

ORIGINAL RESEARCH PAPER

Comparative Evaluation of Bone Marrow Aspiration with Bone Marrow Biopsy in Pancytopenia: A two year experience in a rural teaching hospital

Pathology

KEY WORDS: Pancytopenia, Bone marrow aspiration, Bone marrow biopsy

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Introduction: Bone marrow examination is an indispensible tool in hematology practice in diagnosing pancytopenia patients. It gives a comprehensive bone marrow status and helps to identify the underlying etiology. Bone marrow examination comprises of bone marrow aspiration (BMA) and bone marrow biopsy (BMB).

Aim: Comparative evaluation of BMA with BMB in pancytopenia.

Materials and Methods: This retrospective study was done over a two year period from January 2015 to December 2016 in the department of pathology, Dr RPGMC Kangra at Tanda (HP). All new-onset pancytopenia patients during this period were included in the study. A diagnostic comparison was done between simultaneously performed BMA and BMB procedures.

Results: A total of 56 cases of pancytopenia were identified during this period. Megaloblastic anemia (n=15/46; 32%) was the most common etiology for pancytopenia in adult population (46 patients) and acute leukemia (n=8/10; 80%) was the commonest etiology in pediatric population (10 patients). BMA alone was sufficient to make diagnosis in 40/56 (71%) cases, mainly in nutritional anemias (22), myelo-dysplastic syndrome [MDS] (3) and acute leukemias (6) where the marrow aspirate is adequate. BMB played a pivotal role in 16 (29%) cases. These were dry tap (10 cases i.e. acute leukemia [4], myelofibrosis [3] & aplastic anemia [3]), focal marrow infiltration (4 cases i.e. NHL [2], multiple myeloma [1], granulomatous inflammation [1]) & chronic myeloproliferative neoplasm [CMPN] (2 patients).

Conclusion: Ideally bone marrow examination in patients with pancytopenia must include both BMA and BMB simultaneously. However, in centers with limited resources, the decision to perform BMA alone or in combination with BMB may depend upon the underlying diagnosis being considered. BMA alone is the mainstay of diagnosing nutritional anemias whereas BMB is pivotal in diagnosis of dry tap, focal marrow infiltration and CMPN. In MDS both BMA & BMB complement each other & hence must be performed simultaneously.

Introduction:

Pancytopenia is a relatively common clinico-hematological entity encountered in clinical practice. Pancytopenia is defined as reduction in all three major formed elements of blood (red cells, white blood cells and platelets) below the normal reference range. [1] A spectrum of etiologies is responsible for pancytopenia ranging from benign causes like anemias and infections to serious hematological malignancies like leukemias and lymphomas.¹ Bone marrow examination plays a very important in identifying the underlying pathology. It includes two procedures: BMA followed by BMB. BMA is a simple, easy and rapid diagnostic procedure. On the other hand, BMB provides more comprehensive information regarding the marrow cellularity, architectural patterns and overall hematopoiesis. But BMB is a painful procedure and the processing takes 48-72hrs. [3] So at many low-resource centers, it may not be cost effective in terms of the clinician and laboratory personnel time and patient discomfort to perform bone marrow biopsies in all patients. This retrospective study was done with an aim of comparative evaluation of diagnostic accuracy of BMA and BMB procedures, done simultaneously in pancytopenia patients for a period of two years.

Materials and methods:

This retrospective study was conducted in the Department of Pathology at a Dr Rajendra Prasad Government Medical College, Kangra at Tanda (HP), India. All pancytopenia patients with Hb <10 gm/dl, TLC < 4.00x10³ cells/\(\text{M}\) and platelet count <100x10³ cells/\(\text{M}\) were included in our study retrospectively over a period of two years (January 2015 to December 2016). All the diagnosed cases which were already on chemotherapy were excluded from the study. Written informed consent was obtained from all the patients. All patients had BMA & BMB done simultaneously using 2 needle techniques at the same skin incision site. Pediatric cases were defined as patients 14 yrs or younger.

Bone marrow examination:

Bone marrow examination was done from the posterior superior iliac spine using Jamshidi bone marrow aspiration needle (No. 16 for adults and No. 18 for pediatric patients). At least 4 good smears were prepared from the aspirate material. Two smears were examined with May- Grunwald Giemsa (MGG) and remaining smears were kept for special stains like Perl's stain for iron, reticulin

stain, myelo-peroxidase (MPO) and Periodic acid Schiff (PAS) wherever required. BMA & BMB were reported according to the International Council for Standardization in Hematology (ICSH) Protocol. [4] BMA smears were examined for adequacy of cellularity, megakaryocyte number and morphology, any abnormal cells or granulomas. A minimum of 500 nucleated and intact bone marrow cells were evaluated.

