Correlation of Bone Marrow Aspiration and Trephine Biopsy in Various Haematological Disorders: A Study of 3 Years

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ABSTRACT

Background: The bone marrow examination is an essential investigation for the diagnosis and management of many disorders of the blood and bone marrow. The aspirate and trephine biopsy specimens are complementary and when both are obtained, they provide a comprehensive evaluation of the bone marrow. The present study was conducted to compare the role of trephine biopsy with bone marrow aspiration for effectively diagnosing wide spectrum of hematological diseases. Few studies have compared the relative value of aspirate with trephine biopsy.

Materials & Method: This is a three year observational study undertaken in Dept. of Pathology, MKCG MCH, Berhampur, Odisha. A total of 370 cases presented with haematological disorders, of which only 126 patients had undergone trephine biopsy and correlation was done with aspiration in these patients.

Results: Of a total 370 patients, both BMA & BMB were performed on 126 patients (71 male & 55 female). Commonly encountered diseases were AML (17%), IDA (11%), ALL (9%), others (9%), CML (8%), (6%) accounted for maximum number of cases. Other conditions included TB, NPD, Metastatic Diseases such as SRBCT, NHL & Neuroblastoma. Patients from 2 months to 80 years old were encountered in the study. BMB was diagnostic in 100% cases. In comparison BMA, a positive diagnosis was made in 80% (101) cases, suggestive in 6.3% (8) cases & negative in 13.4% (17) cases. BMB was superior to BMA in diagnosis of MF (2%), ALL (4%), Others (9%) where BMA aspirations yielded a dry tap / diluted marrow. In the present study BMB was advantageous in diagnosis & staging in 19% (25) cases. Additional advantages of BMB noted in the present study were assessment of cellularity, detection of Abnormal localization of immature precursors, assessment of fibrosis, nodular/diffuse/focal patterns of involvement, metastatic deposits, and granuloma could be identified in BMB.

Conclusion: The decision whether to perform a BMA alone or in combination with BMB rests on the diagnostic possibilities. In IDA, ITP & Acute leukaemia’s where cellular morphology is desired aspiration is best. BMM is superior when assessment of cellularity, detection of ALIP, assessment of fibrosis, nodular/diffuse/focal patterns of involvement, metastatic deposits, granuloma, with the use of IHC on BMB samples the accuracy in diagnosis of Lymphoma, AML/ALL, Multiple myeloma & Metastatic Diseases can be made. Thus BMA & BMB should always go hand in hand.

Keywords: Bone Marrow Aspirate, Bone Marrow Biopsy, Myelofibrosis, Niemann Pick Diseases, Gaucher’s Disease, Metastatic Carcinoma, Small Round Blue Cell Tumor.
Berhampur, Odisha to evaluate the complementary role of both the procedures done simultaneously and to see the advantages and disadvantages of these procedures.

Aim of our study was to look for the spectrum of various haematological disorders, examine bone marrow morphology in various bone marrow disorders & to compare the incidence of the underlying pathology and correlate the bone marrow findings with clinical findings and peripheral blood findings. To evaluate the utility and accuracy of BMA & BMB individually and in combination.

**Materials and Method**

This was a prospective study conducted in the Department of Pathology, MKCG Medical College & Hospital, Berhampur over a period of 3 years. During the period 370 bone marrow aspirations were done of which 126 cases had simultaneous bone marrow core needle biopsy. The protocol followed in each case was – [i] recording of detailed clinical history and other investigation findings, [ii] Complete blood counts (CBC) and peripheral smear study in each case followed by [iii] BMA & BMB.

Protocol for BMA[1]: Needle – Salah’s / Klima’s bone marrow aspiration needle; Site – Posterior superior iliac spine was the most common site, others being anterior superior iliac spine, upper end of tibia. Aspirated material was expressed on to multiple slides and wedge smears and crush smears were prepared and air dried. Smears were routinely stained with Leishman stain and Pearl’s stain for iron. Different cytochemical stains performed as per case necessity were myeloperoxidase (MPO), Sudan black B (SBB), Periodic acid schiff’s (PAS), Zeel Nelson stain for acid fast bacilli (AFB), etc.

