DIAGNOSTIC UTILITY OF SIMULTANEOUS BONE MARROW ASPIRATION AND BONE MARROW BIOPSY: A RETROSPECTIVE STUDY

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Abstract

Background: Bone marrow examination remains a corner stone in the diagnosis of various hematological and non-hematological diseases. The correlation between bone marrow aspirations and bone marrow biopsies is important to determine the most diagnostic method in various hematological diseases. The final interpretation requires the integration of peripheral blood, bone marrow aspirate and biopsy findings, together with other ancillary tests such as immunophenotyping, cytogenetic and molecular genetics’ results in the context of clinical picture which can lead to a definitive diagnosis. The aim and objective are to compare the diagnostic utility of simultaneous BMA and BMB for various haematological indications, to study the sensitivity and specificity of BMA as compared to BMB, to find out the concordance between BMA and BMB.

Materials and Methods: This is a retrospective observational study that was performed in the Department of Pathology, Saifee Hospital, Mumbai, to find out the diagnostic utility of BMA and BMB. All the cases where simultaneous bone marrow aspiration and bone marrow trephine biopsy were performed. The study duration was two years, and a total of 118 cases were studied.

Result: A total of 118 cases of simultaneously done bone marrow aspirates and bone marrow biopsies were studied. The age range of the study group was between 2 months and 90 years. Most of cases were of the age between 41 to 60 years. The male to female ratio was 1.51:1. The most common indication for bone marrow examination in our study was anemia (28.8%). 18.6% of the cases were known leukemias for follow up. Out of 118 BMA, 19 (16.1%) were reported as normal. 16 were reported as acute leukemias, including relapse of known cases. 15(12.7%) were reported as plasma cell dyscrasias which included multiple myeloma. 12(10.2%) aspirates were dilute and hence not of any diagnostic help. 17(14.4%) out of 118 BMB were reported as leukemias which included newly diagnosed cases as well as k/c/o leukemias which showed relapse. 16(13.6%) of the cases showed hypoplastic marrow. Single cases each of agranulocytosis, erythroid hyperplasia, essential thrombocythemia, Hemophagocytosis, and storage disorder were reported.

Conclusion: Bone marrow examination is a valuable investigation in hematology practice. BMA and BMB both are important procedures for the diagnosis of hematological and non-hematological conditions. These procedures are also useful for follow up of the patients undergoing chemotherapy. The present study showed higher sensitivity of BMA for Acute leukemia, megaloblastic anemia, PCD and hyperplastic marrows and lower sensitivity for CLPD, hypoplastic marrow and reactive marrow. Higher concordance of BMA and BMA was found in megaloblastic anemia, acute leukemia and PCD and lower concordance was seen in hypoplastic marrow, CLPD, hyperplastic marrow and reactive marrow.
INTRODUCTION

Bone marrow is the principal site of blood cell formation. Bone marrow examination is an important diagnostic procedure for establishing diagnosis of various hematological and non-hematological diseases. It also forms an important prerequisite for follow-up of patients undergoing chemotherapy, bone marrow transplantation and other modalities of medical treatment. Bone marrow examination includes the process of obtaining the soft liquid tissue (aspirate) and solid trephine piece (biopsy) of bone marrow for laboratory analysis and diagnosis. Ideally, a bone marrow examination encompasses examination of bone marrow histology, imprint cytology, bone marrow aspirate (BMA) along with a review of peripheral smear.

There are various indications for BMA and bone marrow biopsy (BMB). Aspiration is particularly useful, and may well be performed alone, when investigating patients with suspected iron deficiency anemia, anemia of chronic disease, megaloblastic anemia and acute leukemia. Aspiration of the marrow is primarily utilized for cytological assessment with analysis directed towards morphology and obtaining a differential cell count. Aspirate can also be useful for additional flow cytometric, immunophenotyping, cytogenetic and molecular studies. Trephine biopsy provides more comprehensive information regarding the marrow cellularity, architectural patterns and overall hematopoiesis. Biopsy is essential for diagnosis in a dry tap or bloody tap which occurs when the marrow is fibrotic or densely cellular as in cases of suspected aplastic or hypoplastic anemia, lymphoma, metastatic carcinoma, myeloproliferative neoplasms and diseases of the bones. Few studies have analyzed the concordance of bone marrow aspirate with trephine biopsy. This study was conducted to study the concordance as well as sensitivity and specificity of bone marrow aspirations as compared with trephine biopsies done simultaneously.

