Print ISSN: 2321-6379
Online ISSN: 2321-595X

Original Article

Correlation of Bone Marrow Aspiration, Trephine Biopsy, and Imprint Cytology in Pancytopenia/ Bicytopenia Cases in Resource-Limited Area

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Abstract

Context: Bone marrow aspiration is a simple method to study hematopoietic disorders whereas trephine biopsy is of greater value in assessing large piece of marrow. At the same time, imprint is helpful in getting morphological details of individual cell.
Aims: The aim of the present study was to correlate the findings of bone marrow aspirate, bone marrow biopsy (BMB), and touch imprints in the diagnosis of pancytopenia/bicytopenia.

Settings and Design: This was a cross-sectional study.

Subjects and Methods: This 2-year cross-sectional study of 50 cases from year 2017 to 2019 is conducted in the Department of Pathology in GMC, Haldwani. The clinical features of pancytopenia/bicytopenia were noted. Bone marrow aspiration (BMA) along with bone marrow imprint (BMI) was evaluated and BMB was performed in all cases. BMA and imprint smears were stained with Leishman stain and biopsy section was stained with H.E., PAS, and reticulin stain. Findings were studied and analyzed.

Statistical Analysis Used: All the data were compiled and entered into MS Excel. Statistical analysis was performed using software SPSS-20.

Results: In all 50 cases, definite diagnosis was made on BMB and strong positive correlation was seen between BMA, BMI, and BMB ($P < 0.00001$). Furthermore, 100% correlation was seen in combined nutritional anemia, immune thrombocytopenic purpura, and metastasis; whereas 82.6% correlation was seen in megaloblastic anemia, 55.6% in reactive marrow, and 50% in acute leukemia.

Conclusions: The present study concludes that BMA and BMI give good cytomorphological details and take lesser time in reporting as compared to BMB. BMB whereas is useful for assessment of cellularity, topography, and fibrosis. BMA alone is usually sufficient to diagnose nutritional anemia, metastasis, and immune thrombocytopenia whereas trephine biopsy is the sole diagnostic tool in myelofibrosis, granulomatous disease, and aplastic anemia.

Key words: Bone marrow aspiration, Bone marrow biopsy, Bone marrow imprint, Combined nutritional deficiency anemia

INTRODUCTION

Cytopenia is a disorder in which production of one or more blood cell types stops or is greatly reduced. Cytopenia is of two types: Pancytopenia and bicytopenia.[1]

Access this article online

www.ijss-sn.com

Month of Submission : 05-2021
Month of Peer Review : 05-2021
Month of Acceptance : 06-2021
Month of Publishing : 07-2021

Etiology of pancytopenia varies according to the geographical distribution and genetic mutations. In developed countries, the most common cause of pancytopenia is aplastic anemia followed by infections. Whereas in developing countries, the most common cause is nutritional deficiency.

Bone marrow aspiration (BMA) is a simple method to study hematopoietic disorders whereas trephine biopsy is of greater value in assessing large piece of marrow. At the same time, imprint is helpful in getting morphological details of individual cell.

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The aim of the present study was to correlate the findings of BMA, bone marrow biopsy, and touch imprints in the diagnosis of pancytopenia/bicytopenia.

SUBJECTS AND METHODS

This 2-year cross-sectional study of 50 cases from year 2017 to 2019 was conducted in the Department of Pathology in GMC, Haldwani. The clinical features of pancytopenia/bicytopenia were noted. Peripheral blood smear and aspirate smears were stained by Leishman's stain and imprint smears were stained with May-Grunwald stain. BMA was performed with Salah's needle whereas bone marrow biopsies (BMBs) were performed with the Jamshidi needle from the posterior iliac crest. Biopsies were then fixed using 10% neutral buffered formalin. Decalcification was carried out by 10% formic acid solution for 24 h. About 4–6 μm thick sections were cut and stained with H and E, PAS, and reticulin.

Criteria for pancytopenia include:
1. Hemoglobin <10 g/dL
2. Platelet count $<100 \times 10^9$/L
3. Absolute neutrophil count $<1.8 \times 10^9$/L.

Inclusion Criteria

All the cases of pancytopenia/bicytopenia in which bone marrow examination was done using all three modalities that are BMA, bone marrow imprint (BMI), and trephine biopsy (BMB) are taken for this study.

