COMPARATIVE STUDY OF BONE MARROW ASPIRATION AND BONE MARROW TREPHINE BIOPSY IN HAEMATOLOGICAL AND NON-HAEMATOLOGICAL MALIGNANCIES

Shobha Dwivedi¹, Sanjay Kumar Nigam²

HOW TO CITE THIS ARTICLE:

Shobha Dwivedi, Sanjay Kumar Nigam. "Comparative study of bone marrow aspiration and bone marrow trephine biopsy in haematological and non-haematological malignancies". Journal of Evolution of Medical and Dental Sciences 2013; Vol2, Issue 31, August 5; Page: 5773-5798.

ABSTRACT: INTRODUCTION: During the last two decades, bone marrow examination has become an indispensable adjuvant to diagnose the malignant diseases of the blood and other body systems. The ease with which a marrow trephine biopsy can now be combined with aspiration, it is suggested that this can be used more frequently with suspected neoplastic diseases. This will obviate the need for many other expensive and time-consuming investigations. **AIMS & OBJECTIVES:** We decided to study the comparison of relative efficacy of bone marrow aspiration and trephine biopsy, and cytological and histological features in different haematological and non-haematological malignancies. **METHODOLOGY:** Total 52 patients with clinical suspicion or diagnosis of any haematological or non-haematological malignancies were studied from March 2011 to Feb 2012. Patients were enquired about their presenting complaints along with clinical course and duration, and past illnesses. Aspiration smears were examined for cellularity, M: E ratio, megakaryocytes, erythropoiesis, myelopoiesis, plasma cells and abnormal cells. Trephine was also examined for trabecular structure; intertrabecular spaces; cellularity; number, distribution pattern and morphology of megakaryocytes; myelopoiesis; erythropoiesis; fibre content; and lymphoid tissue morphology and distribution. Imprint smears aided cytomorphological characterization of immature cells, observing the pattern and reticulin grading from 0 to IV. **OBSERVATION AND RESULTS:** Of the total 52 cases 67.3% (35) were haematological and 32.7% (17) non-haematological malignancy cases. Of these 11.5% were AML, 23.0% ALL, 13.5% CML, 7.7% CLL, 5.8% NHL, 1.9% MDS, 3.9% MM, 21.2% CAB, 7.7% CAP, and 3.9% CAS. 75% of 12 ALL cases were in between 11-20 years and M: F was 11:1. Majority had anemia and fever; and 91.6% were classified as ALL-L₂ and the remaining as ALL-L₁. 16.6% cases had 'dry tap' and were diagnosed on imprints. 42.9% of 7 CML cases belonged to 31-40 years and M: F was 6:1. All had anemia and splenomegaly; and 28.5% yielded 'dry tap' and diagnosed on trephine and cellularity & topographic relationship was also better appreciated. 50% of our 4 CLL cases belonged to 61-70 years and M: F was 3:1. All had anemia as the only clinical presentation. Trephine was done only in 3. In MM 50% belonged to 61-70 years while the other 50% belonged to 71-80 years and both were male. Both had bone pain and 50% also had fracture hip. Rouleaux formation was seen in one while other showed leukoerythroblastic peripheral blood picture. One was classified as plasmacytic type while the other as plasmablastic type. Both aspiration and trephine was done in all non-haematological malignancy cases and bone marrow was normoblastic normocellular. 45.4% showed fibrosis in CAB of which 60% were grade II. Similarly 75% showed fibrosis in CAP of which 33% were grade II. Only one case of CAS showed grade III fibrosis. The 'test of proportion' performed between success rates of Trephine biopsy and Aspiration provided a significant Z value of 2.0008 ($Z > 1.96$ and $p \leq 0.05$). **CONCLUSION:** It is concluded that Aspiration is the most effective method for studying cellular morphology and detection of leukemias,

MDS and identification and classification of lymphomas. Imprints however facilitate the study of cellular details; assessment of marrow infiltration and aid in 'dry tap'/scanty material when aspirate cannot be obtained because of malignant infiltration of marrow. Although trephine sections provide maximum information, all three procedures were found complementary to each other and should be evaluated simultaneously for complete bone marrow interpretation.

KEYWORDS: Bone Marrow Aspiration, Bone Marrow Trephine Biopsy, Hematological Malignancies, Non – Hematological Malignancies.

INTRODUCTION: During the last two decades, bone marrow examination has become an indispensable adjuvant to diagnose the malignant diseases of the blood and other body systems.

It has been proved that bone marrow trephine biopsy yields better results as compared to aspiration smears in diagnosing lymphomas, or non hematological neoplastic diseases; and is more sensitive and reliable than aspirate for detection of bone marrow metastasis.

Some studies (1, 2, 3, 4) in the literature indicate that trephine biopsy is more sensitive and reliable than aspirate for detection of bone marrow metastasis. However, other studies (5, 6, 7, 8, 9, 10) state that for most hematological malignancies, aspiration alone is sufficient and ensure more clear cellular details than trephine biopsy.

The ease with which a marrow trephine biopsy can now be combined with aspiration, it is suggested that when a 'bone marrow examination' is requested, this can be utilized much more frequently. This will obviate the need for many other expensive and time-consuming investigations, particularly with suspected neoplastic diseases.

AIMS AND OBJECTIVES: 1. Comparison of relative efficacy of bone marrow aspiration and trephine biopsy in haematological and non-haematological malignancies.

2. To study the cytological and histological features of bone marrow in different haematological and non-haematological malignancies.

METHODOLOGY: A total number of 52 patients with clinical suspicion or diagnosis of any haematological or non-haematological malignancies was studied.

Exact nature of complaints and clinical course of the patients was recorded. After an informed consent and a thorough clinical examination, patients were subjected to peripheral blood examination, bone marrow aspiration, bone marrow trephine biopsy and imprint preparation.

Patients were enquired about their presenting complaints along with the clinical course and their duration. Significant past illnesses were also recorded.

After an informed consent all patients were subjected to a complete general and systemic examination. Anemia, fever, lymphadenopathy, splenomegaly, hepatomegaly, bleeding tendencies and bone pain were specially looked for. Any other significant finding was also noted.

