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# Utility of simultaneous assessment of bone marrow aspirates and trephine biopsy sections in various haematological disorders

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## Abstract:

**BACKGROUND:** Bone marrow examination is crucial diagnostic modality for evaluation of various hematological and nonhematological disorders. However, marrow aspirate smears and biopsy sections, even though performed simultaneously, are often assessed at different points of time due to different processing methods. This sometimes results in discordance in diagnosis which adds to the diagnostic dilemma and delays the treatment.

**AIM:** This study aims to compare the diagnostic accuracy and the rate of concordance between the two modalities of bone marrow examination.

**MATERIALS AND METHODS:** Three hundred simultaneously performed bone marrow aspirates and BM trephine biopsies were retrospectively analyzed over a period of 1 year. The presence or absence of concordance was recorded. The reasons for inconclusive reports were also recorded. The concordance rates for different hematological disorders were calculated and recorded as high for >80%, moderate for 50%–80%, and low for <50%. The findings of discordant cases and reasons for discordance were also tabulated.

**RESULTS:** A high concordance was found in cases of megaloblastic anemia, leukemias, non-Hodgkin's lymphoma, and multiple myeloma; moderate concordance was found in hypoplastic marrow and concordance was low in Hodgkin's lymphoma, chronic myeloid leukemia (CML) in blast phase, metastatic, and granulomatous involvement of bone marrow.

**CONCLUSION:** Bone marrow aspiration alone is sufficient for the diagnosis of megaloblastic anemia and most of the hematological malignancies. Bone marrow biopsy is more appropriate for detection of disorders with focal marrow involvement such as lymphoproliferative disorders, metastatic cancer, focal blast crisis in CML, granulomatous lesions, and hypoplastic marrow. However, it is strongly recommended that both should be reviewed simultaneously to ensure maximum diagnostic accuracy.

## Keywords:

Aspirate, biopsy, bone marrow, concordance

## Introduction

Bone marrow examination is crucial diagnostic modality for evaluation of various hematological and nonhematological disorders. It is an indispensable prognostic modality and an essential component of the follow-up schedule of patients undergoing chemotherapy or bone

marrow transplantation. There are three basic preparation of bone marrow (BM) examination: BM aspiration (BMA) cytology, BM touch imprint cytology, and BM trephine biopsy. While BMA is a simple and rapid technique of marrow evaluation allowing excellent visualization of cell morphology, trephine biopsy provides a more comprehensive information about the marrow cellularity, trilineage hematopoiesis,

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and architectural pattern of infiltrative marrow diseases. The diagnostic accuracy of the two diagnostic modalities differs for different conditions. Simultaneous assessment of marrow aspirate and biopsy allows a more detailed marrow assessment which may be impossible to achieve with the use of any one approach alone.

However, marrow aspirate smears and biopsy sections, even though performed simultaneously, are often assessed at different points of time. Processing of BM biopsy (BMB) is a lengthy and tedious procedure, while aspiration is a rapid technique of marrow evaluation. The hematopathologist often views the aspirate in isolation from the biopsy as it is processed rapidly. The marrow sections are received in the Department of Histopathology and likewise reported by a trained histopathologist who may not have a steady access to the aspirate smears. Most of the time, the results are concordant, but discordant results can occur. This discordance leads to perplexity about the diagnosis and delays treatment.

We attempted to compare the diagnostic accuracy and determine the rate of concordance between the two modalities to ascertain whether simultaneous assessment of marrow aspirates and biopsy sections serves as a better approach to solve the diagnostic dilemma and improves the diagnostic accuracy or not.

## Materials and Methods

Three hundred simultaneously performed BMA and BM trephine biopsies reported at our institution over a period of 1 year, i.e., July 2013 to September 2014 were retrospectively analyzed. Bone marrow examination was carried out for a variety of indications and patients belonged to a wide age range from 1 to 65 years [Figure 1]. Relevant clinical details and results of previous investigations such as complete blood count, peripheral blood smear, reticulocyte count, and coagulation profile were also reviewed.

BMA and BMB had been performed simultaneously for all the patients. BMA had been done using Salah's marrow puncture needle and 20 ml syringe from PSIS. The aspirated material was smeared on clean glass slides. Thereafter, trephine biopsy was taken using Jamshidi's needle through the same incision. The biopsy was taken approximately 0.5–1 cm away from site of aspiration to avoid hemorrhagic biopsy. A peripheral blood film was also prepared simultaneously. PBS and BMA were stained with Wright's stain.

