

## Original Research Article

# Bone marrow aspiration cytology in the diagnosis of hematologic and non-hematologic diseases in a multi-specialty hospital in Nepal

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### ABSTRACT

**Background:** Peripheral blood smear examination and other routine laboratory assays are not always sufficient to diagnose various diseases which affect the blood and bone marrow. A bone marrow aspirate examination is essential in most of the cases.

**Methods:** This work was a hospital-based cross sectional analytical observational study carried out in the department of pathology at the Chitwan Medical College, Teaching Hospital, Nepal, over a period of 3 years from January, 2013 to December 2015. Bone marrow was aspirated from posterior superior iliac crest under local anesthesia; sternum was the alternative site in obese patients. Univariate analysis was performed for each variable using frequency distribution and means with the help of Statistical Package for Social Sciences 20.0 (SPSS 20.0) software.

**Results:** One hundred and fifty-nine patients were included in the study. Eight cases of 'dry tap' were excluded, for whom bone marrow biopsy was advised. Anemia was the largest group followed by malignancy, infection and miscellaneous diseases. Among anemia, megaloblastic anemia was the most common, followed by aplastic anemia, dyserythropoietic anemia and myelophthisic anemia. The category 'malignancy' consisted of leukemia, multiple myeloma and myelodysplastic syndrome. Regarding individual diseases, megaloblastic anemia was the most common haematological disorder followed by immune thrombocytopenic purpura, leukemia and aplastic anemia.

**Conclusions:** Bone marrow aspiration cytology is a mildly invasive technique which can diagnose many haematological and non-hematologic diseases that can be confirmed by more advanced investigations, if needed: serological, biochemical or molecular. However, bone marrow sample cannot be obtained (dry tap) in a proportion of cases. In such cases, a bone marrow biopsy needs to be performed.

**Keywords:** Bone marrow aspiration cytology, Nepal, Spectrum of diseases

### INTRODUCTION

Bone marrow aspiration cytology is a proven method for the evaluation of hematologic conditions, malignancies, storage disorders and some chronic systemic conditions.<sup>1</sup> Peripheral blood smear examination and other routine laboratory assays are not always sufficient for the diagnosis of various diseases which affect the blood and bone marrow.<sup>2</sup> Iron deficiency, thalassemia and some cases of anemia of chronic disease present as microcytic hypochromic anemia.<sup>3</sup> Bone marrow aspiration and

staining the smear by Pearl's stain is the gold standard method for the diagnosis of iron deficiency anemia. Nevertheless, iron profile is preferred now-a-day because it is a non-invasive test.<sup>4</sup> Megaloblastic anemia presents as macrocytic normochromic anemia. Megaloblasts are found in bone marrow aspirate smear which display nucleocytoplasmic asynchrony.<sup>5</sup> Various types of leukemia present with blast cells in the peripheral blood smear. Bone marrow examination should be done for morphological classification of leukemia. More advanced investigations viz. Conventional Cytogenetics and FISH

can be done on a bone marrow aspirate sample subsequently.<sup>6</sup>

Pancytopenia has varied causes, some of which are as follows: megaloblastic anemia, aplastic anemia, aleukemic leukemia, leishmaniasis, hypersplenism, drug-induced, multiple myeloma, metastatic infiltration of the marrow by malignant cells and myelodysplastic syndrome. Preliminary diagnosis of all these disorders can be made by the examination of bone marrow aspiration sample.<sup>7</sup> Bone marrow aspirate examination is also useful in the diagnosis of thrombocytopenia. Absence of megakaryocytes in the marrow indicates marrow failure as the cause of thrombocytopenia. On the contrary, if megakaryocytes are increased in number the cause of thrombocytopenia is peripheral destruction of platelets or primary hematologic disease.<sup>8</sup>

Bone marrow sample can be obtained by aspiration or trephine biopsy, the former being less invasive of the two. There are different sites from where marrow can be sampled. The most preferred site is the posterior superior iliac crest and the selection of this site has the advantage that, if no material is aspirated, a trephine biopsy can be performed immediately. Other sites are: anterior iliac crest, manubrium or the first or second parts of the body of the sternum. In infants, marrow can be obtained from the medial aspect of the upper end of the tibia just below the level of the tibial tubercle.<sup>2</sup>

The aim of this research was to find out the spectrum of diseases which were diagnosed by bone marrow aspiration cytology at Chitwan Medical College Teaching Hospital. The objectives were as follows:

- to find out the spectrum of diseases diagnosed by bone marrow aspiration cytology.
- to find out the prevalence of diseases in relation to age-groups and sex.
- to find out the proportion of nonneoplastic and neoplastic lesions.
- to categorize both nonneoplastic and neoplastic lesions

