ABSTRACT
Aims: The study was carried out to assess the diagnostic value and utility of bone marrow aspirate and biopsy in bone marrow disorders and the role of both procedures to achieve correct diagnosis when done simultaneously.

Methods and Material: A prospective study was conducted to correlate the findings of bone marrow examination with clinical parameters. The study included 42 patients in whom bone marrow evaluation was carried out in bone marrow aspirate and marrow biopsy. Cases, where bone marrow aspirate was absent or inadequate for opinion were excluded from the study. Statistical analysis was not performed as no significant association was studied.

Results: Most cases were in the age group 30 to 50 years, with male preponderance. The most common finding was nutritional anemia; the features of both megaloblastic and iron deficiency anemia were seen in 38.1% of cases; iron deficiency was seen in 11.9% cases, and 7.1% cases showed megaloblastic anemia. In addition, leukaemia (both acute and chronic) were seen in 4.8% of the cases. Other cases were necrotizing granulomatous inflammation of bone marrow, aplastic anemia, metastasis to bone marrow, plasma cell dyscrasias, Immune Thrombocytopenic Purpura (ITP), the gelatinous transformation of marrow, hemophagocytic lymphohistiocytosis, congenital dyserythropoietic anemia and myelofibrosis etc.

Conclusions: The bone marrow aspirate and biopsy are essential in the diagnosis of marrow diseases. Bone marrow examination plays an important role in diagnosing diseases when correlated with clinical examination and ancillary studies.

KEYWORDS
Bone marrow aspirate, Bone marrow biopsy, Marrow diseases
nias, marrow aspiration alone are sufficient, but for detection of disorders with focal marrow involvement, marrow biopsy is a must. [2] Bone marrow biopsy (BMB), a tedious procedure, also plays a very important role in cases of dry tap in conditions like myelofibrosis, hairy cell leukaemia and aplastic anemia or dilution aspirate by blood. [3] This study aims to discuss the findings of bone marrow aspirates and biopsy and their correlation with other clinical and laboratory parameters.

Material and Methods

In six months, a prospective study was conducted to study the findings of bone marrow examination in the department of Pathology in a tertiary care hospital. Total 66 bone marrow aspirates were received during the period of six months from January 2018 to June 2018, out of which we included 42 cases of adequate samples. Cases, where aspirates were absent or diluted, were excluded. Among these 24 excluded cases, in six cases, BMA were not available, and in 18 cases, the aspirate was either dilute or inadequate for opinion. The data was recorded from the case history sheet and electronic record system of the hospital. Clinical and laboratory parameters were evaluated. Bone marrow aspirates were stained with Leishman stain and biopsies with H & E, special stains like Reticulin, AFB and GMS were done whenever needed and possible. The statistical analysis was not performed as we did not study any significant association.

Results

Among 42 cases, 33 (78.5%) were males, and 9 (21.4%) were females. The male to female ratio was 3.7:1. Age ranged from 11 years to 70 years. Most of the cases were in the fifth decade. (Table 1) On clinical examination, although mixed signs, symptoms and presenting complaints were noted in all patients, pallor was the most common clinical finding, which was present in 36 (85.7%) of the patients. Mild pallor was present in 15 cases, moderate in 17 and severe in 4 cases. Other clinical features observed were splenomegaly in 13 (31%) cases, jaundice in 9 (21.4%) cases, hepatosplenomegaly in 7 (16.7%) cases, hepatomegaly in 1 (2.3%) case and lymphadenopathy in 2 (4.7%). This study observed 11 (26.2%) HIV positive cases, out of which 6 cases were under treatment.

In the present study, 13 (30.9%) cases presented with fever, out of which 12 cases had pyrexia of unknown origin (PUO), and one case had fever due to falciparum malaria. Among 12 cases of PUO, 5 cases were HIV positive. Pancytopenia was the main indication for bone marrow examination seen in 9 (21.4%) cases. Other indications are presented in Table 2. Erythroid hyperplasia was the predominant finding seen in 29 (69%) cases. Micronormoblastic maturation due to iron deficiency was seen in 5 (11.9%) cases. Both megaloblastic change and micronormoblastic maturation were present in 16 (38.1%) cases in aspirates (Figure-1).