Aims and Objectives
1. To compare the diagnostic utility of simultaneous BMA and BMB for various hematological indications.
2. To study the sensitivity and specificity of BMA as compared to BMB.
3. To find out the concordance between BMA and BMB.

MATERIALS AND METHODS

Study Population: All the cases during a span of two years wherein simultaneous BMA and BMB were performed were studied.

Study Design: This study is a retrospective observational study which was done to find out the diagnostic utility of BMA and BMB.

Sample size with justification: All the cases where simultaneous bone marrow aspiration and bone marrow trephine biopsy were performed. The study duration was two years, and a total of 118 cases were studied. Sample size has been calculated using “Period sampling technique”.

Time frame- Two years.

Inclusion Criteria
- All the cases where simultaneous bone marrow aspiration and bone marrow biopsy was performed in the department of Pathology were included in the study.
- The BMA slides and BMB slides or paraffin blocks of the case should be available with the hospital.

Exclusion Criteria
- Cases where only bone marrow aspiration or only bone marrow trephine biopsy was performed were not included.
- Referred slides and blocks were not included.
- Cases where study slides were missing were not included.

Methodology

The Clinical information was obtained from electronic medical record system and studied. Leishman-stained slides of bone marrow aspirations and hematoxylin and eosin-stained slides of bone marrow biopsies were reviewed. Special stains like Reticulin and Prussian blue and immunohistochemistry were studied whenever necessary. Wherever indicated, histochemistry was performed. Gómöri’s reticulin was performed to grade marrow fibrosis. In cases where tuberculosis was suspected, Ziehl Neelsen was performed to stain for acid fast bacilli (AFB). PAS was done to look for glycogen and fungal hyphae.

Statistical Analysis

In groups with sufficient sample size, Cross tables were made and kappa agreement values were calculated. Diagnostic test properties such as sensitivity, specificity, positive predictive value and negative predictive value were calculated. Concordance rates were calculated.

RESULTS

A total of 118 cases of simultaneously done bone marrow aspirations and bone marrow biopsies were studied. The age range of the study group was between 2 months and 90 years. Most of cases were of the age between 41 to 60 years. The male to female ratio was 1.51:1. The most common indication for bone marrow examination in our study was anemia (28.8%). 18.6 % of the cases were known leukemias for follow up. Out of 118 BMA, 19 (16.1%) were reported as normal. 16 were reported as acute leukemias, including relapse of known cases. 15(12.7%) were reported as plasma cell dyscrasias which included multiple myeloma. 12(10.2%) aspirates were dilute and hence not of any diagnostic help. 17(14.4%) out of 118 BMB were reported as leukemias which included newly diagnosed cases as...
well as k/c/o leukemias which showed relapse. 16(13.6%) of the cases showed hypoplastic marrow. Single cases each of agranulocytosis, erythroid hyperplasia, essential thrombocythemia, Hemophagocytosis, and storage disorder were reported.

Normal marrow- All the 12 BMB reported as normal were also reported as normal on BMA. In addition 7 BMA were falsely reported as normal. These were reported as hyperplastic, reactive, granuloma and osteomyelosclerosis on BMB.

Validity parameters for acute leukemia

Acute leukemias accounted for 14.4% (17/118) of all BMB. Of these 11 were newly diagnosed and 6 were relapse of known cases. BMA was in agreement with biopsy in 16 cases but was inadequate in a k/c/o leukemia which had a dilute aspirate. Degree of agreement for BMA and BMB for leukemia was 96.5%. Sensitivity and specificity of BMA was 94.12% and 100% respectively.

Validity parameters for Plasma cell Dyscrasias

Plasma cell dyscrasias were diagnosed in 11.9 % of BMB. On immunohistochemistry, these cells showed evidence of monoclonality by κ or λ light chain restriction BMA was able to diagnose 13 out of those reported on BMB and one was dilute aspirate due to fibrosis with focal aggregation of myeloma cells on biopsy sections. In addition to 14 cases diagnosed on BMB, additional case was reported as MM on BMA which was reported as reactive on BMB. Degree of agreement of biopsy and aspirate was 91.9% in patients of multiple myeloma. Sensitivity and specificity of BMA was 92.86% and 99.0 % respectively.