## METHODS

This was a hospital-based cross sectional analytical observational study carried out in the department of pathology at the Chitwan Medical College, Teaching Hospital, Bharatpur, Chitwan, Nepal, over a period of 3 years from January, 2013 to December, 2015. Patients with dry tap were excluded from the study. Fourteen cases out of 173 cases were 'dry tap', which is about 8% of the total. Bone marrow was aspirated from posterior superior iliac crest under local anesthesia using lignocaine solution. In obese patients, sternum was used as the aspiration site. Saline bone marrow aspiration needle was used for the procedure. To avoid dilution of the yield by peripheral blood, only approximately 0.3 ml bone marrow was aspirated from each patient. At the

same time, peripheral blood was also obtained to complement information obtained from the bone marrow aspiration cytology. The smears were fixed in methanol for 20 minutes after air-drying them thoroughly. The fixed smears were stained with Giemsa stain.

## Data collection and analysis

The data were entered into the Statistical Package for Social Sciences 20.0 (SPSS 20.0). Univariate analysis was performed for each variable using frequency distribution and means.

## RESULTS

One hundred and fifty-nine patients were included in the study (Table 1). 56.0 % patients were female and 44.0 % patients were male. The age range of patients was from 15 years to 86 years. Mean age was 44.6 years. Mean age of female patients and male patients was 43.1 years and 46.4 years respectively. About half of the total patients were in the age group of 26 to 40 years.

**Table 1: Age-group and sex of patients.**

Age-group	Sex= n (%)		N=159 (%)
	M	F	
10-25	19 (41.3)	27 (58.7)	46 (28.9)
26-40	36 (46.2)	42 (53.8)	78 (49.1)
41-55	11(37.9)	18 (62.1)	29 (18.2)
>56	04 (66.7)	02 (33.3)	06 (03.8)
Total	70 (44.0)	89 (56.0)	159 (100.0)

Diseases which were diagnosed on the basis of bone marrow examination are broadly categorized into four groups (Table 2).

**Table 2: Broad categories of diseases diagnosed by bone marrow aspirate cytology.**

Disease group	N=159 (%)
Anemia	122 (76.7)
Malignancy	30 (18.9)
Infection	3 (1.9)
Miscellaneous	4 (2.5)

Anemia was the largest group (60.4%) followed by malignancy, infection and miscellaneous diseases. Among anemia, megaloblastic anemia was the most common type (44.0%) followed by aplastic anemia (11.9%), dyserythropoietic anemia (3.1%) and myelophthisic anemia (1.3%). The last one was due to tuberculosis. The category 'malignancy' consisted of leukemia (80%), multiple myeloma (13.3%) and MDS (6.7%). In leukemia, the most common diagnosis was 'acute myeloblastic leukemia'. Leukemia was classified according to French American British (FAB) system. It was of following types in the descending order of frequency: AML M2 (34.8%), M1 (26.1%), M0 (8.7%),

M3v (8.7%), M3 (4.3%), M4 (4.3%), M6A (4.3%), ALL L1 (4.3%) and CLL/PLL (4.3%). Megaloblastic anemia was the most common hematological disorder (Table 3). At least 10% erythroblasts showed megaloblastic changes viz., larger than normal size, nucleocytoplasmic asynchrony and sieve-like chromatin pattern. Female patients (63.6%) were significantly more in number than the male patients (36.4%). Most of the patients were found in the age-group 26 to 40 years (53%). The cumulative percentage for 10-25 years and 26 to 40 years was 78.8%. Mean age of patients was 45.1 years. Biochemical investigations were advised to confirm the diagnosis of megaloblastic anemia.

**Table 3: Diseases diagnosed by bone marrow aspirate cytology.**

Disease	N= 159 (%)
Megaloblastic anemia	70 (44.7)
Immune thrombocytopenic purpura	26 (16.4)
Leukemia	24 (15.0)
Aplastic anemia	19 (11.9)
Dyserythropoietic anemia	05 (3.1)
Multiple myeloma	04 (2.5)
Myelophthisic anemia due to tuberculosis	02 (1.3)
Eosinophilia	02 (1.3)
Myelodysplastic syndrome	02 (1.3)
Visceral leishmaniasis	02 (1.3)
Infection-associated myelo-suppression	01 (0.6)
Thrombocytosis due to megakaryocytic hyperplasia	01 (0.6)
Hypersplenism	01 (0.6)

The second most common disorder was immune thrombocytopenic purpura. The disease was diagnosed based on thrombocytopenia in peripheral blood and megakaryocytic hyperplasia in the bone marrow smear. Female patients constituted 61.5% and male patients 38.5%. Most of the patients were found in the age-group of 26-40 years (42.3%). Mean age of the patients was 34.9 years. Serological investigations were advised to confirm the diagnosis of ITP.