BMB was taken in all cases with Jamshidi trephine biopsy needle (No. 11 for adults and no. 13 for pediatric cases). A trephine length of 1.5 – 2cm was considered to be adequate. It was fixed in 10% buffered formalin. Biopsies were subjected to decalcification in 5.5% EDTA solution for 24 hrs. After decalcification, the biopsies were subjected to routine tissue processing method and sections were studied using hematoxylin and eosin staining. All BMA and BMB cases were re- evaluated. Reporting protocol for trephine biopsy was: adequacy of the biopsy, overall cellularity, distribution and maturation of marrow elements, trabecular bone structure and evidence of any bone marrow fibrosis on reticulin stain. BMA & BMB findings were compared. The results were collected and analyzed on a Microsoft Excel 2010 sheet and percentages were calculated.

Results:

Patient Characteristics

A total of 56 cases of pancytopenia were included in the study (Table 1). Age of the patients included varied over a wide range (2-86 years) with a median age of 30 years. The male/female ratio (M: F) was 0.64:1. Among all pancytopenia cases, 46 cases were adults (range, 14-86 years; median 50 years) with M: F ratio of 0.5:1. Ten cases were in pediatric age group with M: F ratio of 1:1 (Figure 1).

Most of the cases affected were in the age group of 11-20 years (23%) followed by 61-70 years (21%). In the adult population, most common cause of pancytopenia was found to be megaloblastic anemia (14/46, 30%). In the pediatric population, most common cause of pancytopenia was acute leukemia (8/10, 80%). Remaining two cases in pediatric age group were of megaloblastic anemia and iron deficiency anemia, each. (Figure 1)

Bone marrow biopsy (BMB)

Among all 56 cases, 16 cases (29%) were diagnosed on BMB alone

(Table 2). These were dry tap (10 cases), focal marrow infiltration (4 cases) & chronic myeloproliferative neoplasm [CMPN] (2 cases). Out of 10 cases with dry tap final diagnosis (as made on BMB) were acute leukemia (4 cases), aplastic anemia (3 cases) and myelofibrosis (3 cases) as shown in figure 2a and 2b. Among 4 cases of focal marrow infiltration, 2 cases (as diagnosed on BMB) revealed infiltration by Non Hodgkins Lymphoma (NHL), one case showed infiltration by multiple myeloma (Fig.2c and 2d) and one case showed granulomatous inflammation (Fig.3a). Two cases were finally reported as chronic myeloproliferative neoplasm (CMPN) on BMB (Fig.3b). These cases were reported as erythroid hyperplasia on bone marrow aspirate.

Bone marrow aspiration (BMA)

In remaining cases (40/56; 71%), BMA alone was sufficient to make a diagnosis. These were nutritional anemia (22 cases), acute leukemia (6 cases), MDS (3 cases), aplastic anemia (2 cases), multiple myeloma (3 cases), and one case each of NHL, visceral leishmaniasis, sideroblastic anemia and hypersplenism.

Out of all nutritional anemias (22 cases), BMA identified 15 cases of megaloblastic anemia (Fig.4a) and 7 cases of dimorphic anemia. Three cases of MDS were also identified on BMA (Fig.4b) whereas BMB could identify only one case as MDS. A case of leishmaniasis with hemophagocytosis was identified on BMA which was missed on BMB (Fig.4c). Among cases of acute leukemia (6 cases), 4 cases were of acute lymphoblastic leukemia (ALL) and 2 cases of acute myeloid leukemia (AML), based on MPO positivity. Both AML cases were in adults (Fig 4d).

Correlation

In our study, the correlation between BMA and BMB results in diagnosing nutritional anemia cases was 27% (BMB; 6 & BMA; 22). In diagnosing MDS (total 3 cases) BMB had correlation of 33% only (BMB; 1, BMA; 3).

Out of 10 cases with acute leukemia, the correlation between BMA & BMB was 60% (BMA: 6 [remaining 4 dry tap], BMB; 10). Aplastic anemia (5 cases) had 40% correlation for BMA (BMA: 2, BMB; 5). There were 4 cases of multiple myeloma with positive correlation of 75% for BMA (BMA; 3 cases, BMB; 4 cases). In NHL correlation was 33% (BMA; 1, BMB; 3).