However, trephine biopsies revealed similar findings in ten cases. Among the rest of the six cases, in two (4.7%) cases, biopsies were unavailable while it was fragmented and scanty in one case.
Table 1 Distribution of cases according to age of patient.

| Age (in years) | Number of cases | Percentage (%) |
|---------------|----------------|----------------|
| 11 to 20      | 7              | 16.67          |
| 21 to 30      | 3              | 7.14           |
| 31 to 40      | 9              | 21.42          |
| 41 to 50      | 10             | 23.8           |
| 51 to 60      | 7              | 16.67          |
| 61 to 70      | 6              | 14.28          |
| Total         | 42             | 100%           |

Table 2 Indication for bone marrow biopsy.

| Indication                        | Number of cases | Percentage |
|-----------------------------------|-----------------|------------|
| Pyrexia of unknown origin         | 12              | 30.9%      |
| Pancytopenia                      | 9               | 21.4%      |
| Bicytopenia                       | 9               | 21.4%      |
| Anemia under evaluation           | 17              | 40.5%      |
| M-band in serum                   | 2               | 4.7%       |
| Suspected Acute leukaemia         | 1               | 2.3%       |
| Suspected Chronic leukaemia       | 1               | 2.3%       |
| Thrombocytopenia                  | 1               | 2.3%       |

(2.3%) case. Two (4.7%) cases were reported as tuberculosis, and one (2.3%) case was signed out as Congenital Dyserythropoietic Anemia (CDA). One case (2.3%) showed the gelatinous transformation of marrow, which was a known case of retroviral disease. Table 3 summarises the distribution of cases according to diseases in both BMA and BMB.

The two (4.7%) cases reported as tuberculosis revealed necrotizing granulomatous inflammation with large areas of caseation surrounded by epithelioid cell granuloma (Figure 2a); Ziel-Neelsen stain was positive for acid-fast bacilli suggesting tuberculosis (Figure 2b) which is the commonest endemic mycobacterium in India. GMS (Gomori Methenamine-Silver nitrate stain) and Periodic acid-Schiff stain for fungus were negative. Post diagnosis, patients were put on Category II anti-tubercular regime and responded well. The case, diagnosed as CDA, was a 14-year-old male with refractory anemia, the peripheral blood of which showed a macrocytic picture. Bone Marrow Aspirate (BMA) revealed marked dyserythropoietic, multinucleated erythroid precursors, binucleated erythroblast with megaloblastic change, morphological picture suggested CDA (Figure 3).

One case (2.3%) of acute leukaemia revealed 90% blasts on the peripheral blood smear; a similar finding was observed in BMA and BMB, where blast cells replaced almost all native hematopoietic cells. Haemoglobin was 7.4 gm%, platelet 60000/cu mm, total count 13000/cu mm. In addition, the blasts demonstrated Auer rods confirming the morphology of myeloblasts. One case (2.3%) of chronic myeloproliferative disorder likely to be CML in the chronic phase was also reported in this study.

There were two cases (4.7%) of plasma cell dyscrasia, out
Table 3 Distribution of cases according to diseases.

| Bone marrow findings                        | BM Aspirate | BM Biopsy |
|---------------------------------------------|-------------|-----------|
| Megaloblastic change and micronormoblastic maturation* | 16 (38.0%) | 10 (23.8%) |
| Micronormoblastic*                          | 5 (11.9%)   | 5 (11.9%) |
| Normoblastic*                               | 4 (9.5%)    | 4 (9.5%)  |
| Megaloblastic*                              | 3 (7.1%)    | 3 (7.1%)  |
| Aplastic Anemia                             | 2 (4.7%)    | 2 (4.7%)  |
| Plasma cell dyscrasia                       | 2 (4.7%)    | 2 (4.7%)  |
| Idiopathic thrombocytopenic purpura         | 2 (4.7%)    | 2 (4.7%)  |
| Metastasis to bone marrow                   | 2 (4.7%)    | 2 (4.7%)  |
| Congenital Dyserythropoctic Anemia*          | 1 (2.3%)    | 1 (2.3%)  |
| Myeloid and erythroid hyperplasia           | 1 (2.3%)    | 1 (2.3%)  |
| Acute leukaemia                             | 1 (2.3%)    | 1 (2.3%)  |
| CML                                         | 1 (2.3%)    | 1 (2.3%)  |
| Hemophagocytic lymphohistiocytosis          | 1 (2.3%)    | 1 (2.3%)  |
| Necrotising granulomatous inflammation      | -           | 2 (4.7%)  |
| No biopsy                                   | -           | 2 (4.7%)  |
| Fragmented biopsy                           | -           | 1 (2.3%)  |
| Gelatinous transformation of Marrow          | -           | 1 (2.3%)  |
| Myelofibrosis#                              |             | 1 (2.3%)  |
| No definite opinion                         | 1. (2.3%)#  | -         |