Protocol for BMB[1]: Needle – Jamshedi’s needle; Site – Posterior superior iliac spine. Procedure: After marrow was aspirated biopsy needle with trocar in position was introduced through the same skin puncture site, the outer table was punctured perpendicularly at a site close to the aspiration wound. Then the trocar was removed and the needle was directed towards the anterior superior iliac spine, with a constant firm pressure the needle was progressed with a rotatory movement. Approximately 2 to 3 cm of the needle has to be passed to obtain a marrow core of adequate length. Adequate core of marrow is meant by more than 2 cm length and at least 10 well preserved inter trabecular spaces are available for evaluation. Imprint slides were prepared. Subsequently the cores were processed for 12 hours in 10% NBF followed by decalcification in 10% EDTA solution for 24 to 48 hours. Finally the decalcified cores were routinely processed by the automatic tissue processor, embedded and 3 – 4 µm thick sections were prepared. All were routinely stained with Hematoxylin and Eosin (H & E) stain, Reticulin & Pearls stain. Other stains performed were PAS, ZN stain etc.

All biopsies and aspirates were independently studied by two pathologists. The following features were recorded in each case:

**BMA:**
- Nature of material – Dry tap / Bloody tap / Normal marrow particles.
- Adequacy.
- Cellularity.
- Routine microscopic findings – Leishman stain, Pearl’s stain.
- Other ancillary study findings – MPO, SBB, PAS, ZN stain etc.
- Diagnostic or not.

**BMB:**
- Quality of microscopy.
- Adequacy.
- Cellularity.
- Topographic arrangement of cells.
- Routine microscopic findings – H & E stain, Pearl’s stain.
- Other ancillary study findings – MPO, SBB, PAS, ZN stain etc.
- Diagnostic or not.

**Result**

A total of 370 cases were taken for evaluation, of which 126 had undergone both aspiration and biopsy. These cases were further reviewed in our study. The average length of biopsies in our study was 1.2 - 1.6 cm (Mean=1.5cm). Of the 126 cases, 71(56.3%) were male & 55(43.7%) female patients. There was a positive correlation between BMA and BMB in 101 (80.2%) cases whereas in 17 cases (13.4%) diagnosis was made only from BMB (Table 1). No definite opinion could be given either from BMA or BMB in 8 cases (6.4%).

Our study showed a highest correlation rate with erythroid hyperplasia 23/23 (100%) cases and acute myeloid leukemia 22/22 (100%) cases (Figure 1). In those cases where a diagnosis of erythroid hyperplasia cases (18.2%) were given, depending on whether macro-normoblastic or micro-normoblastic, they were further worked up mostly for anaemia and accordingly Perl’s stains for iron was done,
biochemical parameters were taken into consideration and the impression was given. Iron stains were better appreciated in BMA rather than BMB (76% showed iron stain grade 1 & 2 in BMA whereas were negative in BMB).

Other cases with a good positive correlation were hematological malignancies such as Chronic myeloid leukemia 10/10 (100%), Immune thrombocytopenic purpura showed 100% correlation (2/2) and multiple myeloma 6/8 (75%) cases. We also had cases of AML M6 & ALL (Figure 2), CLL (Figure 3). BMA was reported as suggestive of aplastic anaemia in 16 cases but BMB revealed hypo cellular marrow only in 9 cases where as aplastic anaemia in rest of 7 cases.

Amongst the non-hematological malignancies; NHL comprised 4 cases, metastatic to the bone marrow 3 cases, tuberculosis of bone marrow 3 cases, Niemann pick disease were 2 cases. Of the non hematological malignancies only 6 (50%) cases showed positive correlation in both BMA and BMB. The 3 case of metastasis to bone marrow had primaries in breast (Figure 4), neuroblastoma & small blue round cell tumor.

92 cases were having good cellularity on BMA & BMB while 4 hypocellular in BMA, 5 hypocellular in BMB, while 25 cases with dry tap were excluded in the comparison. Reticulin stain for fibrosis was done in BMB samples, in which Myelofibrosis 2/2 cases (Figure 5), CML 2/10 case & hypoplastic marrow 7/16 cases, respectively showed grade 3 fibrosis (Graph 1).

BMA yield was dry tap in 25 cases which included Non hematological malignancies (6), Aplastic anemia (6), Hypoplastic marrow (5), ALL (4) cases, Multiple myeloma and Myelofibrosis 2 each, MDS (1) respectively. (Graph 2) These cases were later diagnosed by BMB.