The trephine biopsies were fixed in 10% neutral buffered formalin and were subjected to decalcification in 5.5% ethylenediaminetetraacetic acid solution for

72 h. Thereafter, the length of biopsy was noted and it was subjected to the routine processing schedule in automated tissue processor and then embedded in paraffin. 2–3 µm thick sections were cut and stained with hematoxylin and eosin. Histochemical stains were done on additional sections wherever indicated. Reticulin and Masson's trichrome stains were performed to grade bone marrow fibrosis. Immunohistochemistry was done by standard streptavidin biotinylated peroxidase method.

The bone marrow smears had been seen and reported by hematopathologist while the biopsy had been reported by histopathologist. Thus, both the pathologists were blinded to each other's reports. The reports of both aspirate and biopsy for each case were reviewed and compared. The presence or absence of concordance was recorded. The reasons for inconclusive reports were also recorded. The concordance rates for different hematological disorders were calculated and recorded as high for >80%, moderate for 50%–80%, and low for <50%. The findings of discordant cases and reasons for discordance were tabulated.

## Results

Out of the total 300 cases analyzed retrospectively, 60 bone marrow biopsies were found to be inadequate for opinion. Out of these, 37 were of insufficient length, 14 comprised of only cartilage and bone while 7 showed only the presence of blood clot.

A total of 240 BMB were evaluated for concordance with BMA. Concordant results were found in 198/240 cases resulting in an overall concordance of 77.1%. The distribution of diagnoses of different cases is shown in Figure 1.

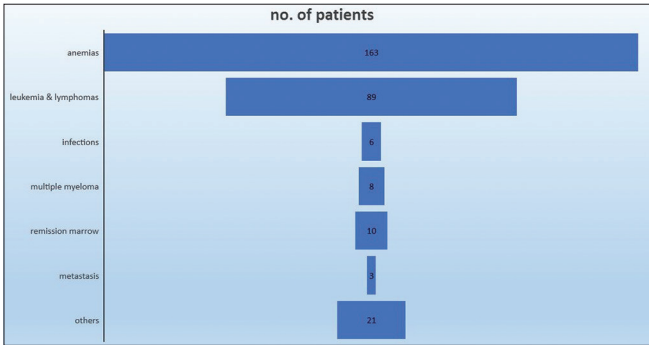
Concordance rates for different hematological disorders are shown in Table 1. Figure 2 shows a comparison of the concordance rates.

A high concordance was found in cases of megaloblastic anemia with 38/44 (86.3%) cases showing concordant results. In the six discordant cases, marrow aspirate showed erythroid hyperplasia alone in 4 cases and was diluted in remaining 2 cases. High concordance was observed also in cases of acute leukemia (88%) of which 25 cases were analyzed and aspirate correlated with biopsy in 22 cases [Figure 3] while it was diluted with blood in the remaining 3 cases. There was a concordance of 92.3% (12/13) among cases of chronic myeloid leukemia chronic phase (CMLCP) and a concordance of 100% in cases of chronic lymphoproliferative disorder [Figure 4]. One case of CML-CP showed myelofibrosis on trephine section and so resulted in dry tap and inadequate marrow aspirate.

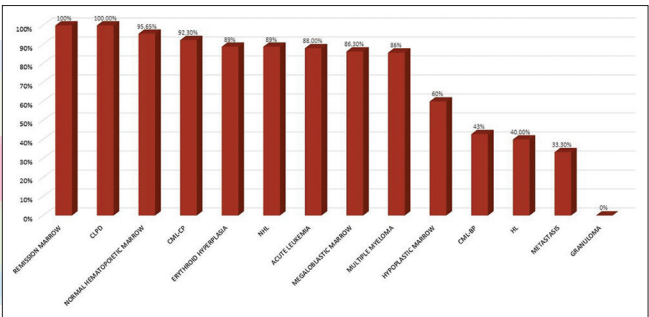
**Table 1: Concordance rates for different hematological disorders**

