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Original Research Article

Bone marrow aspiration and bone marrow trephine biopsy in haematological

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ABSTRACT

Background: Bone marrow study is of fundamental importance in most haematological disorders including haematological malignancies, carried out to permit cytological assessment and for other specialized investigations. Trephine biopsy gives histological details of marrow including cellularity, fibrosis and reveals the causes of dry tap. Finer cytomorphological details of marrow elements can be ascertained by the imprint smears from the biopsy material.

Materials and Methods: A total of 50 cases of haematological malignancies of both sexes within the age group of 4-82 years, were studied by cross sectional, descriptive study. Smears and biopsy sections were stained with routine and special stains. Bone marrow reticulin status was noted. The bone marrow aspiration results were correlated with the trephine biopsy reports and analyzed with standard statistical methods.

Results: Out of 50 cases, diagnostic bone marrow aspiration (BMA) and bone marrow trephine biopsy (BMTB) were done in 44 cases and trephine biopsy alone in rest of the 6 cases. Bone marrow aspiration was non-diagnostic in 1 case (due to focal involvement) and dry tap occurred in 5 cases. Total 21 cases of acute leukaemia, bone marrow aspiration alone could reach to the diagnosis in 17 (81%) cases. Single case of acute myelofibrosis was diagnosed only by bone marrow biopsy. Mild to moderate degree increase in reticulin fibrosis were noted in 18 (36%) cases.

Conclusion: Bone marrow aspiration and bone marrow trephine biopsy act as a complementary to each other so far the diagnosis and management of hematologic malignancies are concerned.

1. Introduction

Bone marrow study is an important medical procedure for the diagnosis of haematological malignancies and other diseases. Trephine biopsy gives histological details of a large piece of marrowalso reveals degree of cellularity, fibrosis and causes of dry taps.[1] Assessment of tumor infiltration pattern, megakaryocytic density and proliferating cell lines in myeloproliferative neoplasm and topographical alterations are also appreciable only on trephine biopsy sections.[2] Haematological malignancies are missed more frequently on aspirate than on the biopsy because of their patchy involvement. However it has got its limitations as the finer cytomorphological detailsof marrow element cannot be studied.[3] This can be overcome by making an imprint smear from the biopsy material. Touch imprintswere used as a reliable diagnostic tool for determining the cellular composition in the neoplastic hematologic diseasesas well as for the assessment of

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marrow cellularity.[4,5] Bone marrow aspiration and bone marrow trephine biopsy act as a complementary to each other so far the diagnosis and management of hematologic malignancies are concerned.[3] This study aims to compare results between bone marrow aspiration and bone marrow trephine biopsy with imprint smear cytology along with correlation of the findings in all cases of haematological malignancies and find out the areas where either method of bone marrow study is superior and where they are complementary to each other.

2. Materials and Methods

2.1. Study area

This study has been carried out in the Department of Pathology, North Bengal Medical College and Hospital, West Bengal.

2.2. Study population

Patients included in the study were from the IPD, OPD of annexed hospital and its oncology clinic.

2.3. Study period

Study of one year from February 2007 to January 2008.

2.4. Sample size

50 Cases.

2.5. Sampling design

All patients those who fulfilling the inclusion criteria during the data collection period. Inclusion criteria- All the consecutive patients of either sex within the age group of 4 to 82 years, who were suspected clinically and /or diagnosed by blood report or by other investigative procedures to be of haematological malignancy and were suffering from, referred to the Department of Pathology for bone marrow examination, were included in the study. Exclusion criteria- Patients who have not given consent, presence of contraindication to trephine biopsy – e.g. bleeding disorder, anticoagulation therapy and persons diagnosed with benign pathology & inadequate tissue sample cases, were excluded.

2.6. Study design

Cross sectional and observational study.

2.7. Parameters to be studied

It includes clinical parameters, detail history, investigation reports, bone marrow aspiration cytology, trephine biopsy & special staining.

