Original Article

DOI: 10.7860/NJLM/2016/20880.2148

Pathology Section

Correlation Between Bone Marrow Aspiration and Bone Marrow Biopsy with Imprint Smears in Hematological Disorders

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ABSTRACT

Introduction: Bone marrow examination is an important diagnostic tool and the cornerstone of hematology. It involves the use of Bone marrow aspiration (BMA), Bone marrow imprint (BMI) and Bone marrow biopsy (BMB).

Aim: The study was aimed to correlate findings of bone marrow aspiration and imprint smears with biopsy in hematological disorders.

Materials and Methods: A retrospective and prospective study was done to correlate bone marrow examination findings by different diagnostic tools. The results were observed and analyzed. 75 consecutive patients in which bone marrow biopsy was done were correlated with aspirate and imprint smears. A single needle technique using Jamshidi needle was used for both aspiration and biopsy. Hematological and histopathological findings were correlated. Statistical analysis was done using SPSS software version 21.

Results: Total of 46 cases showed positive concordance between bone marrow aspiration and biopsy. Maximum cases were those of nutritional anemia followed by aplastic anemia, acute leukemia, MDS, chronic leukemia, multiple myeloma, leukemia/lymphoma syndrome, follow-up case of hematological malignancy and essential thrombocythemia. Biopsy was useful in cases of aplastic anemia, acute and chronic leukemia, multiple myeloma, leukemia/lymphoma syndrome and myelomonocytic leukemia with secondary myelofibrosis where aspiration had yielded dry tap /diluted marrow. Morphological details were better appreciated in aspirate and imprint smears. Perl stain was better appreciated in aspirate smears as compared to biopsy sections.

Conclusion: BMA and biopsy were complementary modalities. Aspiration and imprint smears provided good morphological details/cytologic diagnosis. Biopsy sections were helpful in cases where aspiration yielded dry tap or diluted marrow and in identifying architectural pattern, cellularity and fibrosis. Imprint smears were mainly useful for studying cellular morphology where aspiration yielded dry tap or diluted marrow.

Keywords: Concordance, Dry tap, Imprint cytology, Perl stain

INTRODUCTION

Bone marrow examination is an important tool helpful in diagnosis and management of hematological disorders. Three main modalities to evaluate bone marrow are aspiration, touch imprint cytology and trephine biopsy [1,2].

Bone marrow aspiration and trephine biopsy are complementary to each other. The aspirates are useful in studying the morphology of cells and for obtaining a differential cell count. It is also useful for additional flow cytometric, immunophenotyping, cytogenetic and molecular studies.

Trephine 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 [3,4].

Bone marrow has wide application in clinical medicine. It is used to evaluate patients with malignant lymphomas, acute leukemias, myeloproliferative disorders, myelodysplastic syndromes, metastatic tumour, granulomatous disorders, myelofibrosis and plasma cell dyscrasias. Evaluation of cytopenia, thrombocytosis, leukocytosis, anaemia and iron status can also be done [3,5].

In the present study bone marrow aspiration smears, bone marrow biopsy findings and their imprints were compared and correlated.

MATERIALS AND METHODS

A retrospective and prospective study was conducted in Department of Pathology, Subharti Medical College and associated CSS Hospital, Meerut, on 75 patients over a period of three years (August 2012 to July 2015). Clinical details and informed consent were obtained in all cases. Patients were investigated for routine blood count, reticulocyte count and peripheral blood film examination. Cases where all three modalities, that is, BMA, BMI and BMB were used for diagnosing hematological disorders were included in the study.

Both bone marrow aspiration and biopsy were performed by one needle technique using Jamshidi needle from the same site with aspiration being performed first followed by biopsy. Posterior superior iliac spine (PSIS) was the preferred site. Imprints were prepared and biopsy was put in 10% formalin. Aspiration and imprint smears were air dried and stained with Leishman-Giemsa (LG) stain. Cytochemical stains like Periodic Acid Schiff (PAS), Myeloperoxidase (MPO), Sudan black were done in cases of hematological malignancies and Perl Prussian blue for assessment of iron stores was done in cases of anemia.