Samples for haemoglobin, total leucocyte count, differential leucocyte count, and platelet count and general blood picture were collected. For microscopic evaluation of differential leukocyte count and general blood picture peripheral blood smears were made and stained with Wright's (Romanowsky) stain. Haemoglobin estimation was done by cyanmethaemoglobin (Drabkin's method).

Posterior superior iliac crest (PSIS) was selected for bone marrow sampling in all. Aspiration was done first using Salah's bone marrow needle and 8 or more particulate smears were prepared. Next trephine was done using Jamshidi trephine needle and a biopsy core of average length 1.5 cm was usually obtained.

Wright's (Romanowsky) stained aspiration smears were examined for cellularity, M: E ratio, megakaryocytes, erythropoiesis, myelopoiesis, plasma cells and abnormal cells.

Touch and Roll imprints of the biopsy core were made and the biopsy piece was fixed in Bouin's fluid. Imprints were then air-dried and stained with Wright's stain; mounted in DPX and assessed under microscope.

Trephine biopsy was microscopically also examined for trabecular structure; intertrabecular spaces; cellularity; number, distribution pattern and morphology of megakaryocytes; myelopoiesis; erythropoiesis; fibre content; and lymphoid tissue morphology and distribution.

Imprint smears aided cytomorphological characterization of immature cells located in the marrow.

Working formulation of non-Hodgkins lymphoma (NHL) for clinical usage was followed for classification of NHL cases and FAB classification was followed for diagnosing cases of MDS and acute leukemias. Test of Proportion was used for statistical analysis.

OBSERVATION AND RESULTS: Prospective study: A total number of 52 patients with clinical suspicion of different haematological and non-haematological malignancies were studied

In all the patients, detailed clinical history was taken and physical examination was done. Patients after informed consent were subjected to haematological investigations, bone marrow aspiration and trephine biopsy.

Table 1: Procedures carried out in all 52 patients.

S.No.	Procedure		No. of patients
1	Routine Haematological procedures		52
2	Bone Marrow Aspiration smears	a) Material obtained	48
		b) Dry tap	04
3	Trephine	a) Trephine biopsy	50
		b) Imprints	50

Bone marrow aspiration was done in all 52 cases but there was 'dry tap' in 4 (four) cases. They were diagnosed as CML in 2 (two) cases and ALL in 2 (two) cases.

Trephine biopsy was however done in 50 cases only because two patients were uncooperative. They were diagnosed as NHL in one case and CLL in one case on bone marrow aspiration.

In all 52 cases bone marrow aspiration and trephine biopsy was performed from posterior superior iliac spine.

Graph 1: Distribution of Cases included.

Out of the 6 total 52 cases included in the study Acute Lymphoblastic Leukemia (ALL) comprised of 12 cases (23.0%), Acute Myeloid Leukemia (AML) comprised of 6 cases (11.5%), Chronic Myeloid Leukemia (CML) comprised of 7 cases (13.5%), Chronic Lymphoid Leukemia (CLL) comprised of 4 cases (7.7%), Myelodysplastic Syndrome (MDS) comprised of 1 case (1.9%), Non-Hodgkin's Lymphoma (NHL) comprised of 3 cases (5.8%), Carcinoma Breast (CAB) comprised of 11 cases (21.2%), Carcinoma Prostate (CAP) comprised of 4 cases (7.7%) and Carcinoma Stomach (CAS) comprised of 2 cases (3.9%).

Table 2: Final diagnosis and Procedures carried out.

S.No.	Final Diagnosis	Total Cases	%	Procedures Done		
				Aspiration	Trephine Biopsy	Imprint Cytology
1	ALL	12	23.0	10	12	12
2	AML	6	11.5	6	6	6
3	CML	7	13.5	5	7	7
4	CLL	4	7.7	4	3	3
5	MDS	1	1.9	1	1	1
6	MM	2	3.9	2	2	2
7	NHL	3	5.8	3	2	2
8	CAB	11	21.2	11	11	11
9	CAP	4	7.7	4	4	4
10	CAS	2	3.9	2	2	2
TOTAL		52	-	49	50	50

Table 3: Incidence of abnormal Bone Marrow findings.

S.No	Particulars	No. of patients
1	Total no. of patients under study	52
2	Cases with Normal Bone Marrow finding	17
3	Cases with Abnormal Bone Marrow findings	35

ORIGINAL ARTICLE

Out of total 52 cases in which bone marrow procedure were done, abnormal bone marrow finding was present in 35 cases (67.3%), where as normal bone marrow finding was present in 17 cases (32.7%).

ACUTE MYELOID LEUKAEMIA (AML):

Table 4: Age wise distribution.

Age (yrs)	No. of cases	%
11-20	2	33.3
21-30	1	16.6
31-40	1	16.6
41-50	1	16.6
51-60	1	16.6
TOTAL	6	-

In our 6 (six) cases of AML 2 cases (33.3%) were from 11-20 years and the remaining 4 cases were one case each (16.6%) from 21-30 years, 31-40 years, 41-50 years and 51-60 years age groups.

Graph 2: Sex wise distribution.

Out of the 6 cases of AML 3 cases each (50%) were male and 3 female, making the M: F ratio 1:1.

Graph 3: Clinical presentation of AML patients.

ORIGINAL ARTICLE

In our 6 cases of Acute Myeloid Leukemia (AML), majority had anemia and fever, in 6 cases (100%) and 5 cases (83.3%) respectively. Bleeding tendency was present in 2 cases (33.3%) and generalised lymphadenopathy, splenomegaly, hepatomegaly and bone pain was reported in 1 case (16.7%) each.

OBSERVATIONS: Both aspiration and trephine biopsy was done in all the 6 cases of AML.

As per FAB classification 4 cases were classified as AML-M₁ and the remaining were classified as AML-M₂.

Haemoglobin ranged in between 6.0 to 10.7gm% with a mean of 8.15gm% (SD $\approx$ 1.85), TLC from 45,000 to 1,40,000 cells/cu mm with a mean of 91333.3 cells/cu mm (SD $\approx$ 38779.7) and Platelet count from 56,000 to 1,00,000 cells/cu mm with a mean of 72,500 cells/cu mm (SD $\approx$ 14989.9).

Table 5: Observation table for 6 cases of AML.