Validity parameters for chronic myeloproliferative disorders

7.9 % (9/114) of the BMB were reported as CMPD. This included 5 cases of CML and 3 cases of myelofibrosis. 6 out of 9 cases showed similar findings on BMA. 2 CML cases were reported as chronic phase. 3 cases of myelofibrosis had cellular marrow with grade 3 reticulin fibrosis and collagenization. Out of these, BMA showed 1 hypoplastic marrow. 1 normal marrow and a dry tap after repeated attempts of aspiration. Degree of agreement of biopsy and aspirate was 78.7% in patients of CMPD. Sensitivity and specificity of BMA was 66.67% and 100% respectively.

Validity parameters for chronic lymphoproliferative disorders

Our study had 9 cases of CLPD including 5 cases of NHL, 1 case of marrow involved by NHL, 1 case of marrow involved by HL, 1 CLL and 1 CLPD. Aspirates were diagnostic in only 3 of the cases. Following table shows the findings of non-diagnostic BMA in cases of CLPD. Degree of agreement of BMA and BMB was 47.9% for CLPD. Sensitivity and specificity of BMA was 33.3% and 100 % respectively.
Validity parameters for myelodysplastic syndromes

In the present study 7 (61.4%) cases were reported as MDS on BMB. Of these, BMA was in agreement in 5 cases. Remaining 2 were reported as erythroid dysplasia and megaloblastic anemia. Degree of agreement of BMA and BMB was 82.4%. Sensitivity and specificity of BMA was 71.43% and 100% respectively.

Validity parameters for Megaloblastic anemia - 3 cases were diagnosed as megaloblastic anemia on BMB. All of these were also reported the same on BMA. In addition, 1 case was reported as megaloblastic on BMA but was not appreciated on BMB. Degree of agreement of BMA and BMB was 85.3% for megaloblastic anemia. Sensitivity and specificity were 100% and 99.10% respectively.

Validity parameters for hyperplastic marrow - 6 cases were reported as hyperplastic marrow on BMB. Of these, only 2 were reported the same on BMA. Remaining 4 cases were reported as normal on BMA. Degree of agreement of BMB and BMA was 48.6% for hyperplastic marrow. Sensitivity and specificity were 92.86% and 99.0% respectively.

Validity parameters for hypoplastic marrow - 13 cases (11.40%) were reported on BMB as hypoplastic marrow. Of these, 5 were reported the same on BMA. Rest of the BMA were mostly reported as dilute marrow. Degree of agreement of BMA and BMB was 39.8% for hypoplastic marrow. Sensitivity and specificity were 38.46% and 96.04% respectively.

Validity parameters for reactive marrow - 7 BMB were reported as reactive. Of these, 4 were reported as reactive on BMA. Rest of the 3 BMA were reported as hyperplastic, normal and multiple myeloma. In addition, 2 BMA were reported as reactive on BMA, which were later reported on BMB as hyperplastic and granuloma. Degree of agreement of BMA and BMB was 59.2% for reactive marrow. Sensitivity and specificity were 57.14% and 98.13% respectively.

Granuloma-3 cases were reported as granuloma in BMB only. BMA were not contributory in any of the cases.

Hemophagocytosis - 1 case of Hemophagocytosis was reported on BMA and BMB. In addition, 2 cases were reported on BMA only.

Others - 1 case each of essential thrombocythemia, agranulocytosis and storage disorder and 2 cases of pure red cell aplasia were simultaneously reported on BMA and BMB. There were 6 known cases of leukemias and lymphomas which showed no involvement on biopsy and aspiration. 1 case of Burkitt’s lymphoma was uninvolved on biopsy, but aspiration was dilute and inconclusive. 1 case of NHL was reported as involved on BMB but uninvolved on BMA.