All patients with myelofibrosis (3 cases) & CMPN (2 cases) were diagnosed only on BMB. Furthermore, there was one case each of granulomatous inflammation, visceral leishmaniasis, sideroblastic anemia & hypersplenism. BMB was detrimental in diagnosis of granulomatous inflammation & BMA in diagnosing visceral leishmaniasis & sideroblastic anemia. Hypersplenism was diagnosed on both. [Table 3]

Discussion:

Bone marrow examination is an important tool for identifying the underlying pathology in cases of pancytopenia. A complete bone marrow examination includes BMA followed by BMB. BMA is a simple, reliable and rapid method of marrow evaluation, provided the marrow aspirate is adequate. BMB provides more detailed information regarding the marrow cellularity, architectural patterns and overall hematopoiesis. But BMB is a painful procedure and its processing takes at least 48-72 hours. Pancytopenia is a common hematological presentation due to a variety of underlying etiologies. This study was carried out with an aim of comparative evaluation of results between BMA and BMB cases in pancytopenia patients.

Bone Marrow Biopsy

In our observation, out of 56 cases BMB was pivotal in making the diagnosis in additional 16 cases (28%) which were completely missed on BMA. Of these missed 16 cases, 10 were dry tap, 4 focal marrow infiltration (NHL; 3, multiple myeloma; 1, granulomatous inflammation; 1) and remaining 2 of CMPN.

Dry Tap

Out of 10 cases with dry tap, 4 were of acute leukemia (did not

yield bone marrow aspirate which may be due to packed marrow), 3 of myelofibrosis (with reticulin stain showing grade 3 fibrosis) and remaining 3 of aplastic anemia. All the dry tap cases in our study had a definite underlying abnormality. This highlights the importance of performing BMB especially in failed BMA procedures. This is in accordance with the study done by *Navone R et al* and *Amrish Pandya et al* who also found 100% abnormality in dry tap cases. [5,6]

Focal marrow infiltration

There were 4 cases with focal marrow infiltration i.e. NHL (2), multiple myeloma (1) and granulomatous inflammation (1). Bilateral trephine biopsies were done in all these cases which revealed focal infiltration by the atypical cells in one side trephine biopsy cores only. Our results are in agreement with other studies which also highlight that bilateral trephine biopsy increases the sensitivity of finding a focal pathology.^[7,8] BMB further gives information regarding spatial distribution of lymphoma cells, extent of infiltrate, cellularity of marrow elements and fibrosis which cannot be determined on BMA. This will be especially useful in post chemotherapy patients to assess the residual tumor cell burden. [9] Granulomatous inflammation was diagnosed in one case on BMB. Bone marrow aspirate smears of the same showed normal marrow study. Granulomas have been rarely reported in bone marrow aspirate smears. This may be due to focal involvement of the bone marrow and due to fibrosis in & around the granulomas. This case was confirmed to be tuberculosis on the culture of bone marrow aspirate. Our result is in concordance with other studies which also state that BMB is a better tool in identifying granulomas. [10-12]

Chronic myeloproliferative neoplasm (CMPN)

Two cases of CMPN were identified on BMB. Both cases showed hypercellular marrow with proliferation of all three hematopoietic lineages. The morphological features in both were suggestive of Chronic Myeloid Leukemia and cytogenetics was advised for confirmation. Bone marrow aspirates were diluted in both cases and no opinion was possible. Diluted bone marrow aspirate may be due to packed bone marrow or due to an underlying marrow fibrosis.

Bone Marrow Aspirate

Among all the cases, "BMA alone" was sufficient to make a diagnosis in majority of them (40/56; 71%).

Nutritional anemia

BMA played a pivotal role in identifying nutritional anemia in our study. Megaloblastic anemia was the most common cause of pancytopenia in our study (27%). High prevalence of megaloblastic anemia may be due to nutritional deficiencies and higher incidence of infections in developing countries, as ours. Bone marrow aspirate smears provided a better morphological picture to characterize erythropoiesis, to identify any megaloblastic change and identifying giant myelocytes and metamyelocytes. These features help in making a diagnosis of megaloblastic anemia. BMB, on the other hand, was able to make a diagnosis of megaloblastic anemia in 6 cases only (38%). In remaining 9 cases, 7 were interpreted as normal marrow study and 2 were identified as dimorphic erythropoiesis, on BMB. These results are in accordance with available literature which identifies bone marrow aspirate as a better tool in making diagnosis of megaloblastic anemia. [7,10-13] Study by **P. ch Toi et al** showed that megaloblasts look like leukemic blast on BMB.[14] Additionally in dimorphic anemias, further grading of iron stores was better interpreted on bone marrow aspirates. Also, BMA was a better tool in identifying micro/macro normoblasts. BMB was not an effective tool in identifying and characterizing nutritional anemias in our study as observed by available literature too. [9,12,15,16] Hence, BMA may obviate the need of BMB in diagnosing nutritional anemias in patients with pancytopenia.

