Comparative Analysis of Bone Marrow Aspiration Smears, Touch Imprints and Trephine Biopsy in Haematological Malignancies

Suchita Pant[1], Harshi Dhingra[2], Anshul Gupta[3], RK Misra[4]

ABSTRACT

Introduction: Inspection of the bone marrow is considered one of the most valuable diagnostic tool to evaluate hematologic malignancies. This study compares all three techniques of bone marrow aspiration (BMA), bone marrow imprint (BMI) and bone marrow biopsy (BMB) in morphological diagnosis of hematological malignancies.

Material and methods: The study was conducted on 63 selected cases of various hematological malignancies. Only those cases in which bone marrow examination was done by using all the three techniques were included in the study.

Results: Out of total 63 cases,53 cases were diagnosed on bone marrow aspirate smears with diagnostic accuracy of 84.12%, 60 cases were diagnosed on bone marrow imprint smears with diagnostic accuracy of 95.23% and all 63 cases were diagnosed on bone marrow biopsy with diagnostic accuracy of 100%.

Conclusion: It is concluded that bone marrow imprints are equally useful as bone marrow biopsy in diagnosing hematological malignancies. Imprint cytology should therefore be a standard practice for evaluating bone marrow in cases of hematological malignancies.

Keywords: Touch Imprint, Trephine Biopsy, Bone Marrow Aspiration, Malignancy

INTRODUCTION

Hematological malignancies encompasses a broad spectrum of malignancies affecting blood, bone marrow and lymph nodes including leukemia, lymphoma, myeloma and progressive life threatening form of myelodysplastic syndrome. Hematological malignancies account for 9.5% of the new cancer diagnosis.[1] These represent the fifth most commonly occurring cancers and the second leading cause of cancer death.[2]

The diagnosis of hematological malignancies is complex, expensive and evolving rapidly. There are a myriad of diagnostic tests that can be applied to the analysis of malignant hematological diseases. In the present day, inspection of the bone marrow is considered one of the most valuable diagnostic tools to evaluate hematological disorders.[3]

Sampling of the marrow consists of either aspiration of the cellular component and/or acquirement of tissue fragment. Aspiration of the marrow has been utilized for cytological assessment, with analysis directed toward morphology and obtainment of a differential cell count. Further sampling allows for material to be directed toward other ancillary tests. Biopsies on the other hand allow study of the marrow's overall cellularity, detection of focal lesions and extent of infiltration by various pathological entities.[4,5]

Bone marrow trephine biopsy is carried out to assess various hematological problems, particularly in cases where assessment of marrow cellularity, cell distribution and relationship between cell types is crucial. It is also important in processes that are focal in nature. In some disorders like lymphomas, the pattern of infiltration seen in bone marrow biopsy provides additional prognostic information. Their role is critical in cases of "dry tap" where examination of an aspirate has been unsuccessful owing to a fibrotic or infiltrative process.[6]

Touch imprints were useful for studying cell morphology, where aspiration yielded dry tap.[7] The touch imprint proves to be a reliable diagnostic tool for determining the cellular composition of normal bone marrow and more reliable for the diagnosis of bone marrow involved by a neoplastic hematological disease.[8] Appropriately prepared imprint cytology smears not only provide cellular composition of marrow but also define the topographical architecture of marrow.[9]

Thus, although trephine sections provide maximum information, all three preparations complement each other and should be evaluated simultaneously for complete bone marrow interpretation.[10]

The present study aimed to correlate the findings of morphological examination of bone marrow aspiration smears, touch imprints and bone marrow trephine biopsy in diagnosis of hematological malignancies.

MATERIAL AND METHODS

The present study was conducted on a total of 63 selected patients of hematological malignancies attending the

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out patient department and indoor department of Nehru Chikitsalaya, B.R.D Medical College, Gorakhpur. Only those cases in which bone marrow examination was done by using all the three methods of bone marrow aspiration [BMA], bone marrow imprint [BMI] and bone marrow biopsy [BMB] were included in the study.