Biopsy specimen was fixed in 10% formalin overnight and then decalcified in 10% formic acid for 2 to 3 hours. It was processed routinely and Haematoxylin-Eosin (H & E) staining was done. Reticulin and Perls' Prussian blue stain were done.

RESULTS

The age of the patients ranged from 6 months to 76 year with male to female ratio of 1.3:1. The most common indication for bone marrow examination was pancytopenia (23 cases; 30.3%), followed by bicytopenia (15 cases), acute leukemia (9 cases), MPD (7 cases), evaluation of anaemia (6 cases) and osteolytic lesions (4 cases). Bone marrow was also indicated in 3 cases each of follow up of hematological malignancy and for evaluation of hepatosplenomegaly/ lymphadenopathy, 2 cases of unexplained leucocytosis and 1 case each of chronic lymphocytic leukemia, thrombocytosis and thrombocytopenia.

The diagnostic yield of BMB was highest (80%), followed by BMA (77.3%) and BMI (74.6%).

3 out of 75 cases in the study were inconclusive where diagnosis was not possible by any of the 3 modalities due to either diluted marrow on aspiration or inadequate biopsy [Table/Fig-1].

As both techniques are complementary to each other,

Diagnosis	Number of cases	BMA		BMI		BMB	
		DD	NDD	DD	NDD	DD	NDD
Nutritional anaemia	19	17	02*	17	02*	17	02*
Hypoplastic/aplastic anaemia	14	10	04	07	07	11	03
Anaemia of chronic disease	02	01	01	02	-	02	-
Congenital Dyserythropoietic anaemia	02	02	-	-	02	-	02
Idiopathic thrombocytopenic purpura	01	01	-	-	01	-	01
Acute leukemia	10	08	02*	08	02*	08	02*
Chronic leukemia	05	04	01	05	-	05	-
Leukemia/lymphoma syndrome	04	03	01	04	-	04	-
Essential thrombocythemia	01	01	-	01	-	01	-
Chronic myelomonocytic leukemia with secondary myelofibrosis	01	-	01	01	-	01	-
Myelodysplastic syndrome	06	06	-	05	01	05	01
Multiple myeloma	04	03	01	04	-	04	-
Follow up of hematological malignancy	03	02	01*	02	01*	02	01*
Inconclusive	03	-	03	-	03	-	03
Total	75	58	17	56	19	60	15
Diagnostic yield %	-	77.3	-	74.6	-	80	-

[Table/Fig-1]: Cases diagnosed on bone marrow aspirate, imprint and trephine biopsy.
DD- Definite diagnosis provided; NDD – no definite diagnosis provided
In these three categories [*] the number of cases with definite diagnosis (DD) and no definite diagnosis (NDD) is the same. However there is no overlapping of cases and final diagnosis could be provided by either one of the three modalities.

diagnostic accuracy was calculated taking both methods as gold standard. With bone marrow biopsy as gold standard the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of BMA was 76.7%, 20%, 79.31%, 17.65% and 65.3% and of BMI was 93.3%, 100%, 100% 78.95% and 94.7%. With bone marrow aspiration as gold standard the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of BMI was 79.3%,41.18%, 82.14%,36.84% and 70.7% and of BMB was 79.31%,17.65%,76.67%,20% and 65.3%.

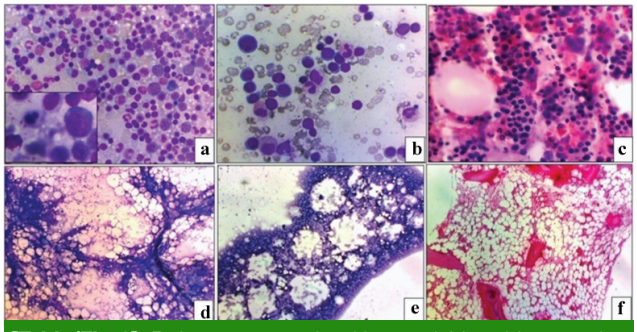
Comparison of cellularity and morphology of BMA and BMI was done by taking the opinion of two pathologists. Interobserver variation was analyzed. In case of gross difference, a third opinion was gathered. Scoring was done as-Excellent, Good, Average and Poor.