Leishmania donovani

BMA helped in identifying intracellular and extracellular amastigotes of *Leishmania donovani*. Furthermore, BMA was also helpful in the diagnosis of haemophagocytosis in this particular case. These findings were missed on BMB. Clinical picture, biochemistry and bone marrow findings were consistent with acquired hemophagocytic lymphohistiocytosis (HLH) associated with pancytopenia in this patient.

Myelodysplastic syndrome

There were 3 cases of MDS diagnosed on BMA. Only one case was interpreted as MDS on BMB. This particular case had dysplastic changes in the megakaryocytes in the form of hypolobation and lobe separation. There was abnormal localization of immature precursors (ALIP) which helped to clinch the diagnosis. This case highlights the fact that dysplastic changes can be easily identified in megakaryocytes on BMB along with ALIP. However, it was difficult to identify dysplastic features in erythroid cells (binucleation, multinucleation or nuclear bridging) and myeloid cells (hypogranulation, hyposegmentation or hypergranulation) on BMB. BMA is a better tool in identifying dysplastic features in marrow cells and BMB better helps in identifying the ALIP. Hence, in patients suspected with MDS both BMA & BMB should be done for better characterization and diagnosis.

Acute Leukemia

BMA was able to diagnose only 60 % cases (6 out of total 10 cases). However, BMA played an important role in typing of leukemia (AML/ALL) based on MPO and PAS positivity (2 cases of AML & 4 cases of ALL). This characterization is important in smaller centers where sophisticated techniques like flowcytometry (FCM) are not available. However, it is prudent to mention that due to poor correlation (only 60%) all suspected patients should simultaneously have BMB too.

There were few limitations in our study. Firstly, the absolute number of cases in all the subgroups is less. Furthermore, our study lacked the sophisticated ancillary techniques like FCM, cytogenetics and RT-PCR in hematological malignancies.

Nonetheless, in low resource settings with very high prevalence of nutritional anemias our study may obviate the need of BMB as a first line of investigation in these patients, thereby providing a rapid, cheap & easy diagnosis with minimal patient discomfort. This may help in early institution of treatment thereby providing rapid relief. However, caution should be advised in patients not responding to conventional treatment for nutritional anemias. These patients should definitely undergo detailed evaluation including BMB.

Conclusion:

BMA and BMB are complementary to each other. The superiority of one procedure over the other depends on the underlying pathology. BMA is an easy, safe and rapid diagnostic procedure which plays a valuable role in diagnosing nutritional anemias. Additionally it plays a significant role in identifying dysplastic changes of marrow cells in MDS cases provided the bone marrow aspirate is adequate. On the contrary BMB is the procedure of choice in suspected cases of focal marrow infiltrations like lymphoma infiltration, myeloma infiltration, granulomatous inflammation and aplastic anemia. Additionally BMB is the sole procedure for the diagnosis of myelofibrosis.

To conclude, ideally both the procedures (BMA & BMB) must be done simultaneously for better bone marrow interpretation in patients with pancytopenia. However, in centers with limited resources, the decision to perform BMA alone or in combination with BMB may depend upon the underlying diagnosis being considered.

Conflict of interest:

There is no conflict of interest with any individual or organization.

Table 1: Distribution of all the patients with pancytopenia enrolled in the study (n=56)

Causes of Pancytopenia (n=56)	
Megaloblastic Anemia	13
Acute Leukemia	10
Aplastic Anemia	05
Dimorphic Anemia	05
Iron Deficiency Anemia	04
Multiple Myeloma	04
Myelodysplastic Syndrome	03
Myelofibrosis	03
NHL infiltration	03
Chronic myeloproliferative Neoplasm	02
Visceral Leishmaniasis with hemophagocytic Syndrome	01
Sideroblastic Anemia	01
Hypersplenism	01
Granulomatous Inflammation	01

Table 2: Patients diagnosed only on Bone Marrow Biopsy (n=16) who were missed on bone marrow aspirate

	Diagnosed on BMB	Diagnosis on BMA
Dry Tap (n=10) Acute Leukemia (4) Aplastic anemia (3) Myelofibrosis (3)	4 3 3	DRY TAP (no aspirate)
Focal marrow infiltration (n=4) NHL (2) Multiple myeloma (1) Granulomatous Inflammation (1)	2 1 1	NORMAL MARROW STUDY
CMPN (n=2)	2	MEGALOBLASTIC ERYTHROID HYPERPLASIA