*Cases with erythroid hyperplasia hyperplasia

#No definite opinion was derived in one case of myelofibrosis on BMA

of which one case was of multiple myeloma. The patient had M-band in serum electrophoresis and hypocalcemia, anaemia and diffuse marrow plasmacytosis. Another case was suggestive of MGUS, as monoclonal paraprotein was 1.2 g/dl (less than 3g/dl) and plasma cells 7% (less than 10 g/dl) in the marrow.

In the present study, there were two cases (4.7%) of aplastic anemia where both aspirate and biopsy showed only fat spaces visualized no hematopoietic tissue. Two cases (4.7%) presented with thrombocytopenia, and bone marrow biopsy revealed increased hypo-lobated megakaryocytes consistent with peripheral destruction of platelets; diagnosis was ITP. One case (2.3%) had dilute marrow aspirate, and bone marrow biopsy showed myelofibrosis with grade 3 reticulin staining. (Figure 4a and 4b).

There was a 35 years old male whose peripheral smear showed pancytopenia and ring forms of Plasmodium falciparum. He had a fever, hepatosplenomegaly, raised serum ferritin (1520 µgm /L) and triglyceride (242 mg %) levels and bone marrow aspiration smears revealed histiocytes with phagocytosed lymphocytes, erythroid cells and platelets. The diagnosis was hemophagocytic lymphohistiocytosis (HLH) following Plasmodium falciparum infection (Figure-5).

There were two cases (4.7%) of metastasis to the marrow in the present study. In the first case, a 50-years-old male bone marrow biopsy revealed the replacement of marrow tissue by atypical cells and fibrosis. The diagnosis of metastasis of malignant epithelial cells to bone marrow was made based on morphology. The primary tumour site was unknown in this case. Another case, a 14-years-old known case of Ewings tumour, demonstrated metastasis of a small round blue cell tumour to the marrow.

Discussion

Bone marrow is commonly involved in patients presenting with haematological and non-haematological disorders. The bone marrow aspirate and biopsy are two separate but interrelated techniques used to diagnose bone marrow diseases. Both procedures complement each other, can be done on an outpatient basis and are cost-effective.

Bone marrow aspiration and bone marrow biopsy are important procedures for diagnosing various disorders, especially haematological and non-haematological malignancies. Usually, both procedures are done at the same time and preferably same site. [1]

In this study, 42 cases were evaluated in which most of the cases were in the fifth decade, and the male to female ratio was 3.7:1. The age of patients ranged from 11 years to 70 years. In a study by Kaur et al. [4] sex ratio was 1.6:1 and a wide range of age from 4 years to 74 years and most common age group were 10-30 years; in a study by Mehra et al. [5], the age range was 4-75 years, and most common age group was 31 to 50 years, male to
female ratio was 1.2:1. As compared to other studies, this study had male preponderance.

Unexplained anemia was the most common indication seen in 40.5% of cases, followed by pyrexia of unknown origin and pancytopenia. Unexplained anemia was also the most common indication of bone marrow examination (32%), followed by pancytopenia (20%) in a study by Mehra et al. [5], whereas the most typical indication was pancytopenia in a study by Kaur et al. [4]

In this study, 52.4% of cases showed features of nutritional anemia on aspirate, which was similar to 50% in a study by Atla et al. [6] and 52.21% in a study by Gohil et al. [7]. However, it was 36% in a study by Mehra et al. [5]. In this study, concordance with biopsy was found in 70% of cases. In contrast, in a study by Mehra et al., BMA alone was diagnostic in 94.7% of cases of nutritional anemia and a positive correlation was seen with Bone Marrow Biopsy (BMB) in all these cases. [5] In a study by Verma et al. [8], 15 out of 19 cases (78.9%) of nutritional anemia showed positive concordance of bone marrow aspirate with biopsy. These cases showed erythroid hyperplasia with either megaloblastic or micronormoblastic maturation. In the current study, 38.1% of cases had erythroid hyperplasia with megaloblastic change and micronormoblastic maturation in BMA, comparable to the study by Kaur et al. [4] where 32% of cases showed erythroid hyperplasia with megaloblastic change or micronormoblastic maturation. This study had 11.9% cases of micronormoblastic maturation and 7.1% cases of megaloblastic change in isolation, and one case (2.3%) of CDA, while Parmar et al. [9] reported 9% cases of both micronormoblastic maturation with megaloblastic change, 10% cases of micronormoblastic maturation 17% cases of megaloblastic change in isolation and 1% cases of CDA. Patel et al. [10] reported 19.6% cases of megaloblastic anemia, 5.8% cases of dimorphic anemia and 1.9% cases of microcytic hypochromic anemia.