Diagnosis	Aspiration Smear	Trephine Biopsy	Imprint
M ₂	Hypercellular. M: E was 28:1 & 35:1 in the 2 cases respectively. Blasts 56 & 60 % respectively. Large cells with moderately basophilic cytoplasm with diffuse chromatin & 2-4 nuclei. Few promyelocytes & myelocytes seen. Erythroid, myeloid and megakaryocytes series depleted.	Hypercellular marrow packed with immature blast cells. Other cell series depleted. Reticulin grade III in both cases.	Same as Aspiration.
M ₁	Hypercellular. M: E ranged from 20:1-35:1. Blasts ranged from 80-90%. Erythroid, myeloid & megakaryocytes series depleted.	Hypercellular marrow packed with Blast cells. Other cell series depleted. Reticulin grade I in 3 cases.	Same as Aspiration.

In all the 6 cases marrow was hypercellular and myeloid to erythroid ratio was increased. Bone marrow aspirates in 4 cases showed sheets of myeloblasts with few maturing cells (<10%) and were classified as AML-M₁. In the remaining 2 cases good number of myeloblasts are seen with promyelocytes and myelocytes (>10%) and were classified as AML-M₂. Erythroid and megakaryocytic cell series were depleted in all the cases.

Trephine showed hypercellular marrow with depletion of fat spaces. Marrow was packed with immature cells in all the cases. Differential count done on imprint smears gave almost same results as on aspiration smears. Reticulin stain in 2 cases showed grade III, in other 3 cases grade I and in one case grade 0 fibrosis.

Cellularity was better commented on trephine biopsy.

ORIGINAL ARTICLE

ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL):

Table 6: Age wise distribution.

Age (yrs)	No. of cases	%
0-10	1	8.3
11-20	9	75.0
21-30	1	8.3
31-40	1	8.3
TOTAL	12	-

In our 12 (twelve) cases of ALL 9 cases (75%) were in between 11 to 20 years and one case each (8.3%) was in between 0-10 years, 21-30 years, and 31-40 years age groups respectively.

Graph 4: Sex wise distribution.

Out of the 12 cases of ALL 11 (91.6%) were male and one (8.3%) was female, making the male to female (M: F) ratio 11:1.

Graph 5: Clinical presentation of ALL patients.

In our 12 cases of ALL 12 cases (100%) had anemia and 11 cases (91.7%) had fever, followed by generalised lymphadenopathy and splenomegaly in 7 cases (58.3%) and 6 cases (50%) respectively. Hepatomegaly was present in 2 cases (16.7%) and bleeding tendency and bone pain was reported in 1 case (8.3%) each.

OBSERVATIONS: Out of 12 cases of ALL aspiration smears were available in 10 cases only as in 2 (two) cases it was 'dry tap'. Trepine was however done in all the 12 cases.

As per FAB classification one case was classified as ALL-L₁ and the remaining 11 cases were classified as ALL-L₂.

Haemoglobin (Hb) ranged from 5.8 to 8.6 gm% with a mean of 7.2gm% (Standard Deviation (SD) $\approx$ 1.05). Total leucocyte count (TLC) ranged from 20,000 to 1,52,000 cells/cu mm with a mean of 78675.0 cells/cu mm (SD $\approx$ 50930.4).

Platelet count in ALL patients ranged from 38,000 cell/cu mm to 90,000 cells/cu mm with mean of 62,000 cells/cu mm (SD $\approx$ 18075.6).

Table 7: Observation table for the 12 cases of ALL:

Aspiration Smear	Trepine Biopsy	Imprint
Hypercellular. M: E = 3: 1 – 6: 1. Sheets of Blast = 38-85% Large cell with scant Basophilic cytoplasm, coarse nuclei with 1-2 small nucleoli.	Hypercellular with diffuse infiltration of Blasts. Other cell series depleted. Reticulin grade ranged from I-III	Same as Aspiration.

Erythroid, myeloid and megakaryocyte series depleted.		
---	--	--

In all the 12 case marrow was hypercellular and myeloid to erythroid ratio (M: E) was normal. Bone marrow aspirate in one case showed homogeneous population of lymphoblasts and was classified as ALL-L₁. In the remaining 9 cases aspirate showed heterogeneous population of lymphoblasts and were classified as ALL-L₂. Erythroid, myeloid and megakaryocyte cell series were depleted in all the cases.

Trephine biopsy showed hypercellular marrow with depletion of fat spaces. Marrow was diffusely infiltrated by blasts in all the cases. Differential count done on imprint smears gave almost same results as that on aspiration smears. Reticulin fibrosis was reported as grade III in 5 cases, grade II in another 5 cases and grade I in the remaining 2 cases.

In the 2 cases (16.6%) with 'dry tap' diagnosis was made on imprints and in the remaining 10 cases (83.3%) diagnosis was made by aspiration smear examination but cellularity and fibrosis was better commented on trephine biopsy examination.

CHRONIC MYELOID LEUKAEMIA (CML):

Table 8: Age wise distribution.

Age (yrs)	No. of cases	%
11-20	1	14.3
21-30	1	14.3
31-40	3	42.9
41-50	2	28.6
TOTAL	07	-

In our 7 (seven) cases of CML 3 cases (42.9%) belonged to 31-40 years age group, 2 cases (28.6%) were from 41-50 years age group and one case each (14.3%) belonged to 11-20 years and 21-30 years age groups respectively.

Graph 6: Sex wise distribution.

Out of the 7 cases of CML 6 (85.7%) were male and one (14.3%) was female making the M: F ratio 6:1.

Graph 7: Clinical presentation of CML patients.

All our 7 cases of CML had anemia and splenomegaly. Fever and generalised lymphadenopathy was present in 3 cases (42.9%) each, hepatomegaly was present in 2 cases (28.6%) and bleeding tendency and bone pain was reported in 1 case each (14.3%).

Observations:

Out of the 7 cases of CML aspiration smears were available in 5 (five) cases only as 2 (two) cases yielded 'dry tap'. Trepine was however done in all 7 cases.

Haemoglobin ranged from 5.9 to 11gm% with a mean of 7.3gm% (SD $\approx$ 1.8), TLC ranged inbetween 75,680 to 2,70,550 cells/cu mm with a mean of 1,35,757.0 cells/cu mm (SD $\approx$ 65486.8) and platelet count ranged from inbetween 1,90,000 to 6,00,000 cells/cu mm with a mean of 4,70,000 cells/cu mm (SD $\approx$ 141067.4).