Diagnosis	Total number of cases	Inadequate	Adequate with findings	Aspirate consistent	Aspirate inconsistent	Concordance rate (%)
Normal hematopoietic marrow	56	10	46	44	2	95.65
Erythroid hyperplasia	25	7	18	16	2	88.80
Megaloblastic marrow	49	5	44	38	6	86.30
Micronormoblastic marrow	5	2	3	2	1	66.60*
Hypocellular marrow	28	8	20	12	8	60
Acute leukemia	27	2	25	22	3	88
CML-CP	13	0	13	12	1	92.30
CML-BP	7	0	7	3	4	42.80*
CLPD	4	0	4	4	0	100*
NHL	11	2	9	8	1	88.80
HL	27	2	25	10	15	40
Granuloma	6	0	6	0	6	0*
MM	8	1	7	6	1	85.70
Metastasis	3	0	3	1	2	33.30*
Remission-acute leukemia	4	0	4	4	0	100*
Remission-CML	3	0	3	3	0	100*
Remission-multiple myeloma	3	0	3	3	0	100*
Inconclusive	21					

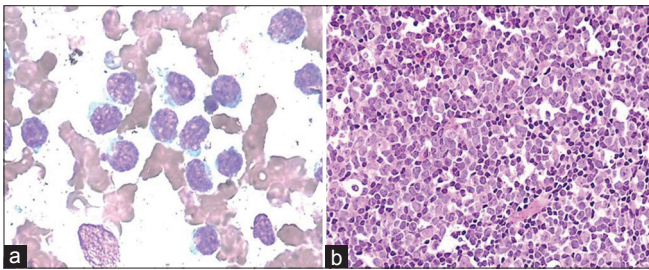
\*Due to small number of cases, exact concordance rate is unknown. CML-BP=Chronic myelogenous leukemia blast phase, CML-CP=Chronic myelogenous leukemia chronic phase, CLPD=Chronic lymphoproliferative disorders, NHL=Non-Hodgkin's lymphoma, HL=Hodgkin's lymphoma, MM=Multiple myeloma



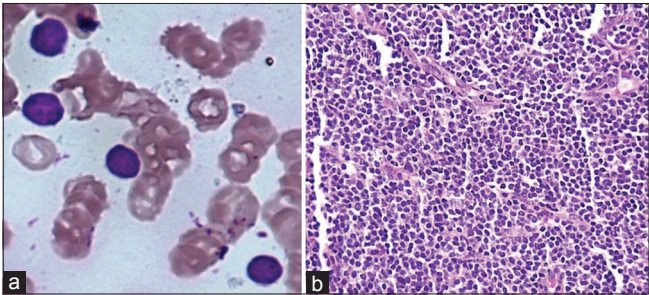
**Figure 1:** Various indications for bone marrow examination



**Figure 2:** Concordance rates for different hematological disorders



**Figure 3:** High magnification photomicrograph of (a) bone marrow aspiration and (b) bone marrow biopsy of a case of acute leukemia



**Figure 4:** Bone marrow aspiration smear (a) and trephine biopsy section (b) of a case of chronic lymphoproliferative disorder

Cases of non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM) also showed high concordance of 88.9% (8/9) and 85.7% (6/7), respectively. The reason for discordance is focal deposits in BMB in a case of NHL [Figure 5].

In cases of hypoplastic marrow, the BM aspirates were concordant with trephine biopsy in 60% (12/20)

of the cases [Figure 6]. Aspirates were reported as normocellular marrow in 7 cases due to the presence of focal hypercellularity.

The concordance rate was observed to be low for cases of CML in blast phase which was diagnosed in 7 cases on BM trephine biopsy while aspirates showed concordant findings in only 3 of these cases resulting in a low



concordance rate of 42.8%. Two of the aspirates were reported as CML-CP while remaining two aspirates were diluted due to the presence of marrow fibrosis causing dry tap.

Concordance rates were low also in cases of Hodgkin's lymphoma [Figure 7] where aspirates correlated with biopsy in only 10 of the 25 cases resulting in a low concordance rate of 40%. Reasons for discordance were recorded as focal infiltration of bone marrow by lymphoma and inadequate bone marrow sampling.