Detailed clinical examination was performed on each patient with particular emphasis on hematological examination. Blood tests were performed on every patient. Total leucocyte count and platelet count was done by automated cell counter as well as manually. Differential cell count was done by visual method after staining the blood film with Leishman and May Grunwald Giemsa (MGG).

Bone marrow aspiration and biopsy were performed from posterior superior iliac spine. The standard technique was employed for obtaining aspirate samples using Salah's needle. The trephine biopsy was performed using Jamshidi's needle with length of the core ranging from 1-3 cms.

The biopsy imprints were made by gently touching and rolling the core on slides, which were then fixed and stained as bone marrow aspiration smears. This was performed before placing the specimen in AZF (acetic acid, zinc, formalin) fixative for histological processing.

All the aspirate and touch imprints were routinely stained by Leishman and May Grunwald Giemsa, while trephine biopsy sections were stained by routine Haematoxylin and Eosin and reticulin stain after bone marrow processing.

Bone marrow processing was done as per the Hammersmith Protocol.[6] In this the bone marrow core biopsy specimens are fixed in AZF fixative for 20-24 hrs and then the biopsy specimens are decalcified in Gooding and Stewart's fluid (10% formic acid and 5% formaldehyde) for 6 hrs before being processed and embedded in paraffin wax, with procedures similar as for other specimens.

The cellularity, differential count and megakaryocyte density were done on all three preparations of bone marrow samples and recorded in a proforma. They were assessed subjectively by two observers. The cellularity was graded as hypocellular, normocellular and hypercellular. Multiple areas of each slide were screened and an estimate made.

RESULTS

Bone marrow examination was done on 63 selected cases of various hematological malignancies with comparative evaluation of all three bone marrow preparations for diagnosing hematological malignancies.

Among the hematological malignancies, acute leukemias formed the largest group accounting for 45(71%) cases of which 25(39.7%) cases were categorized as acute lymphoblastic leukemia (ALL) and 20 (31.7%) as acute myeloblastic leukemia (AML).The next in descending order of frequency were 7(11%) cases of chronic myeloid leukemia (CML), followed by chronic lymphoid leukemia (CLL), multiple myeloma, non hodgkin lymphoma (NHL), 3(5%) cases each and idiopathic myelofibrosis in 2(3%) cases.

The patients varied between 2 years to 73 years of age, with male to female ratio of 1.5:1.In our study, maximum cases were seen in first decade (27% of all cases), where ALL was most common, followed by seventh decade (21% of all cases) where AML was most common.Pallor,fever and generalized weakness were the most common presenting symptoms.

Out of total 63 cases,53 cases were diagnosed on bone marrow aspirate smears with diagnostic accuracy of 84.12%, 60 cases were diagnosed on bone marrow imprint smears with diagnostic accuracy of 95.23% and all 63 cases were diagnosed on bone marrow biopsy with diagnostic accuracy of 100%.

Ten (16%) cases which yielded dry tap or in which bone

	No. of cases	BMA Cytology	BMI Cytology	Bone marrow biopsy(BMB)
ALL	25	24	25	25
AML	20	18	20	20
CML	07	06	06	07
CLL	03	03	03	03
Multiple myeloma	03	01	03	03
Myelofibrosis	02	-	-	02
Lymphoma	03	01	03	03
Total cases	63	53	60	63

Table-1: Cases diagnosed on bone marrow aspirate, touch imprint and trephine biopsy

	Positive correlation of BMA cytology and BMB (%)	Positive correlation of BMI cytology and BMB (%)
ALL	96%	100%
AML	90%	100%
CML	85.7%	85.7%
CLL	100%	100%
Multiple myeloma	33.33%	100%
Myelofibrosis	00	00
Lymphoma	33.33%	100%
Total	84.12%	95.23%

Table-2: Cases showing positive correlation of bone marrow aspirate cytology and imprint cytology with trephine biopsy

