiltration in the form of paratrabecular nodular, interstitial or diffuse pattern. 24 out of these 25 aspirates were reported as positive for lymphoma infiltration, 1 was reported as negative. In 40 cases, both biopsy and aspirate were negative for lymphoma infiltration.

Of 31 patients,28 were positive on bone marrow biopsy. 8 aspirates were discordant and did not show positivity whereas 20 (71.42%) were concordant.Evidence of marrow infiltration with metastases was seen in 7 out of 12 patients (58.33%),the major primaries in the cases were adenocarcinoma from prostate, breast,gastrointestinal tract and lung. Aspirate was negative in only 2 cases and showed concordance with biopsy in 5 cases (71.42%). 41 patients were received, 33 (80.48%) showed positive biopsy and concordant aspirate was detected in 32 (96.96%) cases.Concordance rates were

calculated between BMA and trephine biopsy. In larger subgroups we also calculated validity parameters taking trephine biopsy as gold standard. We had 67 adequate biopsies for lymphoma staging in our study. Of these 38.46% (25) were positive for lymphoma infiltration after examining three sections at deeper levels. The mean length of trephine core was 14 mm, ranging from 1-36 mm. We found that lymphoma positivity showed a rising trend with length of trephine core, with maximum positivity (85.71%) seen in 25-28 mm group, but no further improvement beyond 30 mm Based on this, two groups were made, taking 18mm as cut-off. Fischer's exact test was applied and the difference in both the groups was found to be statistically significant (lymphoma positivity ≤16mm=40.3% and ≥17mm=66.1%, p=0.0011).

In a study by Goyal S et al., (2014)[13]megaloblastic anemia was the most common (37%) cause of pancytopenia.Aspirate had a high sensitivity for acute leukemia (89.4%) and multiple myeloma (88.5%), moderate for NHL (67.6%) and nonhematopoietic metastases (58.3%) and low for aplastic anaemia (38.5%) and Hodgkin lymphoma (5%). Aspirate has no role in granulomatous myelitis and myelofibrosis. Lymphoma positivity increased with trephine length, with maximum positivity (68.9%) seen in 17-20 mm group and no further gain beyond 20 mm. (lymphoma positivity ≤16mm=40.3% and ≥17mm=66.1%, p=0.0011).

In their study, overall incidence of marrow involvement by Hodgkin and Non Hodgkin lymphoma was quite high (42.5% and 51.8% respectively). Various studies have reported marrow infiltration in lymphoma ranging from 27.1 to 55.1%.[14]

No diagnostic gain was achieved above a length of 20 mm, with maximum percentage positivity (68.9%) obtained in biopsies 17-20 mm long. Trephine biopsies ≥17mm had significantly higher lymphoma positivity as compared to those of ≤16mm. The National Cancer Institute has recommended a trephine length of ≥20 mm for NHL staging.[11] Campbell et al has supported this recommendation and emphasized the role of examining multiple sections.[12] Bain suggested a minimum trephine length of 16 mm, based on the findings of Bishop set al. in which a plateau was achieved in the rate of detection of metastatic tumour after trephine length exceeded 16 mm.[15,16]

Peripheral smear and BMA may show overlapping findings in Myeloproliferative neoplasm(MPNS). Role of trephine biopsy is not only in differentiation of MPNs, but also to assess the overall marrow cellularity, histotopography and morphology of megakaryocytes and blasts (CD34 positive precursors) and degree of myelofibrosis. 26 Non diagnostic aspirates in CML patients, who had grade 2 marrow fibrosis highlights the importance of trephine biopsy in CML. Also, focal collection of blasts occupying significant intertrabecular space in biopsy clinched the diagnosis of blast crises, irrespective of blast count in peripheral smear and BMA as was seen in our case.[17]BMA does not have much role in diagnosis of primary myelofibrosis(PMF) because diffuse osteomyelosclerosis, intrasinusoida lhematopoiesis and vascular proliferation, which are characteristic of fibrotic PMF, can be confirmed and graded on biopsy sections only.[18]

CONCLUSION

The study emphasizes the limited role of bone marrow aspirate in certain conditions, such as Hodgkin lymphoma, granulomatous myelitis, aplastic anemia, and myelofibrosis, where trephine biopsy remains mandatory. Additionally, in non-Hodgkin lymphoma, metastases, and chronic myeloproliferative neoplasms , it is highlighted that aspirate alone is insufficient, and biopsy serves as a complementary diagnostic tool. The optimal length for trephine biopsies, specifically in the range of 17-20 mm, examined at multiple deeper levels, emerges as crucial for assessing lymphoma infiltration, providing maximal sensitivity. It is noteworthy that biopsies longer than 20 mm do not offer any added advantage, suggesting a practical limitation in biopsy length.

In conclusion, the study advocates for a nuanced approach in the diagnosis of hematological disorders, recognizing the strengths of bone marrow aspirate while acknowledging the indispensable role of trephine biopsy in specific scenarios. The determination of optimal biopsy length, particularly

in lymphoma assessment, contributes valuable insights for clinicians aiming to enhance diagnostic accuracy in the management of haematological malignancies.

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