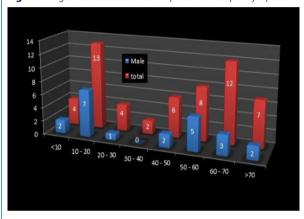
Table 3: Comparative evaluation of diagnosis on BMA & BMB in all patients in study (n=56)

Diagnosis		On Bone marrow biopsy
	aspirate	
Megaloblastic Anemia (13)	Megaloblastic erythroid hyperplasia(13)	Megaloblastic erythroid hyperplasia (5) Normal Marrow study (6) Dimorphic erythropoiesis (2)
Dimorphic Anemia (5)	Dimorphic Erythroid hyperplasia(5)	Normal Marrow study (3) Micronormoblastic erythroid hyperplasia(2)
Iron deficiency Anemia (4)	Micronormoblastic erythroid hyperplasia (4)	Micronormoblastic erythroid hyperplasia (2) Normoblastic erthroid hyperplasia (2)
Acute Leukemia (10)	Acute Leukemia (6) Dry tap (4)	Acute leukemia (10)
Myelo Dysplastic Syndrome (3)	MDS (3)	MDS (1) Normoblastic erythroid hyperplasia (2)
Aplastic Anemia (5)	Hypoplastic/Aplasti c Anemia (2) Diluted Bone marrow (1) Dry tap (2)	Aplastic Anemia (5)
Myelofibrosis (3)	Dry tap (2) Diluted marrow (1)	Myelofibrosis (3)
Multiple Myeloma (4)	Multiple Myeloma (3) Normal marrow study (1)	Multiple myeloma (4)
NHL infiltration (3)	NHL infiltration (1) Normal marrow study (2)	NHL infiltration (3)

CMPN (2)	Megaloblastic erythroid hyperplasia(2)	CMPN (2)
Granulomatou s Inflmm. (1)	Normal marrow study (1)	Granulomatous Inflammation (1) Culture: Tuberculosis
VL with hemophagocy tic syndrome (1)	VL with hemophagocytic syndrome (1)	Lymphoplasmacytic response (1)
Sideroblastic Anemia (1)	Sideroblastic Anemia	Normal marrow study
Hypersplenis m (1)	Hypercellular bone marrow with hyperplasia of all three lineages	Hypercellular bone marrow with hyperplasia of all three lineages

Figure Legends:

Figure 1: Age wise distribution of all patients with pancytopenia



(x-axis: in years, y-axis: number [n])

Fig.2a: Bone marrow biopsy shows aplastic anemia (H and E, x

Fig.2b: Bone marrow biopsy shows myelofibrosis (Hand E, x 400)

Fig.2c: Bone marrow biopsy shows infiltration by NHL (Hand E, x 100)

Fig.2d: Bone marrow biopsy showing sheets of plasma cells (Hand E, x 100) [Inset]: Higher magnification of myeloma cells

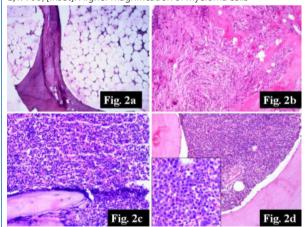


Fig.3a: Bone marrow biopsy shows granuloma (H and E, x 400) {arrow showing granuloma}

Fig.3b: Bone marrow biopsy shows CMPN (H and E, x 400)

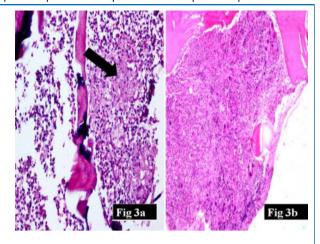
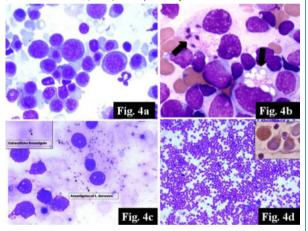


Fig. 4a: Bone marrow aspirate shows megaloblastic anemia (MGG stain \times 400)

Fig.4b: Bone marrow aspirate shows myelodysplastic changes as multi-vacuolations in myeloid cells, pseudo chediak hegashi granules (MGG stain x 400)

Fig.4c: Bone marrow aspirate shows intracellular amastigotes of L.donovani. [Inset]: Extracellular amastigotes of L.donovani (MGG stain x 400)

Fig.4d: Bone marrow aspirate shows Acute Myeloid Leukemia (MGG stain x 100) [Inset]: MPO positivity in blast cells



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