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Diagnostic utility of bone marrow aspiration, imprint and biopsy in evaluation of various haematological disorders

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Abstract

Introduction: Bone marrow examination is an important tool for the diagnosis of various haematological disorders. It involves the use of bone marrow aspiration (BMA), bone marrow imprint smears (BMI) and bone marrow biopsy (BMBx).

Aim: The study was conducted with an aim to compare the findings of BMA, BMI and BMBx in the evaluation of various haematological disorders.

Material and methods: A prospective study was conducted to correlate the findings of bone marrow examination by different methods. The study included 50 patients, in whom bone marrow evaluation was done using all the three methods. All the observations were evaluated using simple and basic statistical tool, i.e. percentage.

Results: A total of 38 cases showed positive correlation between BMA, BMI and BMBx. Maximum cases were of nutritional anemia followed by Leukemia (Both acute and chronic), Myelodysplastic Syndrome (MDS), Multiple Myeloma, Idiopathic Thrombocytopenic Purpura (ITP), Hypereosinophilia, Hypersplenism, NHL invasion of marrow. Of all the methods, BMBx was useful in cases of aplastic anemia and in those cases where aspiration had yielded dry tap/diluted marrow (NHL invasion of marrow, acute leukemia, chronic leukemia, nutritional anemia and Juvenile Myelomonocytic leukemia with secondary myelofibrosis). Cellular morphology was better appreciated in aspiration and imprint smears. Aspiration smears were better for assessment of iron stores as compared to imprint and biopsy. Cellularity and pattern of neoplastic infiltration was better appreciated in biopsy sections. Biopsy was also useful for the detection of fibrosis wherever required.

Conclusion: Bone marrow examination is an important and useful investigation for the study of various haematological disorders. All the three methods of bone marrow examination viz. aspiration, touch imprints and biopsy are complementary to each other and advantage of one method over the other depends upon the specific disease process.

Keywords: Bone marrow aspiration, imprint, biopsy, bone marrow examination

Introduction

The examination of bone marrow has wide applications in clinical medicine. It is a useful investigative tool for the diagnosis of many haematological and non-haematological disorders. The bone marrow evaluation may either confirm clinically suspected disease or may provide the previously unsuspected diagnosis.¹

Bone marrow can be evaluated by three ways – Bone marrow aspirate (BMA) smears, Imprints of bone marrow core biopsies (BMI), and histological sections of core biopsies (BMBx).²

Aspiration of the marrow is primarily utilized for cytological assessment with analysis directed towards morphology and obtaining a differential cell count.² Trepine biopsy is of value when aspirate yields dry tap or a blood tap as it provides information on architecture, cellularity, fibrosis and pattern of distribution of abnormal infiltrates.³ As the bone marrow biopsies require 24-48 hr to prepare, evaluation of Bone marrow imprint smears immediately may sometimes be useful in studying the cell morphology and diagnosing the underlying disease process. It helps in initiating a prompt treatment in critically ill patients without waiting for the histopathological report of core biopsy.²

Materials and Methods

The present prospective study was conducted for the evaluation of bone marrow in 50 cases presenting with haematological disorders in the Department of Pathology, Government Medical College, Amritsar. Clinical details and informed consent of the patient was duly taken. The patients were subjected to a complete blood count, reticulocyte count and peripheral blood film examination. Only those cases in which bone marrow examination was done by all the three methods (BMA, BMI and BMBx) were included in the study. The cases yielding inadequate material were excluded from the study. Posterior superior iliac supine was the preferred site in all the cases. Bone marrow aspiration was performed using Salah's needle and biopsy was performed using Jamshidi needle. Imprint smears were

prepared by gentle rolling of bone marrow biopsy in between the two glass slides, followed by fixation of biopsy in 10% formalin and decalcification in 10% formic acid for 2-3 hours. Aspiration and imprint smears were air dried and stained with Leishman stain. The cytochemical stains such as MPO, PAS were done wherever necessary. Prussian blue staining was done in all the cases of anemia and MDS. Routine histopathologic processing of bone marrow biopsy was done followed by staining with H&E and Reticulin staining.