Table 9: Observation table for 7 cases of CML:

Aspiration Smear	Trepine Biopsy	Imprint
Hypercellular. M: E ratio ranged from 22: 1 – 46: 1. Marked granulocytic hyperplasia with shift to left. Erythroid series depleted. Megakaryocytes increased.	Hypercellular. Normal topographic relation maintained. Increased granulocyte precursors with shift to left.	Same as Aspiration.

ORIGINAL ARTICLE

	Erythroid series depleted. Megakaryocytes increased small hypo lobated. Reticulin grade ranged from I-IV	
--	--	--

Marrow was hypercellular and myeloid to erythroid ratio was increased in all the cases. Bone marrow aspirate showed marked hyperplasia of granulocytic cell series with shift to left and increase in eosinophil precursors and basophils. Erythroid cell series was depleted in 3 cases but was hyperplastic in 2 cases whereas megakaryocytic cell series was increased in all the 5 cases.

Trephine biopsy showed hypercellular marrow with marked increase in granulocyte precursors and shift to left. Differential count done on imprint almost gave similar results as on aspiration smears. Reticulin fibrosis was reported in all cases. In 5 cases it was grade III and was grade IV and grade I in one case each.

Cellularity and the topographic relationship were better appreciated on trephine biopsy.

CHRONIC LYMPHOID LEUKAEMIA (CLL):

Table 10: Age & Sex distribution.

Age (yrs)	No. of cases	%
41-50	1	25.0
51-60	1	25.0
61-70	2	50.0
TOTAL	4	-

In our 4 (four) cases of CLL 2 cases (50%) belonged to 61-70 years age group and 1 case each (25%) belonged to 41-50 years and 51-60 years age groups respectively.

Graph 8: Sex wise distribution.

Of the 4 case of CLL 3 (75%) were male and one (25%) was female, making the M: F ratio 3:1. All 4 cases of CLL had anemia as the only clinical presentation.

Observations:

ORIGINAL ARTICLE

Aspiration smears were available in all 4 cases but trephine was done only in 3 cases.

Haemoglobin ranged from 7.4gm 8.4gm% with a mean of 7.9gm% (SD $\approx$ 0.4), TLC ranged from 45,000 to 1,10,000 cells/cu mm with a mean of 80,750 cells/cu mm (SD $\approx$ 27244.3) and platelet counts ranged 80,000 to 1,20,000 cells/cu mm with a mean of 1,00,000 cells/cu mm (SD $\approx$ 18257).

Table 11: Observation table for 4 cases of CLL

Aspiration Smear	Trephine Biopsy	Imprint
Hypercellular. M: E ratio ranged from 1:1-6:1. Good number of nature lymphocytes. Erythroid and myeloid series depleted. Megakaryocytes was depleted in 2 cases whereas normal in other 2 cases.	Hypercellular. 2 cases diffuse infiltration 1 case nodular infiltration. Reticulin grade III in all cases.	Same as Aspiration.

In all 4 cases marrow was hypercellular with myeloid to erythroid ratio within normal range. Bone marrow aspirate showed increased number of mature lymphocytes. Erythroid and myeloid cell series were depleted in all cases while megakaryocytes were depleted in only 2 cases.

Trephine showed hypercellular marrow with decreased fat spaces. Marrow was diffusely infiltrated by mature lymphocytes in 2 cases and showed nodular infiltration in one case. Differential count done on imprint almost gave similar results as that on aspiration smears. Grade III reticulin fibrosis was reported in all 3 cases.

Cellularity and topographical relationship could be better studied on trephine biopsy specimen.

NON-HODGKIN'S LYMPHOMA (NHL):

Table 12: Age wise distribution.

Age (yrs)	No. of cases	%
41-50	2	66.7
51-60	1	33.3
TOTAL	3	

Of our 3 cases of NHL 2 cases were from 41-50 years age group and the remaining one patient was from 51-60 year age group. All the 3 cases were male.

Graph 9: Clinical presentation of CML patients.

All 3 cases (100%) had anemia and generalized lymphadenopathy and fever and splenomegaly was present in 2 cases each (66.7%).

Observations of Non-Hodgkin's Lymphoma (NHL):Aspiration smears were available in all 3 cases but trephine was available only in 2 cases.

Haemoglobin ranged from 5.7 to 9gm% with a mean of 7.4gm% (SD $\approx$ 1.7) TLC ranged from 10,300 to 35,600 cells/cu mm with a mean of 21,000 cells/cu mm (SD $\approx$ 12968.8) and platelet count ranged from 35000 to 2,20,000 cells/cu mm with mean of 1,10,333.3 cells/cu mm (SD $\approx$ 97161.4).

Table 13: Distribution of NHL cases positive for marrow metastasis.

	Final Diagnosis	No. of Cases
I	Low Grade <ul style="list-style-type: none"> Small Lymphocytic Lymphoma CLL type 	1
II	Intermediate Grade	0
III	High Grade <ul style="list-style-type: none"> Large cell Lymphoblastic Lymphoma 	2
	Total no. of Cases	3

According to working formulation one case was classified as small lymphocytic lymphoma and 2 cases were classified as lymphoblastic lymphoma. In 2 cases of lymphoblastic lymphoma marrow showed sheets of lymphoblasts, in other cases majority of cells were small lymphocytes with cleaved nuclei.

Table 14: Observation table for 3 cases of NHL

ORIGINAL ARTICLE

Diagnosis	Aspiration Smear	Trephine Biopsy	Imprint
Small lymphocytic lymphoma (1 case)	Hypercellular. Atypical lymphoid cells 70%. Small cells with depleted nuclei, clumped chromatin and inconspicuous nucleoli. Erythroid, myeloid and megakaryocyte cell series depleted.	Hypercellular Marrow packed with mature lymphocytes. Other cell series depleted. Reticulin grade III.	Same as Aspiration
Lymphoblastic lymphoma (2 cases)	Hypercellular Atypical lymphoid cells 66% and 56% respectively. Small to medium sized cells with irregular large nuclei, coarse chromatin and 1-2 small nucleoli. Other cells series depleted.	Hypercellular. Diffuse infiltration by blast cells. Other cell series depleted. Reticulin grade III	Same as Aspiration

Aspiration was hypercellular in 2 cases but appeared normocellular in one case. However, in all the cases myeloid to erythroid ratio was within normal range and erythroid, myeloid and megakaryocyte cell series were depleted in all the cases.