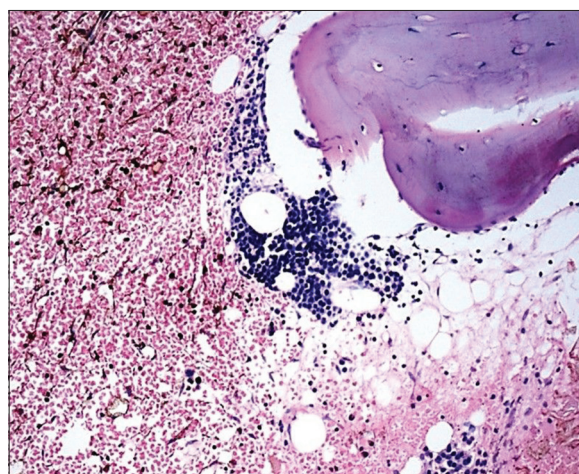
Metastatic involvement of bone marrow was diagnosed in 3 cases on BMB [Figure 8]. However, only one BM aspirate showed metastatic deposits producing a concordance of only 33.3%. Focal involvement of BM by metastasis resulted in low concordance.

Six bone marrow trephine biopsies showed granulomatous involvement of BM while none of the simultaneously performed marrow aspirates showed

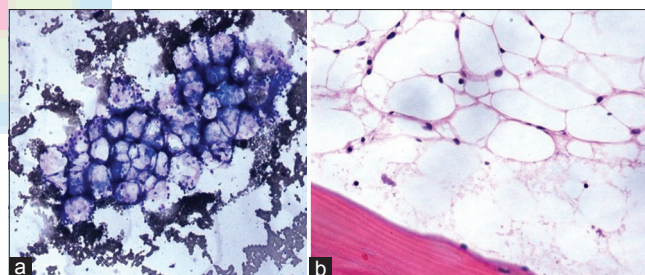
the presence of granulomas [Figure 9]. Hence, in cases of granulomatous myelitis, concordance rate was observed to be 0%.

## Discussion

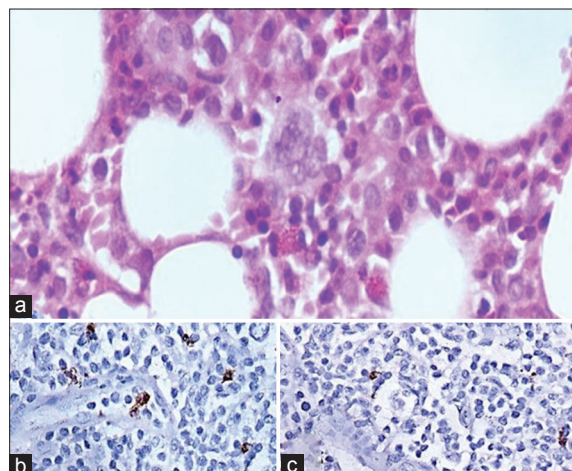
A comparative evaluation of simultaneously performed but separately reported BMA and BMB for various indications showed a positive correlation in 77.1% of the cases. The results obtained are similar to those of other comparable studies which showed concordance rates varying from 61.25% to 80.5%.<sup>[1-3]</sup> However, the sensitivity of BMA as a diagnostic procedure depends on the disease being evaluated. Diseases with diffuse involvement of BM are diagnosed with adequate sensitivity by BMA alone. High concordance rate was obtained in a considerable proportion of cases of acute leukemia, and BMA alone may suffice as a diagnostic procedure in most of the cases. Immunophenotypic analysis of aspirated material can further comprehend the diagnosis and help to further categorize the leukemia. However, a marrow completely replaced by blasts or immature myeloid precursors is often difficult to aspirate and results in a dry tap or inadequate aspirate as seen in 3 of our cases. Thus, BMB is essential in all such cases. BMB complements the peripheral blood and aspirate findings in providing additional information for diagnosis and especially prognosis of acute leukemia.<sup>[4]</sup>



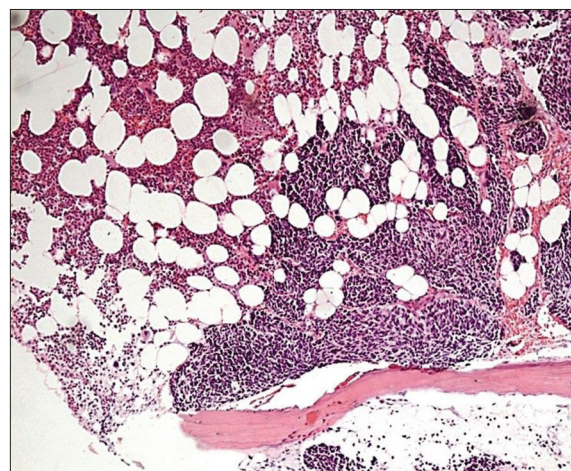
**Figure 5:** Focal paratrabecular lymphoid aggregates in a case of non-Hodgkin's lymphoma