2.8. Study technique

It includesethical clearance, inclusion of study samples according to inclusion and exclusion criteria, history taking as per case record form, and obtaining informed consent from the patient. Aspirated materials were stained with Leishman's stain and Sudan Black B (SBB) stain. Processing of bone marrow trephine biopsy specimen followed by stained with Haematoxylin and Eosin (H&E), Van Gieson and Reticulin stain (Gordon and Sweet's method). The grading system proposed by Bauermeister D.E.[6] of bone marrow reticulin was followed and reticulin graded from grade 0-4. The bone marrow aspiration results were correlated with the trephine biopsy reports including the imprint study.

2.9. Statistical analysis

Statistical evaluation was done using the standard statistical methods.

3. Results

The present studies of 50 cases of haematological malignancies, males (37cases) were more commonly affected (74%) than the females (26%).Maximum numbers of cases were below the age of 10 years of which includes most cases of acute leukaemia including acute lymphoblastic leukaemia (ALL) [Figure 1C], acute myeloid leukaemia (AML) [Figure 1D,E] and one cases each of aleukaemic leukaemia [Figure 3C] and acute myelofibrosis.Two cases of acute undifferentiated leukaemia (AUL) [Figure 1F, Figure 2A,B] in our study were more than 40 years of age. Most cases of chronic myeloid and lymphoid neoplasm occurred in young adults and in middle aged peoples. Only 2 cases (50%) of Hodgkin's disease occurred below the age of 10 years. Most cases of non-Hodgkin's lymphoma were more than 40 years of age. Out of six cases of chronic myeloid leukaemia (CML), 3 (50%) cases occurred below the age of 20 years. All the cases of multiple myeloma were older than 40 years of age. Bone marrow aspiration failed to diagnose 6 cases. Dry tap occurred in 4 (19%) cases of acute leukaemia (out of total 21 cases) and in one case of acute myelofibrosis. Non-diagnostic (non-representative) aspiration occurred in one case of multiple myeloma. In remaining 44 (88%) cases, bone marrow aspiration alone was sufficient for the diagnosis(Table 1). Table 1 shows out of 50 cases of haematological malignancies, total 21 cases of acute leukaemia, bone marrow aspiration alone could reach to the diagnosis in 17 (81%) cases.

Single case of acute myelofibrosis was diagnosed only by bone marrow biopsy and imprint smear [Figure 3D,E,F]. Among 5 cases of multiple myeloma; diagnosis was made by bone marrow aspiration alone in 4 cases [Figure 2C,E]. In one case, aspirate was non-representative