Exclusion Criteria

1. Patients with a history of recent blood transfusion
2. Bleeding and coagulation disorders will be excluded
3. Patients with severe thrombocytopenia with platelet count <20,000/mm^3 or platelet functional defect
4. Prolonged prothrombin time, increased international normalized ratio, and prolonged activated partial thromboplastin time
5. Inadequate biopsy specimen
6. Debilitated patients
7. Aspiration or biopsy will not be performed from infected site.

Table 1: Cellularity on BMA, BMI, and BMB

Cellularity	No. of cases (50) BMA	No. of cases (50) BMI	No. of cases (50) BMB
Normocellular	23 (46%)	23 (46%)	23(46%)
Hypocellular	7 (14%)	7 (14%)	22(44%)
Hypercellular	5 (10%)	5 (10%)	5(10%)
Hemodiluted	15 (30%)	15 (30%)	-

BMA: Bone marrow aspiration, BMB: Bone marrow biopsy, BMI: Bone marrow imprint

RESULTS

In this 2-year study, a total of 50 cases of pancytopenia/bicytopenia were analyzed. The age of patient in this study ranges from 21 to 90 years with maximum number of cases in the age group of 11–20 years. Out of 50 cases, 26 cases were of bicytopenia and 24 cases were of pancytopenia. Females were affected more than males.

BMA and imprint were normocellular in 23 cases (46%), hypocellular in 7 cases (14%), and hypercellular in 5 cases (10%); whereas BMB is normocellular in 23 cases (46%), hypocellular in 22 cases (44%), and hypercellular in 5 cases (10%) [Table 1] [Figures 1 and 2].

Among 50 cases of BMB, 24 cases were of pancytopenia. Cellularity of these cases was compared with BMA; in which 12 cases were cellular on BMA as well as on BMB. Three cases which were hypocellular in BMA and nine cases which were hemodiluted turn out to be hypocellular on BMB [Figure 3] [Table 2].

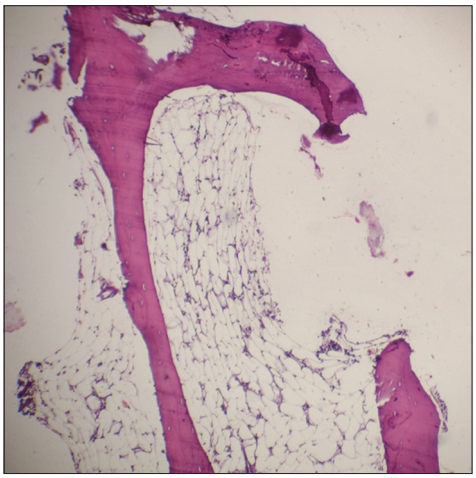

Figure 1: Bone marrow biopsy shows hypocellular marrow (H&E stain, ×100)

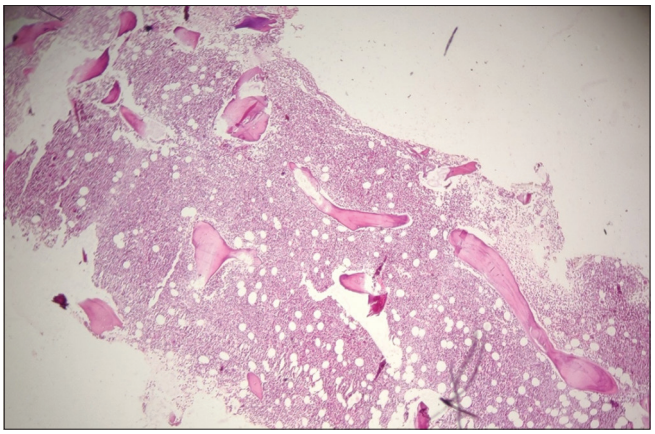

Figure 2: Bone marrow biopsy shows hypercellular marrow (H&E stain, ×100)

Out of 50 cases of BMB, 26 cases were of bicytopenia. Cellularity of these cases is compared with BMA, in which 18 hypocellular and two hemodiluted cases on BMA turned out to be 20 hypocellular on BMB. Two cellular and four hemodiluted cases on BMA turn out to be cellular on BMB [Table 3].

Megaloblastic anemia is the most common finding on BMA, BMB, and BMI and least common cause is metastasis, hairy cell leukemia, and chronic inflammatory disease with gelatinous transformation [Table 4].

About 100% correlation were seen in combined nutritional deficiency, immune thrombocytopenic purpura, metastasis, and hairy cell leukemia followed by 82.06% correlation in megaloblastic anemia, 55.56% correlation in reactive marrow with erythroid hyperplasia, and 50% correlation in acute leukemia [Figure 4] [Table 4].

