Original Research

Diagnostic Utility of Bone Marrow Examination in Pancytopenia Cases in Tertiary Care Centre

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Abstract

Background & objectives: Pancytopenia may be a manifestation of wide variety of disorders which primarily or secondarily affects the bone marrow. Bone marrow aspiration is a minimally invasive, out – patient procedure which provides definitive diagnosis in cases of pancytopenia. The present study was conducted to analyse the various causes of pancytopenia by evaluating bone marrow aspiration and to correlate bone marrow aspiration findings with clinical presentation, complete blood counts and peripheral blood picture.

Methods: This prospective study was conducted in the Department of Pathology, Muzaffarnagar Medical College for a period of 12 months. Total 60 cases were evaluated for pancytopenia.

Results: Out of 60 cases, 25 (41.7%) cases were females and 35 (58.3%) were males with slight male preponderance. Maximum number of cases were in the age group of 31 - 40 years. Most common cause of pancytopenia was megaloblastic anaemia followed by hypoplastic marrow, iron deficiency anaemia, nutritional deficiency and others.

Interpretation & Conclusion: Along with detailed clinical examination and complete blood count, bone marrow examination is still the indispensable diagnostic modality to diagnose the aetiology of pancytopenia.

Keywords: Bone Marrow Examination, Hypoplastic Marrow, Megaloblastic Anaemia, Pancytopenia.

Introduction

Sir William Harvey described blood as "the fountain of life and the primary seat of the soul".[1] Pancytopenia is a significant hematological finding in which three cellular components of peripheral blood diminish in number resulting in anemia, leucopenia, and thrombocytopenia.[2] Diagnosing pancytopenia is difficult because it is associated with many different underlying etiologies which are also influenced by many epidemiological factors. At times, diagnosis is not possible with the single test, hence required battery of investigations thus necessitating

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collaboration between the pathologist and the clinician.[3] Pallor, exhaustion, infection, bleeding, weight loss and organomegaly are the main sign and symptoms. It is possible to identify the causes of pancytopenia with appropriate clinical evaluation and hematological studies, such as peripheral blood counts and bone marrow examination. [4] Typically, pancytopenia manifests as bone marrow failure. Low levels of hemoglobin (≤10.0 g/dl), white blood cells (≤4.0×10^9 /L) and platelet counts (≤150×10^9 /L) are laboratory findings of pancytopenia.[5]

Megaloblastic anemia (61.9%), infections, hypersplenism (25.7%), and aplastic anemia (9%) are the common etiologies of pancytopenia in developing nations like India.[6,7] The cause of pancytopenia determines the cellularity of the marrow, whether erythropoiesis is ineffective, or there is peripheral breakdown of hematopoetic elements or there are problems with the infiltration of bone marrow. Bone marrow aspiration (BMA) is an easy procedure that is safe and diagnose a variety of hematological disorders.[8]

Aim

The present study was undertaken to analyse the causes of pancytopenia by evaluating bone marrow aspiration and biopsy and to correlate them with clinical presentation, peripheral blood picture, as well as serological studies wherever possible.

Material and Methods

This was hospital based observational study conducted in the Department of Pathology, Muzaffarnagar Medical College for 12 months.

Sample size: As this was a hospital based study done for a limited period of time (12 months), purposive sampling was done and 60 patients of pancytopenia were included in the study.

Inclusion criteria:

1. All cases meeting the following criteria –
    i. Hemoglobin level of less than 10 gm/dl.
    ii. Total Leukocyte count of less than 4000 cells/cumm
    iii. Platelet count of less than 1,50,000/ cumm
2. Patients giving informed consent.

Exclusion criteria:

1. Hemorrhagic disorders such as hemophilia and disseminated intravascular coagulopathy.
2. Patients receiving chemotherapy/ radiotherapy.
3. The patients refusing to give informed consent.
4. Pregnant females.

Peripheral smear, reticulocyte count and blood indices were done before procedure. Peripheral smears were stained with Leishman stain and carefully examined for any anisocytosis or poikilocytosis, nucleated RBCs, rouleaux formation or atypical cells.

After taking consent, bone marrow aspiration (BMA) was done from posterior superior iliac spine using Salah needle of number 16 for adults and number 18 for children under aseptic conditions. Bone marrow biopsy was done where additionally indicated or in dry tap using Jamshidi needle.

BMA smears were stained with Leishman stain and bone marrow trephine biopsies were processed and stained by Haematoxylin and Eosin stain.

Perl's stain - on all BMA smears

MPO stain – on suspected myeloid neoplasm

PAS stain – on suspected lymphoblastic leukemia

Reticulin stain – in cases of suspected myelofibrosis necessary.

Statistical analysis

Suitable statistical test was used for statistical analysis using SPPS17/20 software. The p-value <0.05 was considered significant for statistical analysis.