Trephine showed hypercellular marrow with fat spaces in both the cases. In 1 case of lymphoblastic lymphoma marrow was diffusely infiltrated by blasts cell while in another case marrow was packed with mature lymphocytes. Differential count done on imprint smears and aspiration smear correlated well. Reticulin fibrosis was of grade III.

MYELOYDYSPLASTIC SYNDROME (MDS): Our one case of MDS belonged to 21-30 years age group and was a female patient with clinical presentation of anemia and fever.

OBSERVATIONS: Both aspiration and trephine was done in the one case of MDS.

Haemoglobin was 7gm%, total leucocyte count 2100 cells/cu mm and platelet count was 96,000 cells/cu mm. Peripheral blood picture showed leukopenia.

Table 15: Observation table for 1 case of MDS

Aspiration Smear	Trephine Biopsy	Imprint
Hypercellular. M: E = 1:2. Erythroid hyperplasia megaloblastic maturation with dyserythropoietic features. Few binucleated cells seen. Myeloid – ↓ granulation Megakaryocytes – micro megakaryocytes and	Hypercellular megaloblastic maturation. Erythroid – hyperplasia with megaloblastic maturation. Dyshematopoietic features in all the cell series. Reticulin grade III.	Same as Aspiration.

ORIGINAL ARTICLE

multinucleated megakaryocytes.		
--------------------------------	--	--

Bone marrow aspiration showed hypercellular marrow with reverse normal M: E ratio. Erythroid cell series show megaloblastic maturation with dyserythropoietic features; myeloid cell series showed decreased granulation of cytoplasm; and megakaryocyte series showed micro megakaryocytes and megakaryocytes with multinucleation.

Trephine showed hypercellular marrow with dyshemopoietic features in all the cell lines. Reticulin stain revealed grade III fibrosis.

MULTIPLE MYELOMA (MM):

Table 16: Age wise distribution.

Age (yrs)	No. of cases	%
61-70	1	50.0
71-80	1	50.0
TOTAL	2	-

Of our 2 cases of MM one belonged in 61-70 years age group while the other belonged in 71-80 years age groups and both were male.

Graph 10: Clinical presentation of MM patients.

Both the 2 cases (100%) had bone pain and one case each (50%) had fever and fracture hip.

OBSERVATIONS: Aspiration and trephine both were done in both the cases of MM.

ORIGINAL ARTICLE

Haemoglobin was 12.5 and 11.5 gm% respectively with a mean of 12 gm% (SD $\approx$ 9227.7); TLC was 5,600 and 18,650 with a mean of 12125 cells/cu mm (SD $\approx$ 9227.7); and platelet count was 1,20,000 and 1,70,000 cells/cumm with a mean of 1,45,000 cells/cumm (SD $\approx$ 35355.3). In one case rouleaux formation was seen in peripheral blood while other case showed leukoerythroblastic blood picture.

Table 17: Observation table for 2 cases of MM

Aspiration Smear	Trephine Biopsy	Imprint
Hypercellular. Plasma cells and plasma blasts in groups and sheets. Few binucleated and multinucleated plasma cells present. Other cell series normal. M: E was 6:1 and 5:1 in the 2 cases respectively.	Hypercellular. Diffuse sheets of myeloma cells. Reticulin grade III and II in the 2 cases respectively.	Same as Aspiration.

In both the cases marrow was hypercellular. Mature plasma cells were present in one case with few plasma blasts so this was classified as plasmacytic type; while in one case plasma blast accounted for majority of plasma cell population, hence this was classified as plasmablastic type. Other cell series were normal in number.

Marrow trephine biopsy examination was hypercellular and showed diffuse infiltration by plasma cells in both the cases. Differential count done on imprint smears gave almost same results as that of aspiration smears. One case showed grade III fibrosis while in the other case it was of grade II.

Cellularity and topographical relationship could be better assessed on trephine sections.

NON-HAEMATOLOGICAL MALIGNANCIES (CAB, CAP, CAS):

Table 18: Age and Sex distribution in cases of non-haematological malignancies.

Age (in yrs)	CA Breast		CA Prostate		CA Stomach	
	No. of cases	%	No. of cases	%	No. of cases	%
30-35	4	36.4	-	-	-	-
36-40	3	27.3	-	-	-	-
41-45	3	27.3	-	-	-	-
46-50	-	-	-	-	1	50.0
51-55	-	-	-	-	1	50.0
56-60	-	-	1	25.0	-	-
61-65	1	9.1	1	25.0	-	-
66-70	-	-	2	50.0	-	-
TOTAL	11	-	4	-	2	-

Out of our 17 cases of non-haematological malignancies, 11 cases were of carcinoma breast (CAB), 4 cases were of carcinoma prostate (CAP) and 2 cases were of carcinoma stomach (CAS).

Graph 11: Sex wise distribution of CAB cases.

In our 11 cases of CAB 6 cases were from 31-40 years age group, 3 cases were from 41-50 years age group and remaining 1 case each were from 21-30 year age group and 61-70 year age groups respectively. Only one case was male whereas all other 10 cases were female, making the M: F ratio 1:10.

In our 4 cases of CAP 3 cases were from 61-70 years age group and 1 case was from 51-60 years age group.

Both our 2 cases of CAS were from 51-60 years age group and were male.

Graph 12: Clinical presentation of CAB patients.

ORIGINAL ARTICLE

All our 11 cases of CAB presented with swelling/lump over the breast. Lymphadenopathy was also reported in 2 cases, ulcer and swelling was also reported in 1 case, whereas pain and retracted nipple was also reported in another 1 case.

Graph 13: Clinical presentation of CAP patients.

All our 4 cases of CAP presented with dysuria, difficulty in micturition and hematuria. Increase in frequency of micturition was however also reported in one case.

Both our cases of carcinoma stomach (CAS), presented with anorexia, weight loss and vomiting. Pain abdomen was also present in one case.