Cellularity was compared considering the age and disease process. It was found to be slightly better in aspirate smears (40 cases; 64%) as compared to imprint (34 cases; 55%). Morphology was compared taking into account different parameters like nuclear and cytoplasmic details, staining quality, crush artifacts and background staining. It was found to be better in imprint smears (50 cases/81%) as compared to aspirate smears (39 cases/63%).

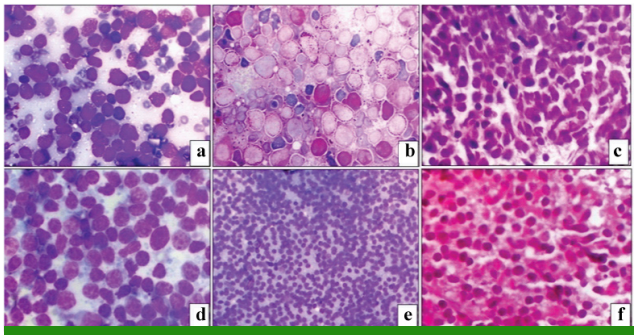
Correlation of BMA and BMB was found in 46 out of 72 cases (excluding the three inconclusive cases) which included cases of nutritional anemia (15/19 cases) [Table/Fig-2a-c], aplastic anemia (7/14 cases) [Table/Fig-2d-f], anemia of chronic disease (1/2 cases), acute leukemia (6/10) cases) [Table/Fig-3a-c], chronic myeloid leukemia (3/4 cases), Chronic lymphocytic leukemia(1/1), Lymphocytic leukemia/lymphoma syndrome (3/3 cases) [Table/Fig-3 d-f], Essential thrombocythemia (1/1 case) [Table/Fig-4a-b], Myelodysplastic syndrome (MDS) (5/6 cases), multiple myeloma (3/4 cases), follow-up of hematological malignancies (1/3 cases). Correlation could not be assessed in congenital dyseyrthropoietic anaemia and Idiopathic thrombocytopenic purpura (ITP) due to inadequate biopsy. Aspiration was dry tap in one case each of Lymphoblastic leukemia/lymphoma syndrome and chronic myelomonocytic leukemia [Table/Fig-4c-d].

We observed that biopsy was essential to make a diagnosis in 11 cases because of dry tap/ diluted marrow.These included 4 cases of aplastic anemia, 2 cases of acute leukemia and 1 case each of chronic leukemia, multiple myeloma, leukemia/ lymphoma syndrome, follow-up of hematological malignancy and chronic myelomonocytic leukemia with secondary myelofibrosis.

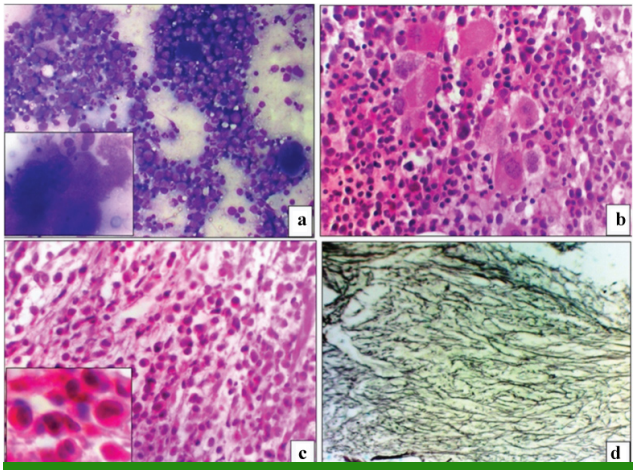
Perls' Prussian blue stain was performed in 44 cases categorized into subtype of anemia and myelodysplastic syndrome. Grading assessment of iron stain on aspirate smears could not be performed in 9 cases due to dry tap, or lack of fragments due to dilution of marrow from blood. In



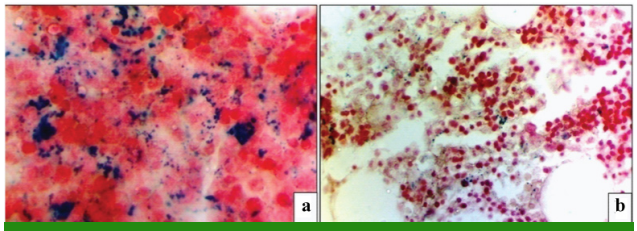
[Table/Fig-2]: Refractory anaemia with megaloblastoid maturation (a) Aspirate LG, x400 (inset shows dyserythropoiesis LG, x1000), (b) Imprint LG, x400, (c) Biopsy H&E, x400. Aplastic anaemia (d) Aspirate LG, x100 (e) Imprint LG, x100 (f) Biopsy H&E, x100.