Results

The patients varied between 4 years to 75 years of age, with male to female ratio of 1.2:1. The majority of the patients belonged to the age group of 31-50 years.

Unexplained anemia was the most common indication of bone marrow examination (32%) followed by pancytopenia (20%), Chronic Leukemia (16%), suspected acute leukemia (12%), staging for lymphoma (10%) and 2% each of hypereosinophilia, multiple myeloma, MDS, thrombocytopenia and unexplained leucocytosis with splenomegaly.

The BMBx was diagnostic in 100% of the cases followed by BMI (97%) and BMA (76%). There were 12 cases of dry tap or diluted marrow.

Of the 12 cases in which aspiration was not successful due to dry tap or diluted marrow, biopsy was helpful in reaching the diagnosis. These cases included Acute Leukemia (3 cases), Chronic Leukemia (3 cases), Aplastic Anemia (2 cases), lymphomatous invasion of marrow (2 cases), Nutritional Anemia (1 case) and JMML with secondary myelofibrosis (1 case).

Cellularity in BMBx samples correlated with BMA in 78% cases and with imprint smear in 96% cases.

Out of the total 50 cases, aspiration was successfully obtained in 38 cases and the correlation between BMA and BMBx was found in all these cases (100%). These cases included 17

cases of nutritional anemia, 5 cases each of acute and chronic leukemia, 3 cases of normal marrow, 2 cases of MDS, and 1 case each of multiple myeloma, ITP, hypereosinophilia, hypersplenism, lymphomatous invasion of marrow.

Out of the total 19 cases of various causes of anemia, BMA alone was diagnostic in 18 cases (94.7%) and a positive correlation was seen with BMI and BMBx in all these cases.

A total of 16 cases of leukemias were diagnosed which included 8 cases of acute leukemia and 8

cases of chronic leukemia. On the basis of morphology and cytochemical stains acute leukemia were further categorised as AML, ALL and acute leukemia (unclassified). 6/8 cases of acute leukemia were diagnosed on BMA and findings of BMI and BMBx correlated with each other, in rest of the 2 cases BMA yielded dry tap/diluted marrow. Cases of chronic leukemia included 6 cases of CML and 2 cases of CLL. BMA was diagnostic in 4/6 cases of CML and 1/2 cases of CLL, and correlated with the findings of BMI and BMBx. BMA was not successful in the remaining cases of chronic leukemia

Table showing the comparison of BMA, BMI and BMBx

Diagnosis (no. of cases)	BMA (no.)	BMI (no.)	BMBx (no.)
Megaloblastic anemia (15)	14	15	15
Dimorphic anemia (1)	1	1	1
Micronormoblastic anemia(2)	2	2	2
Hypersplenism(1)	1	1	1
Normal marrow in HIV positive pateint(1)	1	1	1
Haemolytic anemia(1)	1	1	1
Aplastic anemia (2)	0	0	2
Acute leukemia (8)	5	8	8
CML (6)	4	6	6
CLL (2)	1	2	2
Lymphoma (5)	3	4	5
MDS (2)	2	2	2
JMML with myelofibrosis (1)	0	1	1
ITP (1)	1	1	1
Hypereosinophilia (1)	1	1	1
Multiple myeloma(1)	1	1	1

Bmbx showed diffuse pattern of marrow involvement in all cases of CLL and two cases of lymphomatous invasion of NHL. One case of NHL showed mixed diffuse and interstitial pattern of marrow involvement.

Of the total 50 cases, 21 cases (42%) were of anemia followed by 2 cases of MDS (4%) and 2 cases of normal marrow (4%). In all these cases, aspiration smears, imprint smears and biopsy sections were subjected to Prussian blue staining (Perls' staining) except one case of dry aspirate. BMA smears revealed increased iron stores in 15 cases, normal in 7 cases and decreased stores in 2

cases. Biopsy showed increased iron stores in 6 cases, normal in 13 cases and decreased in 6 cases. On comparison with aspiration, Biopsy showed false negative results in 4 cases. Imprint smears were negative in 10 cases and were positive in 15 cases. On comparison with aspiration, imprint smears showed false negative results in 8 cases.

Of the total 12 cases of dry tap/dilute BM aspirate, 9 cases were of neoplastic pathology. In all the neoplastic cases, reticulin staining showed increased degree of fibrosis of different grades.