**Figure 6:** Bone marrow aspiration (a) and bone marrow biopsy (b) showing concordant findings in a case of hypoplastic marrow



**Figure 7:** Hodgkin's lymphoma (a) H and E (b) CD 15 (c) CD 30



**Figure 8:** Metastatic deposit in bone marrow biopsy



BMA was found to be highly sensitive in diagnosis of NHL and MM. A case of NHL showed focal involvement of bone marrow which was diagnosed on BMB alone by histopathologist. Focal infiltration is more common than diffuse in B-cell lymphomas while diffuse infiltration is relatively more common in T-cell lymphomas.<sup>[5]</sup> Similarly, trephine biopsy helped to identify focal compact masses of plasma cells in a case of MM which could not be diagnosed by the hematologist on BMA alone. The pattern of infiltration can only be fully assessed by examining sections from trephine biopsy specimens.<sup>[6]</sup> Furthermore, biopsy is more helpful for quantifying plasma cell burden (using CD 138 immunohistochemistry), especially in patients with low percentage of plasma cells on aspirate.<sup>[7]</sup> However, cytomorphological classification of myeloma is better done on aspirate or imprint smear (mature, intermediary, immature, and plasmablastic types).<sup>[8]</sup> In a study conducted to evaluate the relative merits and prognostic value of BMA and BMB in the assessment of bone marrow infiltration in B-cell chronic lymphocytic leukemia, it was found that bone marrow histological pattern (BMP) was an independent prognostic factor for survival while percentage of lymphocytes in BMA (lymphocytic infiltration) only had independent predictive value in stage A patients. The interobserver reproducibility and agreement were higher for bone marrow histological pattern than for lymphocytic infiltration.<sup>[9]</sup>

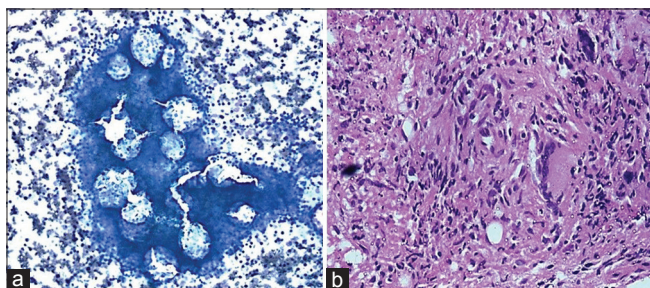
Only 40% aspirates from patients with marrow involvement by Hodgkin's disease were positive. Rest of the aspirates were either inadequate owing to marrow fibrosis or were taken from nonrepresentative area of a marrow which is only focally infiltrated by Hodgkin's disease. Thus, BMA does not have much role in cases of marrow involvement by Hodgkin's lymphoma. On the other hand, BMB is an important part of staging in stage IIB or III cases and hence alters the treatment.<sup>[10]</sup> Foci of fibrosis in the absence of classical or variant RS cells with Hodgkin's lymphoma diagnosed elsewhere are highly suspicious of marrow involvement.<sup>[10]</sup> Furthermore, an additional advantage of trephine biopsy specimen is that

immunohistochemical studies can be performed on sections of paraffin-embedded tissue which is useful in establishing or confirming the nature of lymphoid infiltrates.<sup>[11]</sup>

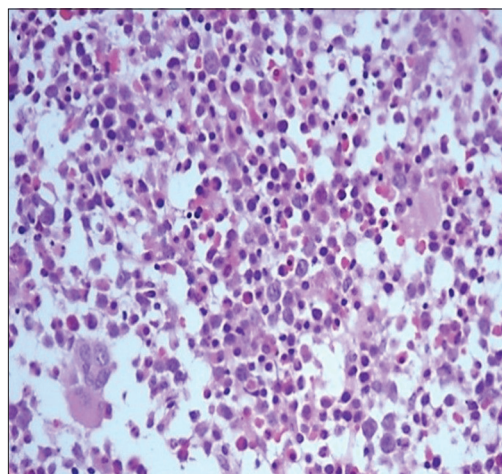
Among chronic myeloproliferative disorders (CMPDs), BMA shows high diagnostic accuracy in cases of CML-CP but low sensitivity for diagnosis of CML-blast phase (BP). Two of the aspirates of CML-BP were nondiagnostic owing to fibrosis while remaining two discordant cases were reported as CML-CP on BMA owing to the presence of focal blast crisis as observed later on BMB by the histopathologist. Role of trephine biopsy is not only in differentiation of CMPDs but also to assess the overall marrow cellularity, histotopography and morphology of megakaryocytes and blasts (CD 34+ precursors) and degree of myelofibrosis.<sup>[12]</sup>