[Table/Fig-3]: Acute Lymphoblastic Leukemia (a) Aspirate LG, x400 (b) Lymphoblasts exhibiting block positivity PAS, x400 (c) Biopsy H&E,x400 CLL/SLL (d) Aspirate LG,x400 (e) Imprint LG,x100 (f) Biopsy H&E,x400.



[Table/Fig-4]: Essential thrombocythemia (a) Imprint showing increased megakaryocytes LG,x100 (inset shows abnormal megakaryocyte LG,x1000) (b) Biopsy showing increased abnormal megakaryocytes (H&E,x400).Chronic Myelomonocytic leukemia (c) Biopsy H&E,x400 (inset shows monocytoid cells H&E,x1000) (d) Increased Reticulin fibres (Reticulin,x400).



[Table/Fig-5]: Refractory anaemia showing increased iron stores (a) Aspirate smear and (b) corresponding biopsy (Perls' Prussian blue stain, x400).

Diagnosis	Grade	Number of Cases	
		Aspirate Smears	Biopsy Sections
Nutritional anaemia (n=15)	0	1	7
	1+	13	8
	2+	1	-
Aplastic anaemia (n=6)	0	-	1
	1+	2	1
	2+	-	2
	3+	3	2
	4+	1	-
MDS (n=5)	2+	-	4
	3+	4	1
	4+	1	-
Anaemia of chronic disease (n=1)	3+	-	1
	4+	1	-
Total		27	27

[Table/Fig-6]: Comparison of Perls' Prussian blue stain on aspirate smears and biopsy sections.

these cases biopsy was available for grading iron stores.

However, there were 8 cases where biopsy was inadequate for assessment of iron stores. Thus, in only 27 cases both aspirate and biopsy were available for correlating between both techniques which showed that grading was better in aspirate in 17 cases compared to biopsy [Table/Fig-5,6].

DISCUSSION

In our study we have done the comparative evaluation of BMA, BMI and BMB to determine their diagnostic utility in various hematological diseases.

The failure rate of BMA and BMB in our study was 22.6% and 20% respectively. The failure rate of aspiration in our study was due to diluted marrow or dry tap and of biopsy was due to inadequate biopsies obtained which comprised of subcortical bony tissue with no marrow elements or only blood clot.

Out of 19 cases of nutritional anemia in our study 15 cases showed positive concordance where both BMA and BMB showed erythroid hyperplasia with either micronormoblastic or megaloblastic maturation. There were 4 cases in which two were inadequate on aspiration and two on biopsy. In a study done by Kaur et al., [6] all 50 cases of anemia showed positive correlation between BMA and BMB. However, there were no inadequate cases in this study.

We had 14 cases of aplastic/hypoplastic anemia, a positive correlation between BMA and BMB was found in 7 cases. In remaining 7 cases, 4 cases were diagnosed on biopsy alone and 3 cases were diagnosed on aspiration alone as in these biopsy were inadequate. One case which was cellular on aspirate was found to be hypocellular on biopsy. This finding agrees with the study by Kaur et al., [6] who stated that use of biopsy avoids misinterpretation of cellularity by smears. We found aspirates to be equally effective in giving a diagnosis of aplastic anemia specially when biopsy could not give definite diagnosis. This was in agreement with a study by Mahajan et al., [3]. They diagnosed 87% cases of hypoplastic anemia on aspiration.

In 10 cases of acute leukemia, concordance was found between BMA and BMB in 6 cases. Of the 4 non-concordant cases, 2 were dry tap on aspiration which could be explained due to the tightly packed marrow by leukemic cells as is also reported by Chandra and Chandra [2] in their study. In two other cases, biopsies were inadequate.