OBSERVATIONS:Both aspiration and trephine biopsy was done in all the cases of non-haematological malignancies.

Haemoglobin ranged from 8.6 to 12gm% with a mean of 10.9gm% (SD $\approx$ 1.1), TLC ranged from 5600 to 8350 cells/cumm with a mean of 6915.6 cells/cumm (SD $\approx$ 859.6) and platelet count ranged from 1,60,000 to 4,12,000 cells/cumm with a mean of 2,66,687.5 cells/cumm (SD $\approx$ 57792.4).

Table 19: Observation table for 17 cases of Non Haematological Malignancies.

Diagnosis	Aspiration Smear	Trephine Biopsy	Imprint
CAB (11 cases) + CAP (4 cases) + CAS (2 cases)	Normocellular. M: E ranged from 3:1-6:1. Erythroid, myeloid and megakaryocyte cell series normal. No atypical cells seen.	Normocellular. Topographic relationship maintained. All the cell series normal. No atypical cell seen. Reticulin grade ranged from grade I-II.	Same as Aspiration.

In all the cases bone marrow was normoblastic normocellular. Out of the 11 cases of CAB only 5 cases showed fibrosis of which 3 were grade II and 2 were grade I. Out of the 4 cases of CAP 3

cases showed fibrosis of which 2 cases showed grade I and one case showed grade II fibrosis. Out of 2 cases of carcinoma stomach only one case showed grade III fibrosis.

Table 20: TEST OF PROPORTION

	Trephine Biopsy	%	Aspiration	%
Success rate	50	100.00	48	92.31
Failure rate	0	-	4	7.69
Total	50	-	52	-
Z = 2.0008 (Z > 1.96; P ≤ 0.05)				

Trephine biopsy was performed in 50 cases of our study and material was obtained in all the cases making the success rate 100%. Aspiration was however performed in all the 52 cases but material was obtained only in 48 cases making the success rate 92.31%. The 'test of proportion' performed provided a Z value of 2.0008 which was significant ($Z > 1.96$ and $p \leq 0.05$).

DISCUSSION: The importance of studying the bone marrow of patients in selected clinical diseases is well known. A variety of techniques utilizing marrow aspirates and biopsy material have been introduced in order to facilitate bone marrow examination and interpretation.

Smears of aspirated marrow are ideal for the study of cytological details of hematopoietic cells and are suitable for cytochemical studies ⁽¹¹⁾.

The aspiration smears contain marrow blood elements from the central intertrabecular regions of the marrow. In the setting of diffuse involvement, aspiration smears are representative preparation of the true marrow picture but not so if there is variable distribution in paratrabecular and central region ⁽¹²⁾.

Trephine imprints are more representative of marrow milieu as the artifacts inherent to aspiration smears are avoided and trephine imprints provide equally good morphological details. Cytochemical stains can be done with equal ease on aspiration smears and trephine imprints. Trephine sections provide maximum information by showing the marrow elements in their exact topographical location ⁽¹³⁾ and by permitting quantification of various cell populations.

In our 6 reported cases of AML, diagnosis was made on blood film and aspiration smear examination. In acute leukaemia FAB (French, American and British) subtype, myelofibrosis and overall cellularity are important factors for prognosis and follow up of patients ^(14, 15, and 16).

Taking into consideration the morphological features, FAB subtyping can be done on aspiration smears and trephine imprints where as trephine section did not prove to be a good preparation for this purpose, similar observation have been made by previous workers as well ⁽¹⁴⁾.

In a study ⁽¹⁷⁾, reticulin fibrosis was seen in upto one third of cases. Out of 6 reported cases of AML in the present study, reticulin fibrosis was reported in 5 cases.

Like other leukemias diagnosis of acute lymphoblastic leukaemia can be made on blood films and aspiration smears alone. In case of dry tap, imprints provide good morphology similar to aspiration smears.

In present study of 12 cases of ALL, aspirates smear were available in 11 cases and in one case it was 'dry tap'. Diagnosis in that case was made on imprint which later confirmed by trephine biopsy.

The assessment of overall cellularity, residual hemopoietic tissues and myelofibrosis which all affect prognosis and treatment of patient can be known only by examination of trephine sections.

In cases of 'dry tap', imprints provide good morphology equivalent to as aspiration smears and sometimes even better identification of cells.

Chronic myeloid leukemia can be diagnosed by examination of peripheral blood and aspiration smears. Trephine biopsy is useful in cases where aspirates fail/dry due to myelofibrosis.

Out of the 7 cases of CML in this study, aspirate could be obtained only in 5 cases. In 2 cases it was 'dry tap'. Quick reporting was given by examination of imprints which was later on confirmed by trephine biopsy.

It has been suggested that cases with marked megakaryocytic proliferation, often accompanied by fibrosis should be distinguished from chronic granulocytic leukaemia and classified as chronic megakaryocytic granulocytic myelosis. However, this distinction is arbitrary and unnecessary since such cases, when they are Philadelphia positive do not differ in any important respect from other cases of chronic granulocytic leukaemia⁽¹⁸⁾.

Reticulin fibrosis is usually increased in CML and it is more common in cases with marked megakaryocytic proliferation⁽¹⁹⁾.

Prognostic features in chronic phase of chronic granulocytic leukaemia include the number of megakaryocytes and the degree of reticulin and collagen fibrosis (indicative of worse prognosis) and the proportion of erythroid precursors (indicative of a better prognosis)⁽²⁰⁾.

In CML the importance of trephine section lies in characterization of proliferating cell line/lines and associated myelofibrosis. Acute transformation can be diagnosed if trephine biopsy sections show extensive focal infiltration by blast cells.

In chronic lymphocytic leukaemia the bone marrow histologic patterns have significant prognostic value as evaluated by some authors. Cases with diffuse bone marrow infiltration had a poor prognosis as compared to cases presenting with a nodular or mixed (nodular and diffuse) pattern. Diffuse pattern of bone marrow histology could be considered as the best criterion for initiation of therapy in CLL patients⁽²¹⁾.

One of the study concluded that trephine sections provide useful information about pattern of involvement and fibrosis which was not forth coming from aspiration smears and trephine imprints in chronic leukemias.⁽²²⁾

In the present study, out of 4 cases of CLL, 2 cases showed diffuse infiltration while one case showed nodular infiltration by malignant lymphoid cells. Marrow was hypercellular in all the 4 cases and grade III fibrosis was observed in all the cases.