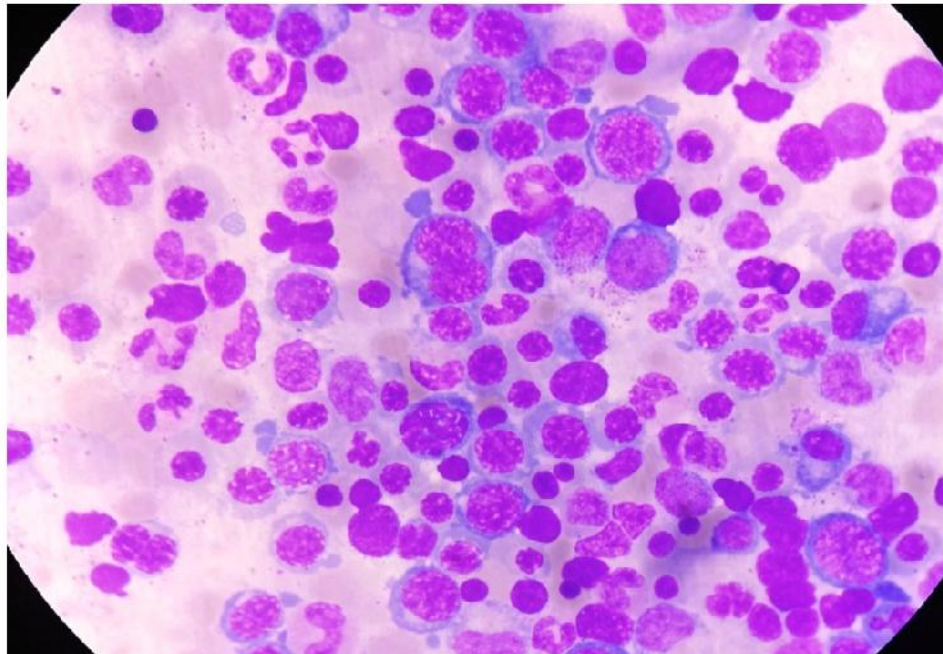


Fig 1 .Photomicrograph showing erythroid Hyperplasia and Megaloblast in BMA smear (Leishman, 1000X)

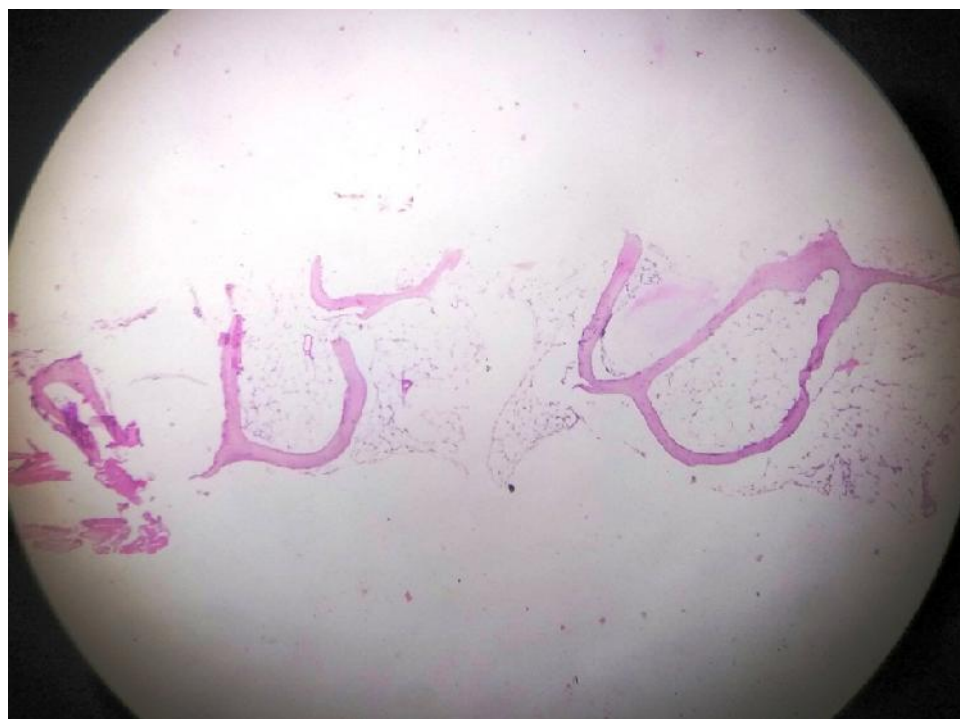


Fig.2. Photomicrograph of BMBx showing hypocellular marrow in a case of Aplastic Anemia (H&E, 40X)