In 5 cases of chronic leukemia 4 cases showed concordance between BMA and BMB. One case was dry tap on aspiration, biopsy showed features of myelofibrosis. Our findings were similar to other studies [2,4].

4 cases of leukemia/ lymphoma syndrome were included in our study out of which 3 cases were CLL/SLL and showed concordance on BMA and BMB with satisfactory findings on aspiration. However, there was an added advantage of biopsy regarding the pattern of involvement of the marrow. These findings were agreeable with those of Sabharwal et al., [7] and Kaur et al., [6]. One case of lymphoblastic leukemia/lymphoma was however dry tap probably because of packed marrow.

In one case of essential thrombocythemia good correlation between aspirate and biopsy was found showing markedly hyperplastic and dysplastic megakaryopoiesis. This was agreeable with a study done by Toi et al., [8].

One case of chronic myelomonocytic leukemia with secondary myelofibrosis was diagnosed only on trephine biopsy as assessment of reticulin is possible only on biopsy sections. Chandra et al., [2] and Sabharwal et al., [8] also stated that diagnosis of fibrosis is possible only on biopsy sections by Reticulin stain.

There were 6 cases of MDS in our study in which there was

concordance in 5 cases and one biopsy was inadequate. The features of dyshematopoiesis were detected on aspirate smears. However, in these cases biopsy additionally detected dysmegakaryopoiesis, abnormal localization of myeloid precursors and assessment of cellularity. These findings were similar to those reported by Sabharwal et al., [7] and Gupta et al., [9]. 3 out of 4 cases of multiple myeloma showed concordance between BMA and BMB. In one case aspirate was inconclusive since it was diluted and showed scattered plasma cells, whereas BMB showed sheets of plasma cells. This was in agreement with the studies done by Tripathy et al., Toi et al., and Sabharwal et al., [4,7,8].

In 3 follow-up cases of hematological malignancies included in our study, concordance was noted in only one case which was a known case of ALL on therapy. One was a known case of CML in blast crisis showing dry tap because of packed marrow. The other case was a follow-up case of AML on therapy which showed therapy induced changes on aspiration but biopsy was inadequate.

While assessing iron status it was seen that aspirate films were more sensitive than formic acid decalcified trephine biopsy sections for the detection of hemosiderin. This was in agreement with Sabharwal et al., [7], Gupta et al., [9] and Stuart- Smith SE et al., [10] who found this to be due to loss of iron during decalcification.

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 nutritional anemia and acute leukemia and 1 case each of anemia of chronic disease, chronic myelomonocytic leukemia, CML, leukemia/lymphoma syndrome, a follow-up case of CML and a case of myeloma. This was in agreement with the study done by Sabharwal et al., [7]. Also while comparing the cellularity and the morphology in cases of aspirate and imprint smears it was found that the cellularity was better appreciated in aspirate smears and morphology of the cells was better appreciated in imprint smears. This was in agreement with a study done by Pasquale and Chikappa [11] and Varma et al., [12] who found that the biopsy imprints are more representative of the marrow milieu as the artifacts inherent to aspirate smears can be avoided.

LIMITATIONS

1. Use of one needle technique instead of the two needle technique for obtaining both aspiration and biopsy. In our study, 15 marrow biopsies were reported as inadequate which can be attributed to this technique.

2. We used 10% neutral buffered formalin for fixation of biopsy but other fixatives like Zenker acetic acid can be used as they provide good cellular details.

CONCLUSION

Bone marrow examination is an important investigation for the diagnosis and management of hematological disorders. Bone marrow aspiration and biopsy are complementary to each other. Aspiration provides good cytomorphological details and has a good predictive value in diagnosing most of the disorders. Also the report for bone marrow aspirate takes lesser time than biopsy. However, if aspiration yields dry tap or diluted marrow, diagnosis is provided by biopsy and imprint smears complement by providing cellular morphological details. Biopsy is also useful for the exact assessment of cellularity, topography, pattern of distribution of any abnormal infiltrate and fibrosis but is less reliable for detecting iron stores than aspirate smears.

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FINANCIAL OR OTHER COMPETING INTERESTS:
None.

Date of Publishing: **Jul 01, 2016**