Leukemia as a group was the third most common disorder. Cytogenetic and flow cytometric investigations were advised to classify leukemia based on WHO/REAL classification system. In cases of aplastic anemia, all three cell lines were suppressed and smears were hypocellular. Mean age of patients was 47.5 years. 52.6% patients were female and 47.4% patients were male.

## DISCUSSION

Anemia was the most common disease diagnosed by bone marrow cytology study (76.7% of total cases) and the proportion was far higher than in other studies (22.7%, 37% and 50%).<sup>9-11</sup> Among the various types, megaloblastic anemia was the most common as in many

other studies. In the research done by Parajuli et al, it was the second most common diagnosis overall.<sup>9</sup> Moreover, it was the most common disease overall too in the present study in concordance with other studies.<sup>11,12</sup> Very young and very old patients were spared by the disease. Most of the patients were found in the age-group 26 to 40 years (53%). The cumulative percentage for 10-25 years and 26 to 40 years was 78.8%. Mean age of patients was 45.1 years. It was more common in females (63.6%). In a study done by Chand et al, peak incidence was found in the age-group of 10-25 years. In similarity to our study, the disease was more common in females (71%).<sup>13</sup> However, megaloblastic anemia was the third most common disease in a study done in Jordan by Momani et al.<sup>10</sup>

Because of the lack of laboratory resources, we could not find out the etiology of megaloblastic anemia. Serum cobalamin level and red cell folate level were advised to confirm the diagnosis of megaloblastic anemia. Although iron deficiency anemia is the most common nutritional deficiency worldwide, almost all cases are diagnosed on the basis of peripheral blood findings and/or biochemical findings. Bone marrow examination is usually not done in these cases. That is the reason why iron deficiency anemia was not featured in the list of diagnoses in our study. Similar finding was observed by other national and international studies.<sup>9,14,15</sup> However, Momami et al from Jordan found iron deficiency anemia as the third most common disorder.<sup>10</sup>

Immune thrombocytopenic purpura (ITP) is an autoimmune disease. Like all autoimmune diseases, ITP is more common in women.<sup>16</sup> In the present study, the female to male ratio was 1.6:1. The proportion of ITP cases in our study was 16.4%. Two studies from Nepal found it to be 9.33% and 10.5%.<sup>9,14</sup> International studies have shown the proportion to be 6.21%, 14.5% and 5%.<sup>17-19</sup> Aplastic anemia is more common in developing countries than in developed countries.<sup>20</sup> It was the second most common anemia (11.9% of all cases) in our study. In the study of Atla et al, 19% cases had aplastic anemia.<sup>11</sup> Some other studies had lower incidence of the disease. It was seen in only 5.3% cases in a study done by Pudasaini et al.<sup>14</sup> The frequency was even lower in a study carried out in Jordan by Momani et al; only one case was found in that study.<sup>10</sup> Although aplastic anemia is an autoimmune disease, its incidence is equal in both sexes.<sup>21</sup> In the present study, the female to male ratio was as good as equal at 1.1:1.

Non-neoplastic diseases consisted of 81.1% and neoplastic diseases consisted of 18.9% of total cases in this study. In one study from Nepal, the incidence of neoplastic diseases was 19.3%.<sup>14</sup> Leukemia as a group was the most common malignant disorder (15%) and acute myeloblastic leukemia was found to be the most common type of leukemia in our study as in other studies.<sup>14,17,22</sup> In contrast, acute lymphoblastic leukemia (ALL) was found to be the most common type of

leukemia in a couple of studies.<sup>13,23</sup> Multiple myeloma was found to be the second-most common malignant disease (2.5%) which is close to the data (3.5%) in one study.<sup>14</sup>

### **Bone marrow tuberculosis**

Tuberculosis is endemic in Nepal as in other developing countries. Two cases (1.3% of total cases) were diagnosed based on the presence of granulomatous inflammation. Supplementary investigations were advised to confirm the diagnosis. Other researchers also found a few cases (1.3 and 6.7%) of granulomatous inflammation in their studies.<sup>9,23</sup> In contrast, some studies did not find any cases of granulomatous inflammation despite being from the Indian subcontinent.<sup>10,14,15</sup> Extrapulmonary tuberculosis accounts for 25% of all cases of tuberculosis. Osteoarticular tuberculosis accounts for only 11% of all extrapulmonary cases.<sup>24</sup> This may be the reason why very few cases of tuberculosis were found in the bone marrow cytology.

We had two cases (1.3%) of visceral leishmaniasis in our study; the data are very close to the data (1.8% and 2.27%) of other studies.<sup>9,14</sup>

### **CONCLUSION**

Bone marrow aspiration cytology is a mildly invasive technique which can diagnose many hematological and non-hematologic diseases that can be confirmed by more advanced investigations viz. serological, biochemical or molecular. However, bone marrow sample cannot be obtained (dry tap) in a proportion of cases. In such cases, a bone marrow biopsy needs to be performed.

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