DISCUSSION

In this study there was a 71.93% concordance between aspirates and biopsies. This is similar to the study published in Pakistan by Khan et al. where positive correlation was 73.8%, as well as a study done in India by Chandra et al where the correlation was 78%. The highest concordance was noted in megaloblastic anemia (100%) as also observed by Ghodasara and Goyal (96%). High concordance was also seen in Agranulocytosis (100%), Erythroid hyperplasia (100%), essential thrombocythemia (100%), Hemophagocytosis (100%), pure red cell aplasia (100%), storage disorder (100%), acute leukemia (94.12%) and Plasma cell dyscrasias (92.86%). Low concordance was seen in chronic lymphoproliferative disorders (33.33%) and hyperplastic marrow (33.33%). Similar findings were observed by Ghodasara, Khan TA and Goyal S. Aspirate has no role in granulomatous disorders and myelofibrosis. Least concordance was observed in granuloma (0%) and myelofibrosis (0%). Tripathy S observed least concordance for lymphoproliferative disorders, myelofibrosis and marrow hypoplasia which is correlating with our findings.

Normal marrow: 12 cases were reported to have normal bone marrow biopsy. All of them have been proven to be normal on aspirate. In addition, 7 cases were reported as normal on aspirate. Of these, 4 were hyperplastic and 1 each was reactive, granuloma and osteomyelosclerosis. This again proves poor diagnostic efficacy of aspirate for granuloma and myelofibrosis. In our study there was 63.16% (12/19) correlation among BMA and BMB for normal marrow. These findings were comparable with the Chandra et al and Aljadayeh MH et al where the correlated normality between aspiration and biopsy was 72.5% and 69.1% respectively.

Inadequate aspirates: 13 cases in our study were undiagnosed by bone marrow aspiration. Of these, 12 (10.2%) were hemodiluted and inadequate samples, 1 (0.85%) was dry aspirate. This is lesser compared to Humphries et al who has reported the frequency of dry aspirate to be 3.9%. 4 (3.39%) biopsies were not diagnostic. Ghodasara J reported 4% aspirates and biopsies to be inadequate. Rehman et al.
reported inadequate specimens in 2007, 2008, and 2009 to be 4.9%, 10.5% and 3.3% respectively. The present study shows the diagnostic efficacy of both bone marrow aspirate and biopsy to be 71.93% and 96.61% respectively. Khan et al have reported the diagnostic efficacy of 73.8% for bone marrow aspiration, and 99% for biopsy, Chandra et al. have reported them to be 77.5% and 99.2% respectively.

**Megaloblastic anemia:** Our study consisted of 3 cases of megaloblastic anemia. All of them were diagnosed on BMA and BMB. In addition, 1 case was reported as megaloblastic anemia on BMA, which was reported as MDS on BMB. We found 100% concordance for our study. Aljadayeh MH et al, reported 89.7% concordance. Ghodasara et al. observed highest correlation for megaloblastic anemia. Most of his cases of megaloblastic anemia were diagnosed on BMA only with trephine biopsy being reported as hypercellular leading to the low concordance.

**Acute leukemia:** We had 17 cases of acute leukemia, all of which were detected on trephine biopsy. Aspiration was diagnostic in only 16 of the cases, as the BMA in the k/c/o ALL was diluted with peripheral blood, hence no opinion could be given. Concordance was 96.5%. Our results were similar to the study done at the King Hussein Medical Center which showed a concordance of 91.1% (92.8% for AML, 89.5% for ALL) for the same. Pampa Ch Toi62 reported a positive correlation of 62.5% in leukemias. Younus U 12 and associates emphasized that although BMA confirms the diagnosis of acute leukemia, bone marrow biopsy specimen complements the peripheral blood and aspirate findings in providing additional information for the diagnosis and especially prognosis of acute leukemia. IHC can be used on biopsy for subtyping of leukemias. For example pan-myeoloid markers are CD13, CD33, CD65, CD117 and anti – myeloperoxidase. Markers of monocyctic differentiation are CD14, CD11b, CD64. Megakaryocytic markers are CD41, CD42a, CD42b and CD61.