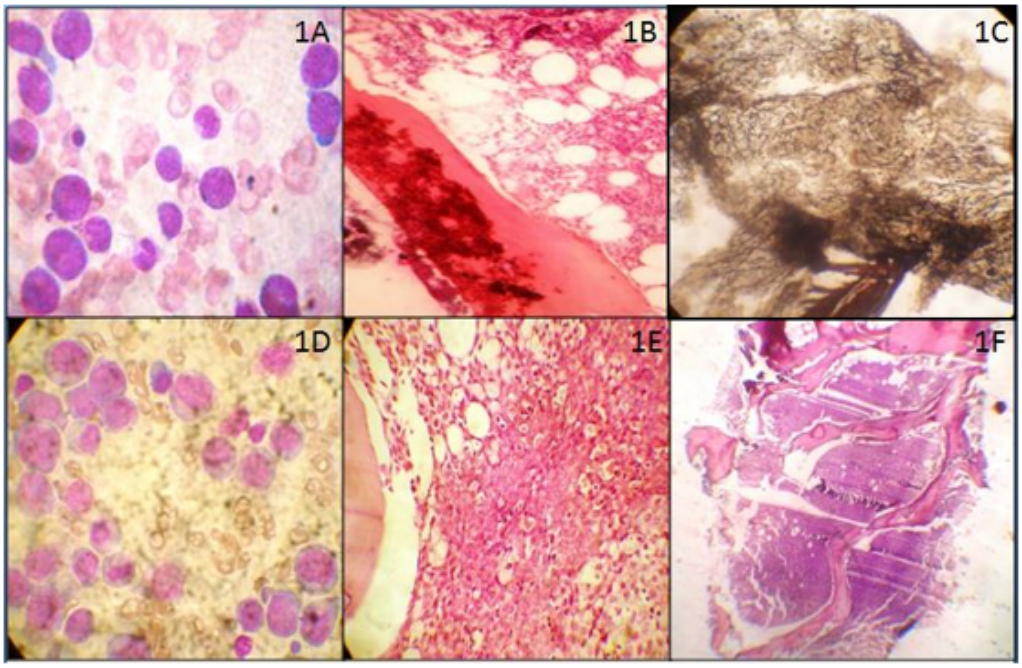

Figure 1: Photomicrograph showing; **A**: All -Incomplete remission, BMA, (1000×, Leishman Stain). **B**: All- Incomplete remission, BMTB, (400×, Hematoxylin and Eosin Stain); **C**: ALL, BMTB, (400×, Reticulin Stain). **1D**: AML- M5, BMA, (1000×, Leishman Stain); **E**: AML- M5, BMTB, (400×, Hematoxylin and Eosin Stain); **F**: Acute undifferentiated Leukaemia (AUL), BMTB, (100×,Hematoxylin and Eosin Stain).

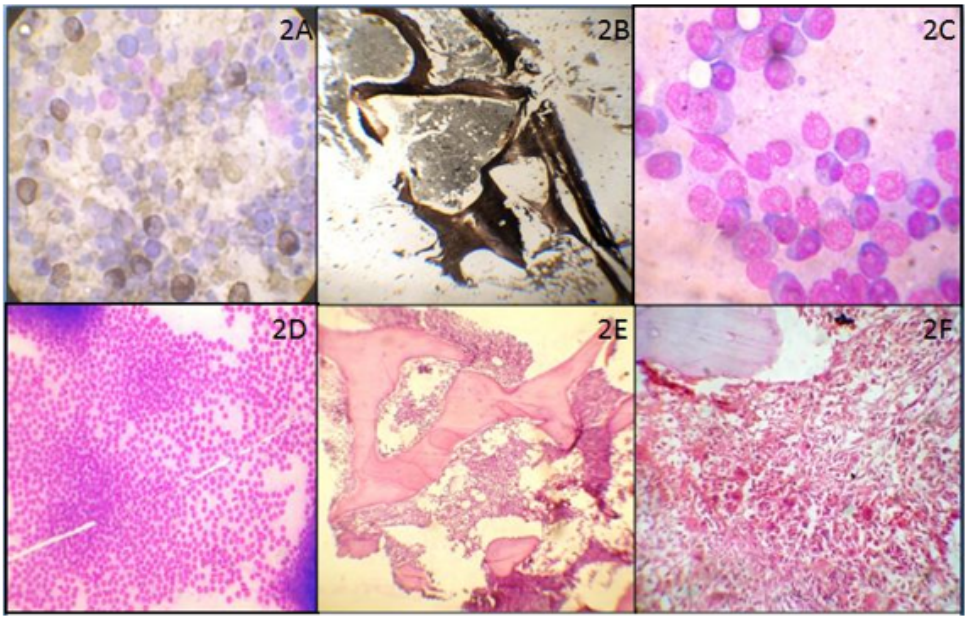

Figure 2: Photomicrograph showing; **A**: AUL, BMA, (1000×, Sudan Black B Stain); **B**: AUL, BMTB, (100×, Reticulin Stain); **C**: Multiple Myeloma (Pleomorphic type), BMA, (1000×, Leishman Stain.); **D**: Multiple Myeloma, Imprint Smear, (100×, Leishman Stain.) **2E**: Multiple Myeloma- Interstitial Infiltrate, BMTB, (100×,Hematoxylin and Eosin Stain); **F:** CML, BMTB(400×, Hematoxylin and Eosin Stain).