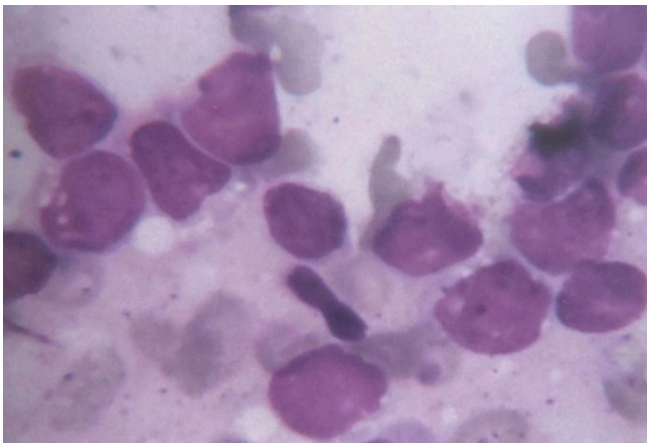

Figure-1: Touch imprint smears shows case of AML with immature blast cells having round to indented nuclei and fine chromatin with prominent nucleoli. [MGG x400]

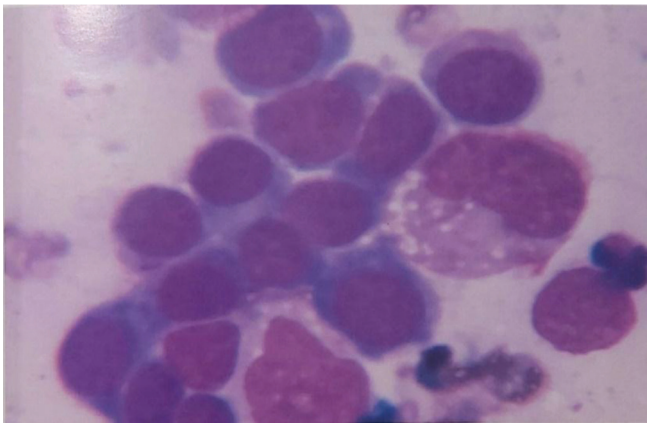

Figure-4: Touch imprint smear shows sheets of immature plasma cells in a case of Plasmablastic Multiple Myeloma [MGG x400]

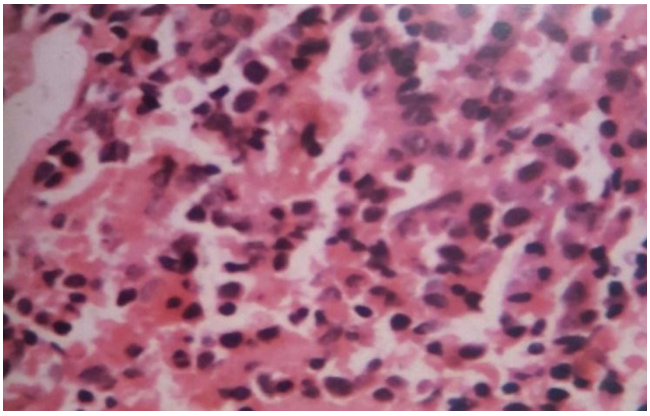

Figure-2: Trephine section of same case shows blasts with abundant cytoplasm [H&E x400]

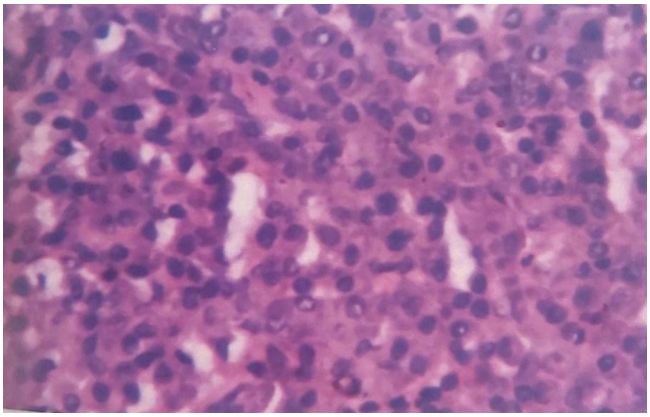

Figure-5: Trephine section of above case of Multiple Myeloma shows diffuse involvement by plasma cells [H&E x400]