This study had 4.7% cases of leukaemia which is lower as compared to Mehra et al. [5], who reported 28% cases of
leukaemia, and Atl et al. [6], who reported 16.2% cases of leukaemia. Concordance of aspirate and biopsy in diagnosing haematological malignancies was 100% in our study (for both acute leukaemia and CML) it was 92.5% in the study by Chandra et al. [11], and it was 88.2% for acute leukaemia and 100% in CML in a study by Tilak et al. [12].

In the current study, 4.7% of cases had plasma cell dyscrasia which was similar to the study done by Atla et al. [6] as they also reported 4.7% cases of plasma cell dyscrasia. In contrast, Mehra et al. [5] reported 2% cases. We found a correlation of BMA with biopsy in these cases, as observed in a study by Chauhan et al. [13]. Charles et al. also noted a similar correlation in their study of multiple myeloma. [14]

In this study, 4.7% of cases had aplastic anemia, and 4.7% had ITP. Mehra et al. reported 4% cases of aplastic anemia and 2% cases of ITP. [5] Moreover, Alta et al. reported 19% cases of aplastic anemia [6], which were on the higher side compared to the current study.

We found 4.7% cases of necrotizing granulomatous inflammation due to tuberculosis (TB). Bone marrow involvement of TB is rare. Granuloma formation results from a complex interaction of cytokines, inflammatory cells with the pathogen [15]. Bone marrow granulomas can occur in infectious diseases, sarcoidosis, other autoimmune diseases, drugs and malignant lesions, and TB is the commonest among infectious causes. [15] The first article which described granulomatous inflammation of bone marrow was published in 1956 by Pease. [16] According to Jeevan et al. [17], the incidence of bone marrow granulomas was 1.4% on trephine biopsies, and most of them were interstitial. Well-formed granulomas were seen in 55.3% of cases, and caseous necrosis was observed in 23.4% of cases in their study. Basu et al. [18] reported that 2.46% of the bone marrow biopsies showed granulomas.

In the current study, there was one case of HLH secondary to Plasmodium falciparum infection that died even after treatment. A similar case was first reported by Ohno T et al. in 1996 in a 24-year-old male [19]. Fewer than 20 cases of HLH secondary to malaria have been reported, thus highlighting the rarity of this condition. Most of the reported cases of HLH secondary to malaria recovered completely after the patient received anti-malarial treatment alone, while few required additional immunosuppression. [20] Metastatic deposits were seen in 4.7% of cases in our study in both aspirate and biopsy, which is similar to 4% of cases reported by Atla et al. [6] Thiyagarajan et al. [21] reported one case (0.6%) of metastasis of adenocarcinoma in their study. Chauhan et. [13] al studied 570 cases, out of which six cases (1%) of metastasis were reported in bone marrow biopsy, and two cases (0.3%) were missed in aspiration. Kaur et al. [4] reported four (8%) non-diagnostic aspirates in patients who had grade-3 marrow fibrosis; however, we found one (2.3%) such case. Bone marrow biopsy revealed a degree of fibrosis in cases where aspirates were thought to be diluted or a dry tap; in such cases, trephine biopsy is compulsory to come to a definitive diagnosis. These cases must not be dismissed as inadequate aspiration due to faulty technique. [13]

Bone marrow examination is invaluable in diagnosing various marrow disorders and remains the basis and essential diagnostic aid despite the recent molecular advances in haematology.

**Conclusion**

This study concludes that both bone marrow aspirate and bone marrow biopsy are essential for diagnosing haematological diseases; as they are easy, rapid, cost-effective, and complementary to each other. If done together, these procedures enable us to study cytomorphology along with architectural patterns and distribution of cells/lesions to achieve a definite diagnosis. This has a tremendous impact on the management of patients.

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**Conflict of interest**

There are no conflicts of interest to declare by any of the authors of this study.

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