For lymphoma bone marrow examination is an essential part of pre-therapy work up and follows up⁽²²⁾. Marrow involvement in the presence of nodal disease indicates stage IV disease. The non-Hodgkin's lymphomas (NHL) that involve the bone marrow most frequently are the low grade categories comprising primarily of small cells (small lymphocytic and small cleaved cell), high grade lymphomas of lymphoblastic and small non cleaved cell types, and the various peripheral T cell lymphomas that span all of the working formulation prognostic groups. Among B-lineage lymphoma,

bone marrow infiltration is more common in low-grade tumours than in high grade. Overall infiltration is probably more common in B cell lymphoma than in T cell.

In our study of 3 cases of lymphoma all were recognized and classified by aspiration smears alone. Following the working formulation, 2 cases were reported as lymphoblastic type (high grade) and one case was reported as small lymphocytic type (low grade). In these cases additional advantages in terms of true cellularity, residual hemopoietic tissue, pattern of involvement and fibrosis were obtained from trephine biopsy.

Diagnosis of myelodysplastic syndrome (MDS) requires consideration of clinical, peripheral blood and bone marrow features. Peripheral blood and bone marrow aspirate findings are most important and in a straight forward case may be all that is required for diagnosis.

However, when the bone marrow aspiration is inadequate, trephine biopsy can establish diagnosis and type of MDS and rules out aplasia or tumour infiltration as possible alternative causes of cytopenia ⁽²³⁾.

Also an excess of blasts or 'abnormal localization of immature precursors' (ALIP) can be detected on trephine sections only. The presence of ALIP predisposes patients to early death with high probability of developing acute myeloid leukemia, whereas the absence of ALIP carries a better prognosis with more long-term survival and low probability of transformation to AML ⁽²⁴⁾.

Histopathological evaluation of bone marrow trephines is therefore strongly advocated as an additional source of information not only to establish diagnosis but also to assess accurately bone marrow cellularity, fibrosis, and the presence of ALIP and to detect the incipient evolution into acute non lymphoid leukaemia.

In case of multiple myeloma usually the diagnosis is made by examination of aspiration smears as cellular details are best appreciated. The importance of aspiration smears lies in recognition of the types of multiple myeloma, as the frequency of a leukaemic blood picture (plasma cell leukaemia) is highest in patients with plasmablastic type and this has an unfavourable prognosis.

It is not always possible to make a diagnosis of multiple myeloma on bone marrow morphology alone. Multiple myeloma characteristically affects bone marrow in a patchy manner and an aspirate from a patient with multiple myeloma will not necessarily contain a large number of plasma cells, nor will the morphology of the myeloma cells necessarily be very abnormal ⁽²⁵⁾.

For non-haematological malignancies the presence of metastasis in the bone marrow alters the outcome for the patient so drastically, that it should be detected as early as possible. Marrow involvement indicates stages IV disease and it alters the clinical course, response to treatment and overall survival of patient.

Though incidence of marrow metastasis in Carcinoma Breast (CAB), Carcinoma Prostate (CAP) and Carcinoma Stomach (CAS) was reported as 30%, 28% and 10%, respectively by Eliva and Lawrence, 19.6%, 20.3% and 3.4% respectively by Anner and Drewinko in their respective studies, whereas in our study we could not find any positive case either due to small sample size or due to early stage of the disease.

In all these cases, aspiration and trephine biopsy findings correlated well however the assessment of cellularity and fibrosis could only be done on trephine biopsy.

After analysing the results test of proportion was done to compare the reliability between aspiration and trephine biopsy. Z value of 2.008 was obtained which was significant ($Z \geq 1.96$ and $P \leq 0.05$) and emphasizes the reliability of trephine biopsy over aspiration.

CONCLUSION: With the aim to study the merits and demerits of aspiration smears, trephine biopsies and imprint cytology, following conclusions were drawn. Bone marrow may be the only positive investigation in non-Hodgkin's lymphoma. Trephine biopsies along with touch preparations answer many queries and aspiration smears act complementary. Aspiration smears in conjunction with touch imprints provide good cytomorphological details and are helpful in morphological classification of lymphomas.

Trephine helps identify patterns of infiltration, state of uninvolved marrow (residual hematopoietic tissue) and effects on bony structure, which all affect prognosis. Diagnosis can be made by aspiration smears alone but additional information about the cellularity and fibrosis is provided by trephine and is also helpful in 'dry tap'.

Imprints facilitate the study of cellular details when aspirate cannot be obtained because of malignant infiltration of marrow.

The differential counts on trephine imprints and aspiration smears correlate well and cytomorphological characterization of immature cells (blasts and promyelocytes) can also be done.

Trephine biopsy not only provides diagnosis in 'dry tap'/scanty material but is also useful for assessment of marrow infiltration in lymphoma, cellularity, megakaryocyte density and proliferating cell lines in myeloproliferative disorders. Bone marrow fibrosis, patterns of bone marrow involvement and topographical alterations are all appreciable only on trephine sections.

Although trephine sections provide maximum information, all three procedures were found complementary to each other and should be evaluated simultaneously for complete bone marrow interpretation. So it would be only practical to conclude that in all suspected cases of metastasis it is worthwhile to perform a single specific informative procedure (Trephine Biopsy) alone rather than doing aspiration alone and risk chances of 'dry tap'/ scanty material, or doing all three and causing additional trauma to the patient and misusing/wasting manual and laboratory resources.

This will further save the patient from the agony of repeated painful aspirations due to failed procedure (Dry Tap/ Scanty Material) and hence will also reduce the duration of hospital stay and the cost involved.

BIBLIOGRAPHY:

1. Bearden JD, Ratkin GA, Coltman CA, Comparison of the diagnostic value of bone marrow biopsy and bone marrow aspirate in neoplastic disease. J. Clin Pathol 1974; 27:738-740.
2. Contreras E, Ellis LD and Lee RE. Value of the bone marrow biopsy in the diagnosis of metastatic carcinoma. Cancer 1972; 29:778-783.
3. Brain W, Kelly, James F. Morris, Brian P. Harwood, Timothy E. Bruya. Methods and prognostic value of bone marrow examination in small cell carcinoma of the lung. Cancer 1984; 53: 99-102.
4. Webb DI, Ubogy G and Silver RT. Importance of bone marrow biopsy in the clinical staging of Hodgkin's disease. Cancer 1970; 26:313-17.