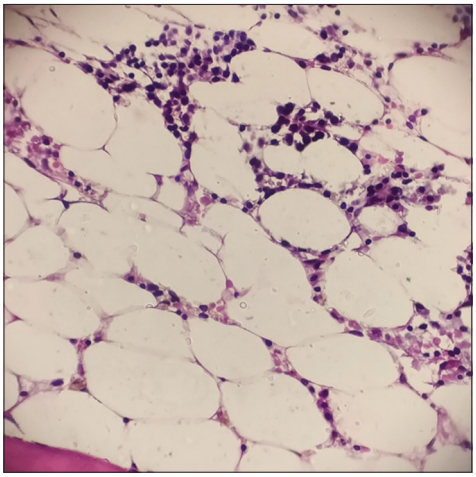

Figure 3: Bone marrow biopsy shows replacement of marrow with fat spaces along with islands of lymphocytes (H&E stain, ×400)

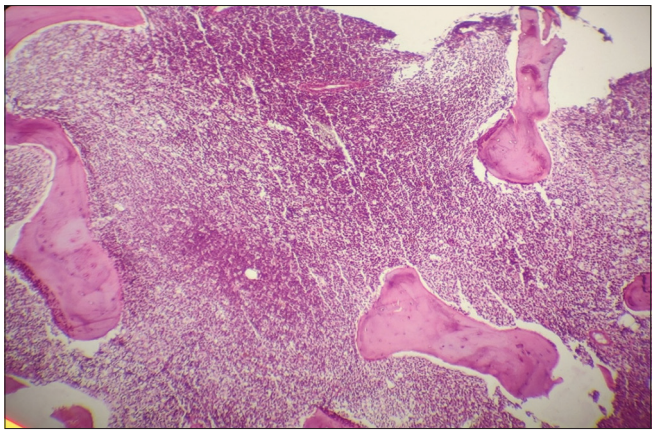

Figure 4: Hypercellular marrow showing diffuse infiltration of marrow with lymphoid cells (BMB; H&E stain, ×100)

In relation to BMB, 100% sensitivity was seen in combined nutritional deficiency, immune thrombocytopenic purpura, metastasis [Figure 5], and hairy cell leukemia.

DISCUSSION

Bone marrow examination is an essential pre-requisite for making diagnosis and the advantage of each procedure differs. These procedures are complimentary to each other and should be performed simultaneously. On all the three modalities, cellularity was assessed and compared according to age. Normocellular and hypercellular marrow was considered as cellular for comparison. Out of 24 pancytopenia cases, BMA and imprint were cellular in 12 cases, hypocellular in three cases, and hemodiluted in nine cases. Whereas in BMB, cellular cases were 12 and hypocellular cases were 12. These findings were in contrast to a study done by Metikurke[2] in which he found 100% correlation between cellularity except in two cases in which they found inadequate BMB.

Table 2: Correlation of cellularity of pancytopenia cases on bone marrow aspirate and biopsy

Cellularity	BMA (*n*=24)	BMB (*n*=24)
Cellular	12	12
Hypocellular	3	12
Hemodiluted	9	-

BMA: Bone marrow aspiration, BMB: Bone marrow biopsy

Table 3: Correlation of cellularity of bicytopenia cases on bone marrow aspirate and biopsy

Cellularity	BMA (*n*=26)	BMB (*n*=26)
Cellular	2	6
Hypocellular	18	20
Hemodiluted	6	-

BMA: Bone marrow aspiration, BMB: Bone marrow biopsy

Table 4: Positive correlation between BMB, BMI, and BMA for various hematological disorders

Diagnosis	BMA	BMI	BMB	Positive correlation (%)
Reactive marrow	5	5	9	55.56
Megaloblastic anemia	19	19	20	82.06
Combined nutritional anemia	3	3	3	100
Acute leukemia	3	3	6	50
Myelofibrosis	0	0	3	0
Immune thrombocytopenic purpura	3	3	3	100
Aplastic anemia	0	0	3	0
Metastasis	1	1	1	100
Hairy cell leukemia	1	1	1	100
Chronic inflammatory disease	0	0	1	0