Additional information such a tumor cell type focal granuloma (Figure 6) & pattern of involvement was better appreciated from BMB in metastatic cases, cellularity in case of hypoplastic marrow, plasma cell percentage & pattern of involvement in multiple myeloma, pattern of involvement of marrow in case of CLL, fibrosis in hairy cell leukemia (Table 2). Our diagnostic accuracy in BMA with BMB was 86%. The remaining 13% cases BMB was only helpful for diagnosis.

### Discussion

BMA and BMB are important diagnostic procedures for diagnosis of hematological, non-hematological malignancies and other diseases. These procedures are also

| TYPE OF CASES    | DIAGNOSIS ESTABLISHED BY | TOTAL |
|------------------|---------------------------|-------|
|                 | BMA+BMB (CONCORDANCE)     | BMB (DISCORDANCE) |
| IDA+MA          | 23 (100%)                 | 0     | 23 |
| AML             | 22 (100%)                 | 0     | 22 |
| CML             | 10 (100%)                 | 0     | 10 |
| CLL             | 6 (100%)                  | 0     | 6 |
| ITP             | 2 (100%)                  | 0     | 2 |
| HYPERSPLEENISM  | 2 (100%)                  | 0     | 2 |
| SA              | 2 (100%)                  | 0     | 2 |
| HPS             | 1 (100%)                  | 0     | 1 |
| HES             | 1 (100%)                  | 0     | 1 |
| HCL             | 0                         | 1 (100%) | 1 |
| MF              | 0                         | 2 (100%) | 2 |
| MDS             | 5 (83.3%)                 | 1 (16.7%) | 6 |
| MM              | 6 (75.0%)                 | 2 (25.0%) | 8 |
| ALL             | 8 (66.6%)                 | 4 (33.3%) | 12 |
| METASTATIC      | 6 (50.0%)                 | 6(50.0%) | 12 |
| HYPOPLASTIC     | 7 (43.0%)                 | 9(57.0%) | 16 |
| TOTAL           | 101 (80.1%)               | 25 (19.8%) | 126(100%) |
Table 2: Additional findings obtained from BMB.

| Type Of Cases | No. | Additional Findings                                      | No. Of Cases With Add. Findings |
|---------------|-----|----------------------------------------------------------|---------------------------------|
| AML           | 22  | Fibrosis & cellularity assessment                        | 5                               |
| IDA           | 14  | Nil                                                      | 0                               |
| ALL           | 12  | Cellularity                                              | 4                               |
| Metastatic    | 12  | Tumor cell type, focal granulomas, pattern of involvement| 12                              |
| CML           | 10  | Fibrosis                                                 | 10                              |
| Hypo plastic  | 16  | Cellularity (hot spot)                                   | 12                              |
| MA            | 8   | --                                                       | 0                               |
| MM            | 8   | Plasma cell %, Pattern of involvement                    | 3                               |
| CLL           | 6   | Pattern of involvement                                   | 6                               |
| MDS           | 6   | Blast %                                                  | 4                               |
| ITP           | 2   | Nil                                                      | 0                               |
| MF            | 2   | Fibrosis                                                 | 2                               |
| Hyperspleenism| 2   | Nil                                                      | 0                               |
| SA            | 2   | Iron stores                                              | 0                               |
| HES           | 1   | Eosinophil %                                             | 0                               |
| HCL           | 1   | Fibrosis & cell character                                | 0                               |
| HPS           | 1   | Nil                                                      | 0                               |

Table 3: Advantages and disadvantages of BMA & BMB

| ADVANTAGES AND DISADVANTAGES OF BMA & BMB | BMA | BMB |
|------------------------------------------|-----|-----|
| ADVANTAGES:                             |     |     |
| Comparatively easy to perform with less discomfort to the patient. |     |     |
| Features like cellularity and topographic relationship of different marrow elements is better appreciated. |     |     |
| Cellular morphology is better appreciated. |     |     |
| Multiple sections will be available for various ancillary stains and their comparative evaluation. |     |     |
| Suitable for Cytochemical stains.       |     |     |
| Suitable for ancillary studies like Immunohistochemistry. |     |     |
| Suitable for other ancillary studies like Flow cytometry, cytogenetics, Culture, etc. |     | --  |
| DISADVANTAGES:                          |     |     |
| Cellularity cannot be properly assessed. |     |     |
| More discomfort to the patient.         |     |     |
| Dry tap can occur.                      |     |     |
| Time consuming.                         |     |     |
| Possibility of missing focal diseases.  |     |     |
| Cellular morphology cannot be assessed. |     |     |