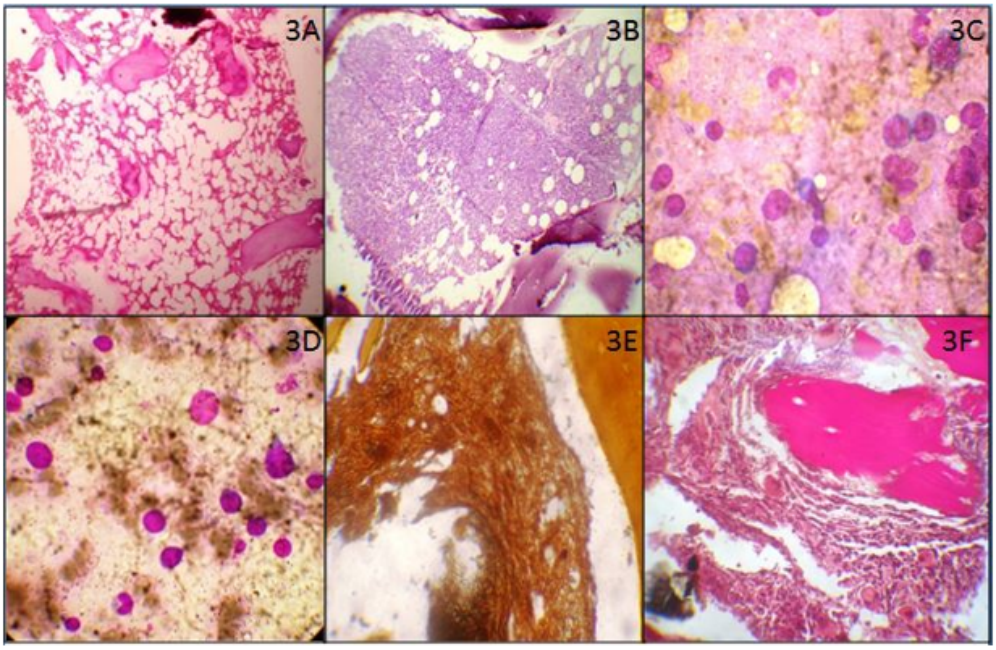

Figure 3: Photomicrograph showing;**A:** Cutaeneous Lymphoma, Normal study, (BMTB, 100×,Hematoxylin and Eosin Stain); **B**: SLL-Diffuse Involvement, BMTB, (100×,Hematoxylin and Eosin Stain); **C**: AleukaemicLeukaemia, Imprint Smear, (1000×, Leishman Stain); **D**: Acute Myelofibrosis, Imprint Smear, (1000×, Leishman Stain); **E:** Acute Myelofibrosis, Reticulin Stain Grade 4, (BMTB, 400×); **F:** Acute Myelofibrosis, BMTB, (400×, Van Gieson Stain).

Table 1: Comparison between bone marrow aspiration and bone marrow biopsy in the diagnosis of haematological malignancies (n=50):

Disease (n)	Bone marrow aspiration	Bone marrow biopsy
Acute lymphoblastic leukaemia (11)	10	11
Acute myeloid leukaemia (7)	6	7
Acute undifferentiated leukaemia (2)	1	2
Aleukaemicleukaemia (1)	0	1
Chronic lymphocytic leukaemia (1)	1	1
Chronic myeloid leukaemia (6)	6	6
Multiple myeloma(5)	4	5
Non-Hodgkin's lymphoma (12)	12	12
Hodgkin's lymphoma (4)	4	4
Acute myelofibrosis (1)	0	1
Total=50	44 (88%)	50 (100%)

Table 2: Assessment of grades of bone marrowreticulin (n=50):

Diseases (n)	Reticulin Grades				
	Grade-0	Grade-1	Grade-2	Grade-3	Grade-4
ALL (11)	2	3	4	1	1
AML (7)	2	3	1	0	1
AUL (2)	0	1	1	0	0
Aleukaemicleukaemia (1)	0	0	1	0	0
CLL (1)	0	1	0	0	0
CML (6)	0	1	4	1	0
Multiple myeloma (5)	2	2	1	0	0
NHL (12)	5	6	1	0	0
Hodgkin's lymphoma (4)	3	1	0	0	0
Acute myelofibrosis (1)	0	0	0	0	1
Total (n) = 50	14	18	13	2	3

Table 3: Assessment of cellularity in bone marrow aspiration, biopsy and in imprint smears (n=50).