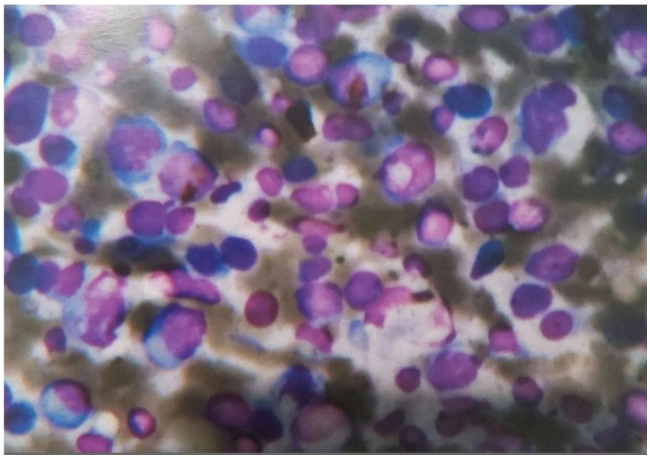

Figure-3: Bone marrow imprint cytology smear shows diffuse infiltration by lymphoid cells in a case of NHL [MGG x400]

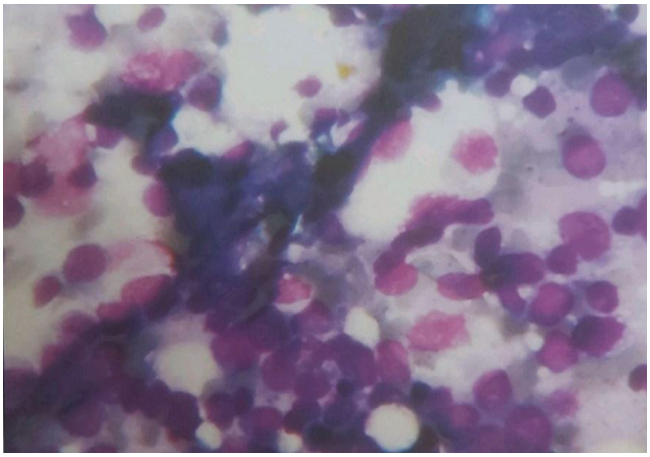

Figure-6: Touch imprint smear shows mainly blast cells, diagnosed as ALL on the basis of cytomorphology [MGG x400]

marrow aspiration smears were inadequate for diagnosis, were diagnosed only on trephine biopsy and touch imprint. These include 2(3%) cases of myelofibrosis which yielded dry tap on aspiration, 2(3%) cases of multiple myeloma where bone marrow aspirate smears were inadequate and diagnosis was made on touch imprint and trephine biopsy, 1(2%) case of CML which yielded dry tap, 3(5%) cases of acute leukemia where aspirate smears yielded dry tap[Fig.1 and 2], 2(3%) cases of NHL [Fig.3] where bone marrow aspirate smears were inadequate and were subsequently diagnosed on touch imprints and biopsy.

All cases were diagnosed on touch imprint which has a high diagnostic accuracy (95.23%), except 2 cases (3.17%) of myelofibrosis and 1(1.6%) case of CML (Table 1).

All the three procedures were found to be complementary in all cases of leukemias,multiple myeloma and lymphomas (Table 2).

On statistical analysis bone marrow biopsy was the standard procedure in diagnosing hematological malignancies than bone marrow aspiration alone and this difference was statistically significant (Z value =3.29 p <.01).