**Plasma cell dyscrasias:** There were 14 cases of Plasma cell dyscrasias, all of which could be diagnosed on BMB. Though it was not difficult to diagnose multiple myeloma in BMA alone where the aspirate was good, there was a case where the plasma cells were scattered and diagnostically difficult. Sabharwal et al, found that in cases where BMA was inconclusive for multiple myeloma, BMB complemented BMA as it helped to identify compact masses of plasma cells with no stroma. This was seen in 1 of our 14 cases and it was a significant histological feature for differentiation between myelomatous and non-myelomatous plasmacytosis and the role of BMB proved invaluable for this. Biopsy is more sensitive method for quantifying plasma cell burden. IHC can be performed on biopsy using CD138, Kappa & Lambda antibodies especially in patients with low percentage of plasma cells on aspirate. Concordance in our study was 91.9%. Similar study conducted by Goyal et al found concordance of 88.5% in these cases. Pampa Ch Toi, reported 88.8% positive correlation in BMA and BMB among multiple myeloma.

**Chronic myeloproliferative disorders:** There were 9 cases of CMPD, 6 of which could be diagnosed on aspirates. All the cases of CML were diagnosed on aspirates. All the cases of myelofibrosis were diagnosed on biopsy alone. Fibrotic marrow prevented adequate aspirates. They were reported either as normal or hypoplastic marrow. This might be due to variability of cellularity from one inter trabecular space to the next. Thus, the use of the biopsy avoids misinterpretation of cellularity by smears. One case was a dry tap, aspiration being not possible. An important limitation of bone marrow obtained by aspirate is the admixing of marrow and sinusoidal blood, which may not allow for reliable estimates of marrow cellularity. Also, it is necessary that finding of a ‘dry tap’ should never be dismissed as being due to faulty technique and always needs a bone marrow biopsy for further evaluation. Role of trephine biopsy is not only in differentiation of MPN but also to assess the overall marrow cellularity and morphology of megakaryocytes as well as blasts, and degree of fibrosis. BMA does not have much role in diagnosis of primary myelofibrosis because diffuse osteomyelosclerosis, intrasinusoidal hematomiesis and vascular proliferation which is characteristic of primary myelofibrosis, can be confirmed and graded on BMB only. Concordance in our study was 78.7% for BMA and BMB. Similar study by Goyal, showed concordance of 76.7% for CML chronic phase with grade 2 fibrosis. Aljadayeh MD reported concordance of 90.9% for CML and 0% for myelofibrosis.

**Chronic lymphoproliferative disorders:** Our study included 9 cases of CLPD, of which only 3 were diagnosed on aspirate. There were 2 post chemotherapy cases of lymphoma which showed involvement on BMB but not on BMA. This also implies that trephine biopsy may be more useful in post chemotherapy patients to assess the residual tumor cell burden and degree of chemotherapy response. There was one case of CLL with focal involvement of marrow, which was seen on biopsy section while aspiration showed only that marrow is hypoplastic. Bone marrow examination in cases of CLL should always include a trephine biopsy because bone marrow aspirate gives very little information beyond that already available from examination of blood. Pattern of marrow involvement by leukemic cells could only be analyzed by trephine biopsy. Also, trephine biopsy permits an accurate assessment of extent of infiltration and gives information of prognostic importance. Concordance for BMA and BMB in our study was 47.9%. Ghodasara reported all the cases to be concordant on BMA and BMB. Khan et al reported concordance of 92.8% for CLL and 16.6% for lymphoma. 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

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Myelodysplastic syndrome: In our study 5 cases were simultaneously reported as MDS on BMA and BMB. 2 cases reported as MDS on BMB were reported on BMA as erythroid dysplasia and megaloblastic anemia. Trephine biopsies proved better than bone marrow aspiration in the diagnosis of Myelodysplastic syndrome since it showed the presence of aggregates of immature myeloid precursor cells. Orazi A,[19] in his study concluded that myelodysplastic/myeloproliferative disorders can only be accurately categorized by a careful multiparametric approach in which the bone marrow biopsy exerts a pivotal role. Features of abnormal erythropoiesis and myelopoiesis were easily detected on aspirate smears as was also observed by Sabharwal et al.[13] Concordance of BMA and BMB was 71.43% in our study. Similar study by Khan et al,[6] showed 80% concordance for MDS.