BMA: Bone marrow aspiration, BMI: Bone marrow imprint, BMB: Bone marrow biopsy

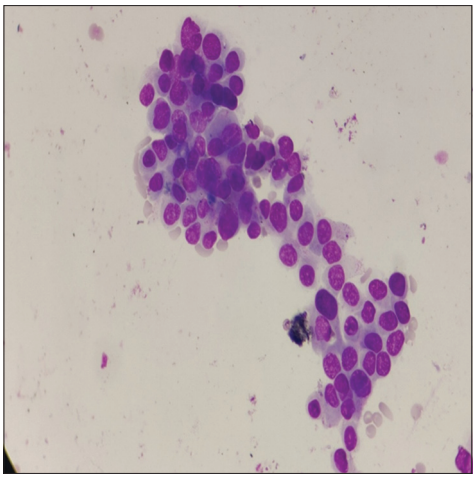

Figure 5: Bone marrow imprint shows sheets of malignant cells (H&E stain, ×400)

Out of 26 bicytopenia cases, BMAs were cellular in two cases, hypocellular in 18 cases, and hemodiluted in six cases. BMB was cellular in six cases and hypocellular in 20 cases. Two cases, which were found hypocellular in BMA, were found to be hypercellular in BMB. Similar finding was made by study done by Gruppo *et al.*[3] in which greatest variance was seen in the hypercellular or normocellular marrows as estimated by biopsy in which 44 of 113 (39%) were interpreted as moderately or severely hypocellular by aspirate.

Out of 24 pancytopenic cases, nine cases were hemodiluted on BMA which turned out to be hypocellular on BMB. Out of six bicytopenia hemodiluted cases on BMA, four cases turned out to be cellular on BMB and two cases were reported as hypocellular. This could be due to faulty BMA technique as our institute being a teaching institute and BMA is mostly performed by postgraduate students. Thus, our study supports that for the assessment of bone marrow cellularity, BMB is the better diagnostic modality.

In all 50 cases, definite diagnosis was made on BMB and strong positive correlation was seen between BMA, BMI, and BMB ($P < 0.00001$).

In our study, combined nutritional deficiency shows 100% correlation which is in contrast to study done Metikurke.[2] Immune thrombocytopenic purpura shows 100% correlation which is similar to study done by Toi *et al.*, Patro *et al.*, and in contrast to a study done by Gilotra *et al.*[4-6]

One case of metastasis diagnosed on BMA/BMI shows acinar arrangement as well as sheets of malignant cells. Furthermore, BMB displayed acinar arrangement and intracellular mucin giving 100% correlation in our study which is in contrast to study done by Toi *et al.*, Tripathy and Dudani, Ghodasara and Gonsai, and Patro *et al.*[5-8]

In the present study, megaloblastic anemia shows 82.6% correlation which is in contrast to a study done by Patro *et al.*, Gilotra *et al.*, and Toi *et al.*[4-6]

Reactive marrow with erythroid hyperplasia shows 55.56% correlation which is in contrast to a study done by Toi *et al.*, Tripathy and Dudani, Ghodasara and Gonsai, and Gilotra *et al.*[4,6-8]

Acute leukemia shows 50% correlation which is in contrast to a study done by Ghodasara and Gonsai, Gilotra *et al.*, and Patro *et al.*[4,5,8]

Our study shows that BMB is the sole diagnostic tool in myelofibrosis (three cases) where BMA yielded diluted marrow or dry tap and BMB demonstrated Grade 3 fibrosis on reticulin stain.

BMB was more helpful in diagnosing aplastic anemia, hairy cell leukemia, and granulomatous lesion. In hairy cell leukemia, BMB demonstrated characteristic spacing of the cells along with Grade 1 fibrosis on reticulin stain. One case of granulomatous lesion, on BMB, demonstrated granuloma with gelatinous transformation which is common finding in chronic inflammatory disease.[9]

CONCLUSIONS

The present study concludes that BMA and BMI give good cytomorphological details and take lesser time in reporting as compared to BMB. BMB whereas is useful for the assessment of cellularity, topography, and fibrosis. BMA alone is usually sufficient to diagnose nutritional anemia, metastasis, and immune thrombocytopenia, whereas trephine biopsy is the sole diagnostic tool in myelofibrosis, granulomatous disease, and aplastic anemia.

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How to cite this article: Singh B, Joshi U, Kaushik A. Correlation of Bone Marrow Aspiration, Trephine Biopsy, and Imprint Cytology in Pancytopenia/Bicytopenia Cases in Resource-Limited Area. Int J Sci Stud 2021;9(4):139-143.

Source of Support: Nil, **Conflicts of Interest:** None declared.