Disease(n)	Aspirate smears				Imprint smears			Biopsy specimens		
	↓	N	↑	DT	↓	N	↑	↓	N	↑
ALL (11)	3	-	7	1	2	1	8	2	-	9
AML (7)	-	-	6	1	-	-	7	-	-	7
AUL(2)	-	-	1	1	-	-	2	-	-	2
Aleukaemicleukaemia (1)	1(2^{nd} attempt)	-	-	1(1^{st} attempt)	-	-	1	-	-	1
CLL (1)	-	-	1	-	-	-	1	-	-	1
CML (6)	-	-	6	-	-	-	6	-	-	6
Multiple myeloma (5)	-	1	4	-	-	-	5	-	-	5
NHL (12)	-	11	1	-	-	11	1	-	11	1
Hodgkin's lymphoma (4)	-	4	-	-	-	4	-	-	4	-
Acute myelofibrosis (1)	-	-	-	1	-	-	1	-	-	1

Note: N=Normocellular marrow, ↓=Hypocellular marrow, ↑=Hypercellular marrow, DT=Dry tap.

Table 4: Comparative evaluation of bone marrow aspiration and bone marrow biopsy in post-chemotherapy acute lymphoblastic leukaemia (ALL) (n=4)

Post-chemotherapy	Bone marrow aspiration		Bone marrow biopsy	
ALL(n=4))	Cellularity	Blast	Cellularity	Blast
Complete remission (1)	Hypocellular	Not found	Hypocellular	Not found
Complete remission (1)	Hypocellular	Not found	Hypocellular	Not found
Incomplete remission [relapse case] (1)	Normocellular	15%	Moderately cellular	Interstitial
Incomplete remission (1)	Hypercellular	40%	Hypercellular	Interstitial

(non-diagnostic), later diagnosed upon biopsy and imprintssmears [Figure 2D]. This happened due to focal involvement of the marrow in plasma cell myeloma. In all other cases including one case of chronic lymphocytic leukaemia, 6 case of CML [Figure 2F], both bone marrow aspiration and bone marrow biopsy independently diagnosed the cases. In all 4 cases of Hodgkin's lymphoma and in 11 cases (out of 12) of non-Hodgkin's lymphoma, both bone marrow aspiration and bone marrow biopsy findings were normal. Bone marrow involvement was seen in single case of small lymphocytic lymphoma (SLL), and positive findings were found in both the procedure.

We have got 12 cases of non-Hodgkin's lymphoma which includes 7 cases of diffuse large B cell lymphoma (DLBCL), 2 cases of SLL and single cases each for marginal zone lymphoma, cutaneous lymphoma [Figure 3A] and maltoma. Focal involvement of bone marrow was seen in one case of multiple myeloma. Diffuse infiltration seen in case of SLL [Figure 3B] and four cases of multiple myeloma.Table 2 shows the grading system of bone marrow reticulin, which was graded from grade 0-4.Normal marrow reticulin (grade 0-1) seen in 32 cases (64%). Increased reticulin fibrosis was seen in rest 36% of cases. Table 3 shows almost all cases, bone marrow aspiration smears and bone marrow biopsy with imprint smears correlated well in assessing cellularity and differential count. In one case of acute leukaemia (incomplete remission), marrow biopsy showed hypercellular marrow but imprint smears were reported as normocellular and bone marrow aspirates were hypocellular. In the case of aleukaemicleukaemia, second aspirate was falsely reported as hypocellular. In all other cases of dry tap, bone marrow biopsy as well as imprint smears correlated well in assessing cellularity and differential count. In a single case of multiple myeloma, bone marrow aspiration findings were absolutely normal. The common complications of both the procedures in our study were pain and shock; neurogenic shock in a patient with AML-M3 and vasovagal attack in patient with CML.Table 4 shows out of the four cases ofpost-chemotherapy ALL, two cases were clinically asymptomatic. Rest incomplete remission cases [Figure 1 A,B], one had relapse and another did not respond to chemotherapy. In the relapse case, during consolidation phase, few blast cells appeared in the peripheral blood and blast cells were also found in the bone marrow aspiration smears and in trephine biopsy sections. The bone marrow was reported as normocellular in bone marrow aspiration (BMA), but bone marrow biopsy (BMB) proved it to be moderately cellular in relapse case. Hypercellular marrow was seen in both BMA and BMB in another patient with incomplete remission. Diagnostic bone marrow aspiration and bone marrow trephine biopsy (BMTB) done in 44 cases and trephine biopsy alone in 6 cases. Bone marrow aspiration was non-diagnostic in 1 case (due to focal involvement) and dry tap occurred in 5 cases. Based on these assessments, the status of bone marrow aspiration in

diagnosis, compared with trephine biopsy was: for all cases-sensitivity: 88%, specificity: 100%, false positive: 0%, false negative: 12%, positive predictive value: 100% and negative predictive value was 16.