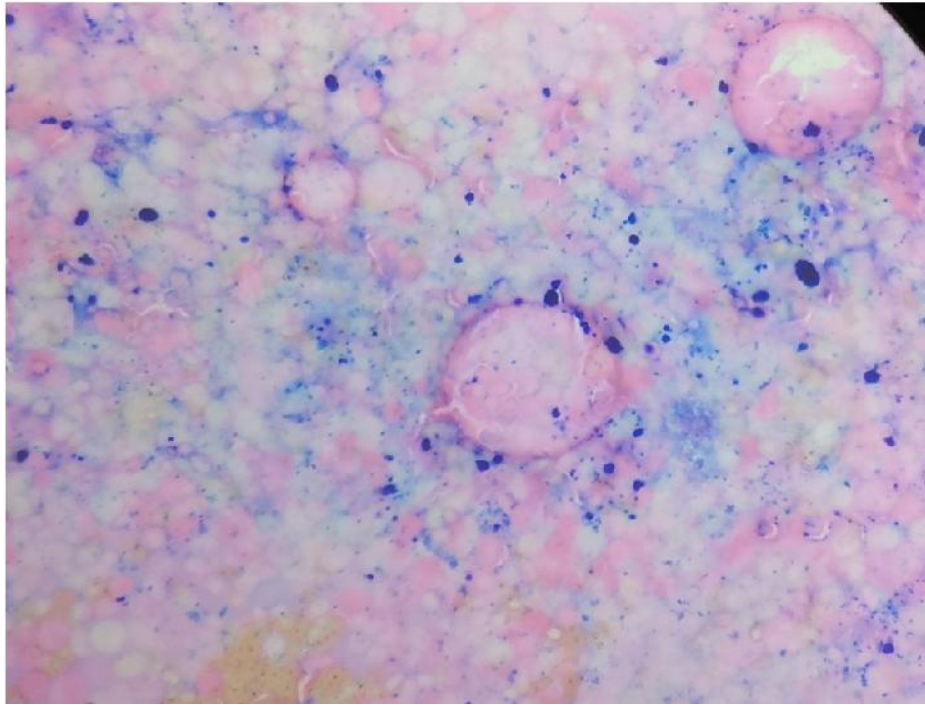


Fig.3 . Photomicrograph of BMA smear showing increased iron stores in a case of megaloblastic anemia (Perls' Stain, 400X)