Graph 1: Grading of fibrosis
Graph 2: Causes of failure in BMA

Fig. 1a: AML M3- PS showing blast with auer rods (arrow); Figure 1b: BMA with blast showing auer rods and MPO positive; Figure 1c,d: BMB cellular with myeloblast and megakaryocyte. (MGG, x40; MPO, x40; H&E, x40).
Fig. 2a: ALL L1 · PS with lymphoblast and smudge cell; Figure 2b: BMA with lymphoblast which are positive for PAS satin (Figure 2c); Figure 32d: BMB with lymphoblast replacing marrow space. (MGG, x40; PAS, x40; H&E, x40).

Fig. 3a,b: CLL · PS & BMA cellular with blast negative for MPO (inset); Figure 3c: cell block with mature lymphoid cells; Figure 3d,e: BMB with mixed pattern of lymphoid infiltrate. (MGG, x40; H&E, x40).
Fig. 4a: Metastasis of Breast carcinoma to BM- PS showed leucoerythroblastic features; Figure 4b: BMA showed clusters of tumor cells; Figure 4c: Cell block with tumor cell and microfilaria; Figure 4d,e: BMB with tumor cell and increased fibrosis and IHC positive for Her-2/neu. (MGG, x40; H&E, Reticulin, IHC x40)

Fig. 5a: Myelofibrosis- PS with leucoerythroblastic features; Figure 5b: BMB showing atypical megakaryocytes with increased fibrosis (Figure 5c). (MGG, x40; H&E, Reticulin, x40).
valuable for follow up of patients undergoing chemotherapy, bone marrow transplantation and other forms of medical treatment.\textsuperscript{[2,4]} It is a well-known fact that bone marrow aspiration and bone marrow biopsy complement each other. Now-a-days both specimens are routinely obtained at the same time and usually same site.\textsuperscript{[4]} In our study, we did a comparative evaluation of all such BMA and BMB, to see the complementary role of both the procedures, to study the advantages and disadvantages of both the procedures done simultaneously (Table 3). Erythroid hyperplasia was the most common diagnosis in BMA accounting for a total of 23 cases (18.25\%) in our study. Similar incidence (14\%) was shown in a study by Khodke et al.\textsuperscript{[5]} Jha et al in their study showed an incidence of 19.6\%.\textsuperscript{[6]} These cases showing erythroid hyperplasia with either macronormoblastic or micronormoblastic proliferation were further worked up. Perl’s stain was done and the biochemical parameters were taken into consideration. The diagnosis was given either as iron deficiency anaemia or megaloblastic anaemia. But iron stained sections of BMB showed differences in iron content from that of BMA smears Ion deficiency anemia cases with Perl’s stain showed better results with aspirate (60\% grade 1) compared to biopsy (13.3\% grade 1). These observations were nearly similar to the findings seen in a study conducted by Ch Toi P et al.\textsuperscript{[2]} Stuart-Smith SE et al., have also shown in a study that aspirate smears reflect bone marrow iron stores more reliably than acid decalcified trephine biopsy sections.\textsuperscript{[7]} 75\% (6/8 cases) of multiple myeloma showed a positive correlation in BMA and BMB. Although it was not difficult to diagnose multiple myeloma in BMA alone, where the aspirate was good, there were cases where the plasma cells were scattered. In such cases BMB complemented the BMA, as it helps to identify compact masses of plasma cells with no stroma as observed by Sabarhwal et al.\textsuperscript{[8]} Our study correlated with the study done by Charles et al.\textsuperscript{[9]} where they detected myeloma in trephine biopsies and all simultaneous bone marrow aspirates.

30 cases (23.8\%) of acute leukemia; acute lymphoblastic leukemia and acute myeloid leukemia, were diagnosed in BMA in our study compared to 4 cases which were diagnosed on BMB. The predominant reason for not diagnosing acute leukemia on BMA in our case was dry tap either due to marrow fibrosis or tightly packed marrow by leukemic cells.

Other haematological malignancy in which we observed good concordance rates were CML 10 cases, 6 cases each of CLL and Multiple myeloma, 5 cases of MDS. Both bone marrow aspiration and trephine biopsy were complementary in all the other cases of malignancies diagnosed in our study. While aspiration smears were observed to be most effective
for studying cellular morphology, biopsy on the other hand, was helpful in assessing marrow cellularity, pattern of involvement by leukemic cells. Our findings are comparable to the study by James et al who observed that combined procedures of aspiration and biopsy gave a higher yield and are essential in patients with leukemia and lymphoma. 