Bone marrow imprint was found to be more useful than bone marrow aspiration alone in diagnosing hematologic malignancies and this difference was significant (Z value=2.05, p <.05)

whereas bone marrow imprints were found to be equally useful as bone marrow biopsy in diagnosing hematological malignancies.

DISCUSSION

In the present study, it was observed that in cases of hematological malignancies, bone marrow imprint (BMI) smears were almost as reliable as bone marrow biopsy (BMB) sections, however with slightly higher detection rate by bone marrow biopsy (100%) in comparison to bone marrow imprint (95.23%), whereas detection rate by bone marrow aspirate smears was 84.12%.

The predominant reason for not diagnosing leukemia on BMA was dry tap due to either marrow fibrosis or tightly packed marrow by leukemic cells. Aboul Nasr et al[8] had also observed that bone marrow imprint (BMI) cytology was reliable for making diagnosis in cases of hematological malignancies especially in cases of dry tap or dilution of aspirate by peripheral blood.

The efficacy of bone marrow imprint(BMI) observed in our study was mainly because of diagnosis of almost all cases of leukemias (except one case of CML), multiple myeloma and lymphoma on imprint cytology.In our study, all three (100%) cases of lymphoma were diagnosed on imprint cytology and biopsy sections, whereas only one case was diagnosed on aspirate smears this is in concordance to the study by Aboul Nasr et al[8] who also observed that lymphoma cells were easily detected in all cases on touch imprints.According to S. Chandra[9], touch imprint cytology considerably increased the chances of detection of lymphoma cells (88.2%) in comparison to bone marrow aspirate (BMA) smears (52.9%) alone,they also suggested that although the topographical arrangement of lymphoma cells are best seen on biopsy sections but this arrangement can also be appreciated in meticulously prepared touch imprint smears.

In the present study, out of three cases of multiple myeloma only one (33.33%) case was diagnosed on aspirate smears, while two (66.67%) cases where bone marrow aspirate(BMA) smears were inadequate subsequently diagnosed on bone marrow imprint smears and bone marrow biopsy [Fig. 4 and 5]. This corresponds to the study by Sabharwal et al[11] who observed that aspiration smears provided inadequate information for accurate diagnosis of multiple myeloma.

The diagnosis of myelofibrosis could only be made from biopsy sections.In our study, two (3.17%) cases of myelofibrosis were diagnosed on biopsy sections only. Hence, a finding of a dry tap should never be dismissed as being due to faulty technique and always need a bone marrow biopsy. Humphries[12] in his study of 87 cases of dry tap on marrow aspiration, obtained trephine biopsies which showed significant pathology.Dry tap with marrow aspiration were due to fibrosis and hypercellularity.

We found no significant difference in the differential count from touch imprints and aspirate smears. According to Mehra V et al[13] also, imprint smears are useful to study the cytomorphological features in cases of hematological malignancies [Fig.6], where aspirate smears are inadequate. They have the advantage of rapid diagnosis in such cases. Varma et al[10] also suggested that the differential counts done on trephine imprints and aspiration smears correlated well and cytomorphological characterization of immature cells (blasts and promyelocytes) could be done on these two preparations.

Many studies have shown the role of bone marrow imprints in hematological diagnosis and concluded that imprints are superior to smears for evaluation of cellularity, and are also better than sections for analysis of cytological changes.[14,15]

The adequacy of touch imprint in diagnosing most of the hematological malignancies is related to properly prepared touch imprint smears, so that impression of the cells are made by almost all aspects of the core biopsy.This procedure enhances the detection of even focal lesions in marrow.In addition, it also avoids the unnecessary delay caused by decalcification and processing of trephine biopsy sections in routine histopathology labs.[9]

CONCLUSION

It is concluded that bone marrow imprints are equally useful as bone marrow biopsy in diagnosing hematological malignancies. The imprints were superior to smears for evaluation of cellularity and are also better than sections for analysis of cytological changes. Meticulously prepared touch imprint smears also define the topographical architecture of marrow. Imprint cytology should therefore be a standard practice for evaluating bone marrow.

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