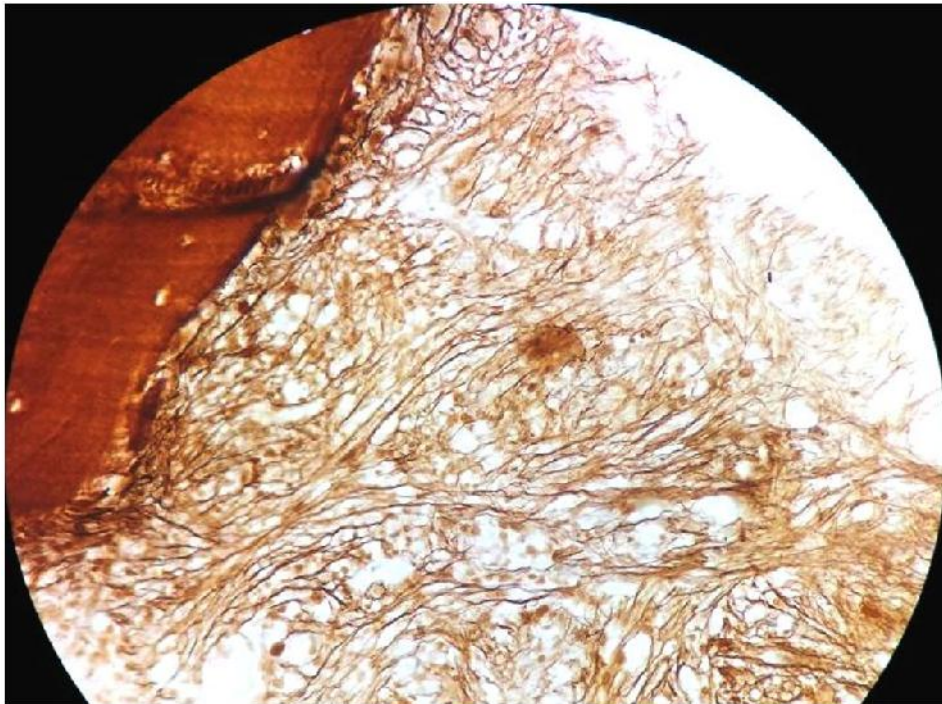


Fig.4. Photomicrograph of BMBx showing fibrosis of grade 3 in the case of CML (Reticulin, 400X)

Discussion

In our study, we have done the comparative evaluation of the different methods of obtaining bone marrow viz. BMA, BMI and BMBx to determine their diagnostic utility in the various haematological disorders.

Of the total cases, aspiration was possible in 40 cases with the failure rate of 20% which is comparable to the other studies in the literature.⁴ The failure was due to dry tap or diluted marrow.

Cellularity in BMBx samples correlated with BMA in 78% cases and with imprint smear in 96% cases which is comparable to a study done by Tilak V et al². The study stated that Bone marrow biopsy is a better tool for the evaluation of the cellularity of the bone marrow in contrast to aspiration and imprint smears.

The present study found nutritional deficiencies to be the most common cause of anemia, seen in 15/50 (30%) of the cases. Similar finding was seen in study done by with Patel K et al⁶. A higher percentage of nutritional anemia observed may be due to the higher prevalence of nutritional deficiencies in our country.⁷

In the present study, BMA alone was diagnostic in 94.7% cases of nutritional anemia and a positive correlation was seen with BMI and BMBx in these cases. Our results were in concordance with Verma S et al⁴ and Kaur M et al⁵ in which they observed 100% correlation between BMA and BMI anemia cases. We found aspirates to be as good as imprint smears and biopsies in the diagnosis of nutritional anemias.

A total of 2 cases were diagnosed as aplastic anemia on bone marrow biopsy which was given as hypocellular marrow on aspiration and imprint. Our results correlated with Shah P et al⁸ and Goyal et al⁹. Milosevic R et al¹⁰ stated that trephine biopsy gives the qualitative and quantitative assessment of cellularity, therefore, is confirmatory in the diagnosis of aplastic anemia.

In our study, BMA smears were found to be better for assessment of iron stores as compared to imprint and biopsy. Our results were in concordance with Chandra S et al.³ High rate of false negative results in imprint smear may be due to lesser number of particles⁶ while in biopsy iron is lost during decalcification.^{3,4,11,12} In our study, 1 case showed dry tap, in which imprint smears and biopsy sections were used for assessment of iron stores. The results correlated with Pasquale D et al¹³, their study demonstrated that biopsy imprint smears allowed definite assessment of iron stores in cases of dry tap. Thus, use of combination of two techniques allowed the assessment of iron stores in more number of the cases.

In cases of acute leukemia, BMA showed positive correlation with BMI and biopsy in 5/8 cases (62.5%). Aspiration had yielded dry tap/diluted marrow in 3 cases. Similar results have been reported in the past literature by Verma S et al⁴ which can be explained due to the tightly packed marrow by the leukemic cells.

In our study 6/8 cases (75%) of acute leukemia were subtyped as AML and ALL on the basis of morphology and cytochemical stains. While remaining two cases (23.7%) could not be subtyped on the basis of morphology and Immunophenotyping was suggested. The use of ancillary techniques is essential to make a final diagnosis as also suggested by Subashchandrabose P et al¹⁴ in their study.