Hyperplastic marrow: 6 cases were reported as hyperplastic marrow on BMB. Of these, only 2 were reported the same on BMA. Remaining 4 cases were reported as normal on BMA. Trephine biopsies are more useful in assessing the cellularity. Concordance of BMA and BMB in our study was 33.33%. Shilpa et al reported more than 90% concordance for hyperplastic marrow.[20]

Hypoplastic marrow: 13 cases were reported on BMB as hypoplastic marrow. Of these, 5 were reported the same on BMA. Rest of the BMA were mostly reported as dilute marrow due to admixture with sinusoidal blood. Concordance of BMA and BMB in our study was 38.46%. Ghodasara et al.[4] observed that bone marrow hypoplasia could only be diagnosed on biopsy. Low concordance was observed for the same. Pampa Ch Toi,[15] in his study has observed that in some cases where aspiration was done prior to core biopsy, cellular aspirates showed hypocellular picture on BMB perhaps due to the BMB being done from the same area. When both the procedures are done at the same time, he advised to use the two-needle technique, change the position of the needle after one procedure to an adjacent site in order to get maximum material.

Granuloma: 3 cases were reported as granuloma in BMB only. BMA was not contributory in any of these cases. They were reported as normal, dilute and reactive marrows. Concordance in our study was 0%. Ghodasara et al found that 75% of granulomatous lesions in bone marrow were diagnosed by BMB alone. Aspirates were diagnostic in only 25% cases. BMB had a higher yield in detecting granulomas as compared to BMA, which is consistent with studies done by Basu et al. and Frisch et al.,[21,22] Pampa Ch Toi.[15] In his study found that 80% cases (8/10) of granulomatous lesion in the bone marrow were diagnosed by BMB alone. He concluded that BMB is a better procedure for detecting granulomas in the marrow.

Marrow involvement by leukemia and lymphoma: The known 22 cases of leukemia/lymphoma were a part of our study. These included 10 ALL, 3 AML, 3 NHL, 3 HL, 1 CML, 1 Burkitt’s lymphoma and 1 CLL. 8 out of 22 BMB were involved by leukemia/lymphoma. Of these 5 showed involvement on BMA, rest showing dilute, hypoplastic or uninvolved marrow.[7] Cases of lymphomas were present in the study – 3 NHL, 3 HL and 1 Burkitt’s lymphoma. Of these, 2 showed involvement on BMB and BMA were not contributory. BMA failure could be due to focal involvement by lymphoma or inadequate aspiration due to fibrosis. Our study showed lower concordance than Gupta N,[23] and Sabharwal et al.[13] who reported a 100% positive result for both aspirate smears and biopsy sections in lymphomas. Khan et al[6] reported 16.6% concordance of BMA and BMB for lymphomas.[1] Case of CLL was wrongly reported as lymphocytosis on BMA, which showed hypoplastic marrow on BMB. Also, various investigators have studied the diagnostic value of immunohistochemistry suitable for use on paraffin wax embedded sections in the Diagnosis of Acute Leukemia in Sections from Bone Marrow Biopsy Specimens.[24]

CONCLUSION

- Bone marrow examination is a valuable investigation in hematology practice. BMA and BMB both are important procedures for the diagnosis of hematological and non-hematological conditions. These procedures are also useful for follow up of the patient’s undergoing chemotherapy.
- The utility of BMA as compared to BMB have been discussed and debated. The answer, though complicated remains essentially the same. Both procedures complement each other with aspiration smears being primarily used for cytological diagnosis and trephine biopsies helpful for histological diagnosis as cellularity, fibrosis and architectural patterns are better visualized.
- Our study showed higher sensitivity of BMA for Acute leukemia, megaloblastic anemia, PCDA and hyperplastic marrows and lower sensitivity for CLPD, hypoplastic marrow and reactive marrow.
- Higher concordance of BMA and BMA was found in megaloblastic anemia, acute leukemia and PCDA and lower concordance was seen in hypoplastic marrow, CLPD, hyperplastic marrow and reactive marrow.
- Despite the growing complexity and dependence on newer methodologies and ancillary assays including immunohistochemistry, cytotogenetic analysis, flow cytometry and molecular assays, the traditional role of examination of BMA and histopathological evaluation of BMB remain the basic investigations to commence the detailed evaluation of the patient.
Both of the procedures should be done simultaneously as they play an important role in primary assessment and are mandatory for final diagnosis.

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