4. Discussion

In all cases touch imprints preparations correlated well with bone marrow trephine biopsy specimens. The imprint smears are also very important especially in five cases of dry tap by helping to diagnose the cases. It also correlates well with observation by Lu et aland James et al, showing imprint smear is important in haematological malignancies when aspirate is inadequate.[5,7] In cases of CML and acute myelofibrosis, megakaryocytic number is increased, with atypia and clustering noted which correlates well with studies of Pu et al and Bain et al.[8,9] In our study, Hodgkin's lymphoma cases were negative in marrow aspiration and biopsy. The maximum incidence of bone marrow involvement by SLL was 50% seen in our study as compared to study ofJuneja et al showing 83% bone marrow involvement.[10] A case of multiple myeloma with marrow aspirate smear showed 30-35% plasma cells of plasmacytic type and biopsy sections showed interstitial pattern of infiltration. Similar observations were reported in the study by Aghai et al which wasassociated more than 70% plasma cells in the marrow.[11] Bone marrow aspiration can distinctively assess the various cytomorphological variants of plasma cell and bone marrow biopsy can assess the plasma cell percentage, extent, infiltration pattern and marrow fibrosis. So, both the techniques complement each other in the diagnosis. Out of 21 cases, 9(43%) cases of acute leukaemia increased bone marrow fibrosis (grade 2-4) noted. Hann et al observed 57% simple marrow fibrosis in acute leukaemia.[12] In our study, incidence of dry tap was 10% with a maximum frequency seen in acute leukaemia (4/21=19%), which correlates well with the observations by Navone et alwhere dry tap occurred in 4.3% cases, with the maximum incidence in cases of acute leukaemia (35/218=16%).[13] Engeset et al found 6.6% dry tap in cases with haematological malignancies.[14] Trephine biopsy was required in conditions of dry tap (either due to increased marrow reticulin fibrosis or in hypercellular packed marrow) or when focal marrow involvement was seen e.g. in multiple myeloma. Results of our study have much similarity with observations ofNandaet al.[15] Hypercellular marrow was seen in both BMA and BMB in the patient with incomplete remission correlated with study of Kiddet al.[16] We had two cases of acute undifferentiated leukaemia; both of them were older than 40 years. This observation correlates well with the observation by Elaine et al.[17] We have got one case of acute myelofibrosis, a 4 years of old boy, with features of pancytopenia, was ultimately diagnosed by bone marrow trephine biopsy. Single case of aleukaemicleukaemia presented with pancytopenia diagnosed by bone marrow examination with grade 2marrowfibrosis and the observation correlates with the findings of Reid et al.[18]

5. Conclusion

The bone marrow biopsy is important for diagnosis, staging procedure as well as assessing prognosis. Touch imprint preparation not only helps in the cytomorphological assessment but also play a great role in assessment of cellularity and differential count. Most of the cases of dry tap, either for hyper cellular packed marrow or extensive marrow fibrosis, bone marrow trephine biopsy alone can diagnose the cases. Increased reticulin deposition (reticulin fibrosis), restricted to the area of marrow infiltration is common in haematological malignancies and assessment of reticulin, collagen fibrosis should be done on biopsy sections with the help of special stains. Bone marrow biopsy was superior for the assessment of cellularity, architecture, topographical alteration (e.g. abnormal distribution and clustering of blasts found in cases of acute leukaemia), and in bone marrow fibrosis. But bone marrow aspiration was better for the assessment of cytomorphological details and for differential count, though it was comparable with imprint smear cytology. So, we conclude that, performing bone marrow aspiration and biopsy together with imprint smear brings the correct diagnosis at the earliest for better patient management.

6. Conflict of Interest

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of this article.

7. Source of Funding

None.

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