Hence, it is important that both the aspirated and biopsy material should be examined together, since the two methods are often complementary. Also, trephine biopsy permits an accurate assessment of extent of infiltration and gives information of prognostic importance.

Our study had 2 cases, where BMA yielded dry tap was diagnosed as myelofibrosis on trephine biopsy. Trephine biopsy was also superior to BMA in diagnosing 6 cases of hypoplastic/a aplastic anemia similar to study by Gupta N et al. Sabharwal et al[18] included 7 cases (23.3%) of myelofibrosis which were diagnosed on trephine biopsy sections. Other cases of dry tap in our study were ALL, metastasis, aplastic anemia, hypoplastic marrow myeloma and HCL. In a study done by Humphries of 87 cases of dry tap on marrow aspiration, obtained trephine biopsies which showed significant pathology. Hence, the finding of a dry tap should never be dismissed as being due to faulty technique and always needs a bone marrow biopsy.

One case of tuberculosis was diagnosed by BMA, while 2 cases detected by biopsy. This could mainly be due to focal involvement of the marrow by granulomas which is very difficult to be detected on aspirate smears. Ch Toi P et al., have mentioned that 80% cases of granulomatous lesions were diagnosed by BMB alone.

There were four cases of NHL in the present study where BMB biopsy rendered information which cannot be determined from aspiration such as spatial distribution and extent of infiltrates, overall cellularity and fibrosis. This also implies that trephine biopsy may be more useful in post chemotherapy patients to assess the residual tumour cell burden and degree of chemotherapy response.

Microfilaria was identified in one case with 100% correlation between BMA and BMB. Similar finding was seen in a study done by Santra et al.

Hence, it was observed from the above discussion that bone marrow evaluation is an important cum effective tool in diagnosing and evaluating hematological and non-hematological disorders. Complete evaluation of bone marrow samples includes a brief patient history, hematological profile, marrow aspirate smears and biopsy sections. A correlation of bone marrow aspiration and biopsy showed that both the procedure were complementary to each other. The BMA generally provides an excellent cytomorphological details which enables hematopathologist in recognising the abnormal hematopoietic cells or the non-native cells in case of non-hematological disorders. Whereas, a bone marrow trephine biopsy demonstrates the topographic arrangement of hematopoietic cells within the marrow framework and hence gives a more representative view of the cellularity of the marrow and allows infiltration to be recognized clearly. BMB examination has definite edge over BMA in the detection of minimal residual diseases, staging of lymphoma and for the diagnosis of acute leukemia in relapse cases which are otherwise clinically silent.

Conclusion
The present study showed that BMA and BMB are easy, rapid, cost-effective and more or less are of equal value in various hematological and non-hematological disorders of bone marrow. The study concludes that bone marrow aspiration cytology and trephine biopsy complement each other and should be performed simultaneously for complete bone marrow workup and evaluation. Though cellular morphology is better understood in marrow aspirates and is equally effective to biopsy in diagnosing anemia and leukemia, it is the histopathological study of trephine biopsy of bone marrow that gives well preserved marrow architecture with its all cellular, stromal components, architectural patterns, grading of fibrosis, pattern of infiltration with lymphomas and granulomatous conditions. So, trephine biopsy becomes mandatory in the diagnosis of aplastic anemia, myelofibrosis and granulomatous involvement yielding dry aspirate on bone marrow aspiration. The evaluation of BMA & BMB comprises the complete work up for proper bone marrow interpretation and to reach final diagnosis.

Ethics Approval and Consent to Participate: All procedures performed in the study involved human participants were in accordance with the ethical standards of the institutional ethics committee.

Consent for Publication: Written consent was obtained from individual participants included in the study.

Availability of Data and Materials: All the data regarding the findings are available within the manuscript.

Authors’ Contributions: TS carried out concepts & design, literature search, participated in clinical study. MK carried out data acquisition, data analysis & manuscript preparation will stand as guarantor also. AB carried out concepts & design, literature search. SB participated in clinical study & manuscript review. BB carried out literature search & data acquisition. DM participated in clinical study & manuscript review. All the authors have...
read & approved the final manuscript.

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