In the present study, BMA showed positive correlation with BMI and BMBx in 4/6 (66.7%) cases of CML. The rest 2 cases were dry tap and eventually diagnosed on imprint smears and biopsy. These 2 cases also showed increased fibrosis on reticulin staining. Our results are comparable to the study done by Chandra S et al.³ who has given the reason of dry tap due to marrow fibrosis.

Of the total, 7 cases were of lymphoid leukemia/lymphoma. Out of 7, 2 cases were diagnosed as CLL and BMA showed positive correlation with BMI and BMBx in one of those and the other had a diluted marrow on aspiration which can be due to the packed marrow by leukemic cells as also reported by Verma S et al.⁴

Of the 5 NHL cases, two cases had a normal bone marrow study and infiltration was seen in 3 cases. The infiltration was detected in all the 3 cases in biopsy specimen and in two cases in imprint smears. The aspiration smears was positive for infiltration in only one case. Our results are in concordance to the studies conducted by *Aboul Nasr R et al*¹⁵ and *Chandra S et al*³ who have observed that imprint smears and biopsies increased the chances of detection of lymphoma cells as compared to aspirate smears.

In the present study, BMBx showed diffuse pattern of infiltration in both the cases of CLL and in cases of NHL, diffuse pattern in two cases and mixed diffuse and interstitial pattern in one case. In correlating all the methods of bone marrow evaluation, BMBx has an added advantage regarding the pattern of involvement in lymphoid leukemia/lymphoma. Past studies have also suggested that BMBx should be done in leukemia/lymphoma cases as it provides additional information about the pattern of involvement and prognosis.^{9,16,17} *Hasserjan RP et al* has also reported that the pattern of infiltration can be seen only on biopsy which has a prognostic significance.¹⁸

There were two cases of MDS in our study. Findings of BMA, BMI and BMBx were concordant with each other, the results of which are comparable to the study done by *Verma S et al*⁴ The literature has mentioned that biopsy may sometimes be more helpful in the assessment of cellularity and finding dysmegakaryopoiesis and abnormal localization of myeloid precursors.¹⁹

In one case of ITP, BMA, BMI and BMBx findings were concordant with each other. Our results correlated with *Hota R et al*¹⁶ and *Chauhan S et al*.²⁰ and have reported that all the procedures of bone marrow evaluation are complimentary to each other in diagnosing ITP, but BMBx may show an additional finding of arrangement of megakaryocytes¹⁶.

Positive correlation was observed in between BMA, BMI and BMBx in one case of multiple myeloma in our study and the results were in

concordance with studies conducted by *Chauhan S et al*²⁰ and *Toi PC*.²¹ A study has reported that bone marrow biopsy is recommended in multiple myeloma even if an adequate aspirate is obtained, since it is needed as a baseline to assess a post treatment response when no adequate aspirate is obtained.²²

In our study, bone marrow imprints were found to be of diagnostic value in cases where aspiration was a dry tap or diluted with blood. This included 2 cases each of CML and acute leukemia and 1 case each of megaloblastic anemia, juvenile myelomonocytic leukemia, CLL and lymphomatous invasion of marrow which are in agreement with the past studies in the literature.^{4,19} Therefore the cases which show dry tap on aspiration, significant role is played by BMI in aiding the diagnosis of the underlying pathology.

In the present study, BMBx showed increased degree of fibrosis in cases with dry tap and diluted marrow. A dry tap or inadequate aspiration should not be dismissed as a result of faulty technique but should always be accompanied by trephine biopsy to arrive at a conclusive diagnosis.²⁰

Conclusion

All the three methods of bone marrow examination viz. aspiration, touch imprints and biopsy are complementary to each other and advantage of one method over the other depends upon the specific disease process. Bone marrow aspiration and imprint smears are equally important to study the cytomorphology and for differential counts. Aspiration smears are best for assessment of iron stores in comparison to imprint and biopsy. Bone marrow biopsy is mandatory for diagnosis in cases with dry tap or diluted marrow on aspiration. Bone marrow biopsy is a better tool for assessment of cellularity, pattern of marrow infiltration, and for detection and grading of fibrosis. Imprint smears are helpful to study the cytomorphological details in cases of hematological malignancies where bone marrow aspirate is inadequate and also have the advantage of early diagnosis in such cases.

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