In cases of megaloblastic anemia, findings of BMA were concordant with those of BMB in significant number of cases. However, morphological identification of megaloblastic change was mistaken for acute leukemia [Figure 10] as the biopsy was reported without reviewing the peripheral blood and marrow aspirate findings.<sup>[6]</sup>

In cases of hypoplastic bone marrow, moderate concordance was found and findings were suggestive of normocellular marrow in the discordant cases. This is explained on the basis of the presence of focal areas of slightly increased cellularity or hotspots in an otherwise profoundly hypocellular marrow.<sup>[13]</sup> Trephine biopsy gives the qualitative and quantitative assessment of cellularity and therefore is confirmatory in the diagnosis of aplastic anemia and overcomes the limitation of dry tap.<sup>[14]</sup> Furthermore, biopsy can provide the number and distribution of megakaryocytes, lymphocytes, plasma cells in



**Figure 9:** Granulomatous myelitis diagnosed on bone marrow biopsy (b) and aspirate (a) showing normocellular marrow



**Figure 10:** Megaloblasts can mimic lymphoblast or myeloblast on trephine biopsy sections

marrow and blasts, all of which are of prognostic value in follow-up of aplastic anemia.<sup>[14]</sup> An important limitation of BMA is the admixing of marrow and sinusoidal blood, which may not allow for reliable estimates of marrow cellularity.<sup>[13]</sup>

The hematologist could not diagnose granulomatous myelitis as well-formed epithelioid cell granulomas were not seen in any of the six aspirates from patients subsequently diagnosed as granulomatous myelitis on biopsy, thereby emphasizing the importance of BMB in diagnosis of focal marrow pathology. Similar results were shown by various studies.<sup>[5,6]</sup>

Frequency of positive BMA in metastatic marrow varies from 23% to 100% in different studies.<sup>[15-18]</sup> Almost two-third of BMA from metastatic marrow disease did not show metastatic deposits. In a comparison of a large series of simultaneously performed BMA and BMB, a correlation of 75% was obtained for detection of metastatic neoplasms.<sup>[19]</sup> Focal deposit of nonhematopoietic malignant cells and tumor-associated desmoplasia and necrosis are the causes of dry tap on aspiration.<sup>[19]</sup>

## Conclusion

BMA and BMB both play distinct roles as diagnostic modalities for marrow examination. They show a good degree of correlation in majority of cases. The decision to perform a marrow aspiration alone or in combination with marrow biopsy depends on the diagnosis being considered. BMA alone is sufficient for diagnosis of megaloblastic anemia and most of the hematological malignancies. However, focal marrow pathology is often missed on BMA and is a major cause of discordant reports. BMB is more appropriate for detection of these disorders such as lymphoproliferative disorders, metastatic cancer, focal blast crisis in CML, granulomatous lesions, and hypoplastic marrow. Marrow fibrosis resulting in dry tap on aspiration is another major cause for discordance and biopsy clinches the diagnoses in most such cases. Trephine biopsy sections render information which cannot be determined from aspiration, such as spatial distribution and extent of infiltrates, overall cellularity, and fibrosis. Ancillary techniques such as immunohistochemistry can be performed with greater ease on a trephine biopsy section than an aspirate. Furthermore, trephine biopsy may be more useful in postchemotherapy patients to assess the residual tumor cell burden and degree of chemotherapy response.<sup>[6]</sup> BMA and BMB when performed simultaneously are complementary to each other. There is more material to study the cytomorphology as well as the pattern of distribution of cells. Furthermore, it is strongly recommended that both

should be reviewed simultaneously to ensure maximum diagnostic accuracy

## Consent

Informed consent was obtained from the patient.

## Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that name and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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