5. Coppala A. Comparison of bone marrow aspiration versus biopsy. *Am J Clin Pathol* 1977; 67(3):309.
6. Beaden JD, Ratkin GA and Coltman CA. Comparison of the diagnostic value of bone marrow biopsy and bone marrow aspiration in neoplastic disease. *J Clin Pathol* 1974; 27:738-740.
7. Slager UT, Reilly EB. Value of examining bone marrow in diagnosing malignancy. *Cancer* 1967; 20:1215-20.
8. Brynes RK, Mckenna RW, Sundberg RD. Bone marrow aspiration and trephine biopsy – An approach to a thorough study. *Am J Clin Pathol* 1978; 70:753-59.
9. Williamson PJ, Smith AG. Bone marrow aspiration and biopsy. *Br J Hosp Med* 1991; 46(5):328-30.
10. Varma N, Dash S, Sarode R and Marwaha N. Relative efficacy of bone marrow trephine biopsy sections as compared to trephine imprints and aspiration smears in routine hematological practice. *Indian J Pathol Microbiol* 1993; 36(3):215-226.
11. James LP, Stass SA and Schumacher HR. Value of imprint preparations of bone marrow biopsies in hematologic diagnosis. *Cancer* 1980; 46:173-77.
12. Block M. Bone marrow examination. *Arch Pathol Lab Med* 1976; 100:454.
13. Frisch B, Lewis SM, Burkhardt R and Bartl R. Biopsy pathology of bone and bone marrow- biopsy pathology series. London: Chapman and Hall; 1985.
14. 14.Bartl R, Frisch B, Burkhardt R. Bone marrow biopsies revisited – a new dimension for haematological malignancies. Basel (Switzerland): 1985.
15. 15.Bennet JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR. Proposals for the classification of the acute leukaemias. *Br J Haematol* 1976; 33:451-458.
16. 16.Ferzi OM, Scribano P, Comperts E, Izadi P, Millin R, Issacs JH et al. Comparative evaluation of the bone marrow by the volumetric method, particle smears and biopsies in pediatric disorders. *Am J Haematol* 1988; 29:144-147?
17. Islam A, Catovsky D, Goldman JM and Galton DA. Bone marrow fibre content in acute myeloid leukaemia before and after treatment. *J Clin Pathol* 1984; 37:1259-1263.
18. Hyun BH, Gulati GL, Ashton JK. Bone marrow examination: Techniques and interpretation. *Hemato Oncol Clin North Am.* 1988; 2; 513-523.
19. Lazzarino M, Morra E, Castello A, Inverardi D, Coci A, Pagnucco G et al. Myelofibrosis in chronic granulocytic leukemia : Clinicopathologic correlations and prognostic significance. *Br J Haematol* 1986; 64:227-240.
20. 20.Thiele J, Kvasnicka HM, Niederle N, Kloke O, Schmidt M, Lienhard H et al. Clinical and histological features retain their prognostic impact under interferon therapy of CML. A pilot study. *Am J Hematol* 1995; 50:30-39?
21. Rozman C, Hernandez-Nieto L, Montserrat E and Bragues R. Prognostic significance of bone marrow patterns in chronic lymphocytic leukaemia. *Br J Haematol* 1981; 47:529-537.
22. Varma N, Dash S, Sarode R and Marwaha N. Relative efficacy of bone marrow trephine biopsy sections as compared to trephine imprints and aspiration smears in routine hematological practice. *Indian J Pathol Microbiol* 1993; 36(3):215-226.
23. Delacretaz F, Schmidt PM, Piguet D, Bachmann F and Costa J. Histopathology of myelodysplastic syndromes- the FAB classification (proposals) applied to bone marrow biopsy. *Am J Clin Pathol* 1987; 87:180-186?

24. Tricol G, Wolf-Peeters C, Vlietinck R and Verwilghen RL. Bone marrow histology in myelodysplastic syndromes-II Prognostic value of abnormal localization of immature precursors in MDS. Br J Haematol 1984; 58:217-225.
25. Bartl R, Frisch B, Burkhardt R, Fateh-moghadam A, Mahl G, Gierster P et al. Bone marrow histology in myeloma: its importance in diagnosis, prognosis, classification and staging. Br J Haematol 1982; 51:361-375.

Bone marrow aspirate, showing good number of myeloblasts with few showing granules in the cytoplasm.

Trephine biopsy, showing mixture of blast cells and maturing cells of granulocytic series

Peripheral smear, showing large pleomorphic blast with condensed chromatin and inconspicuous nucleoli.

BM aspirate, showing heterogeneous population of lymphoblasts with basophilic cytoplasm, clumped chromatin and 1 to 2

BM aspirate, showing granulocytic hyperplastic mainly myelocyte, metamyelocyte and band forms.

Trephine biopsy, showing hypercellular marrow, increased number of megakaryocytic with hypolobated nuclei.

Trephine biopsy, showing nodule of mature lymphocytes.

ORIGINAL ARTICLE

NHL, lymphoblastic lymphoma, peripheral smear showing lymphoblast with nuclei and a mature lymphocyte

Multiple myeloma, BM aspirates showing plasmablasts with single prominent nucleoli and binucleation.

Multiple myeloma, trephine biopsy, showing diffuse infiltration by plasma cells.

Trephine biopsy, reticulin grade IV, showing thick coarse fibres.

AUTHORS:

1. Shobha Dwivedi
2. Sanjay Kumar Nigam

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Pathology, Rama Medical College Hospital & Research Centre, Mandhana Kanpur, U.P.
2. Professor Department of Pathology, Rama Medical College Hospital & Research Centre, Mandhana Kanpur, U.P.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Shobha Dwivedi,
117/H-2/170,
Pandunagar, Kanpur, U.P.
Email – drshobhadwivedi@rediffmail.com

Date of Submission: 27/07/2013.
Date of Peer Review: 29/07/2013.
Date of Acceptance: 30/07/2013.
Date of Publishing: 31/07/2013