Consent and ethics

Ethical approval was taken from the institutional ethical committee of Muzaffarnagar Medical College, Muzaffarnagar. (IEC no. – MMC/IEC/2022/126)

Results

Total 60 patients were evaluated. Age group of patients varied from 3 to 90 years of age Mean age was 52.3 years. Most commonly affected age group was 31-40 yr with 13 cases (20%) followed by 51- 60 years age group having 11 (18.3%) cases. (Table 1)

Table 1. Age - wise distribution of the pancytopenia cases

Age Groups	Total cases (n)	
	No. of cases (n)	Percentage (%)
0 - 10 yrs	3	5.0%
11 - 20 yrs	5	8.3%
21 - 30 yrs	10	16.7%
31 - 40 yrs	13	20.0%
41 - 50 yrs	09	16.7%
51 - 60 yrs	11	18.3%
>60 yrs	9	15.0%

Out of 60 cases, there were 25 females (41.7%) and 35 males (58.3%), indicating a male predominance with M : F ratio of 1.4 : 1.

Generalized weakness was identified in all patients (100%), emerging as the predominant symptom. Fever was the second most prevalent symptom affecting 54 patients (90%) and then dyspnoea in 18 patients (30%) only. On examination, pallor was the most prevalent feature observed in 52 cases (86.7%), petechiae in 25 cases (41.7%). 13 cases (21.7%) had hepatomegaly, 11 cases (18.3%) had splenomegaly, and 7 cases (11.7%) had pedal odema. In our study, concurrent hepatosplenomegaly was seen in 5 patients.

RBC morphology was observed on peripheral blood smear. Twenty one patients (35.0%) had macrocytic morphology, while 19 patients (31.7 %) had normocytic normochromic blood picture. (Table 2)

Table 2. Table showing blood picture of pancytopenic patients

Peripheral smear	No. of cases (n)	Percentage (%)
Macrocytic	21	35.0%
Normocytic normochromic	19	31.7%
Microcytic hypochromic	14	23.3%
Normocytic hypochromic	3	5.0%
Dimorphic	3	5.0%
Total	60	100%

Decreased total leukocyte count and platelet count was seen in all cases.

Hypersegmented neutrophils was seen in 22 cases (36.7%). Immature WBC was found in 7 cases only (11.7%).

Hypercellular bone marrow was seen in 33 aspirates (55.0%), hypocellular in 11 cases (18.3%) and normocellular were 09 cases (15.0%). However, in 7 cases (11.7%), there was dry tap.(Table 3)

Table 3. Cellularity of bone marrow aspiration

Bone Marrow Cellularity	No. of cases (n)	Percentage (%)
Hypercellular	33	55.0%
Hypocellular	11	18.3%
Normocellular	09	15.0%
Dry tap	07	11.7%
Total	60	100%

Perl's Prussian blue stain was done in all aspiration smears and revealed increased iron stores in 45% cases, decreased in 31% and was normal in 24% cases.

Bone marrow aspiration showed Megaloblastic Anemia as the most common cause in 25 (41.7%) patients. Megaloblasts were the large cells with basophilic cytoplasm and peculiar sieve like chromatin. Other findings were the presence of giant myelocytes and metamyelocytes. Hypoplastic Marrow was diagnosed in 10 (16.6%) patients. The bone marrow of 5 (8.3%) patients was normal showing trilineage hematopoiesis. Iron deficiency anemia and nutritional deficiency were seen in 3 (5.0%) patients each. Diagnosis of nutritional deficiency was made after correlating the BMA finding with serological iron studies and Vitamin B12 and folate levels. Myelodysplastic Syndrome and Acute Myeloid Leukemia were identified in 2 (3.3%) of the cases. One case (1.7%) each were diagnosed as of metastasis, acute lymphoblastic leukemia and multiple myeloma. (Table 4)

Table 4. Diagnosis on BMA of pancytopenic patients

Diagnosis	No. of cases (n)	Percentage (%)
Megaloblastic Anemia	25	41.7%
Hypoplastic Marrow	10	16.6%
Trilineage hematopoiesis	5	8.3%
Iron Deficiency Anemia	3	5.0%
Nutritional Deficiency	3	5.0%
Metastasis	1	1.7%
Acute Lymphoblastic Leukemia	1	1.7%
Acute Myeloid Leukemia	2	3.3%
Myelodysplastic Syndrome	2	3.3%
Multiple Myeloma	1	1.7%
Dry tap	7	11.7%
Total	60	100%

Perl's Prussian blue stain was done in all aspiration smears and revealed increased iron stores in 45% cases, decreased in 31% and was normal in 24% cases.

Dry tap was in 7 cases. Bone marrow biopsy (BMB) was done in these 7 cases and diagnosed

3 cases as hypoplastic marrow, 1 case as acute lymphoblastic leukemia, 1 case as hairy cell leukemia. A single case of metastasis was also found in BMB which was from Prostatic adenocarcinoma.(Image 4) Single case of myelofibrosis was diagnosed which was confirmed on Reticulin staining (Grade III). Reticulin stain demonstrate varied fibrosis and collagen. As the grade of fibrosis increases, patient clinical outcome worsens and in higher grade, requires the need for bone marrow transplantation. (Image 5) **(Reticulin Stain, 400 X)**(Table 5)

Table 5. Diagnosis ofBone Marrow Trephine Biopsy in dry tap cases

Diagnosis	No. of cases (n)
Hypoplastic marrow	03
Myelofibrosis	01
Metastasis	01
Acute lymphoblastic leukemia	01
Hairy cell leukemia	01
Total	07

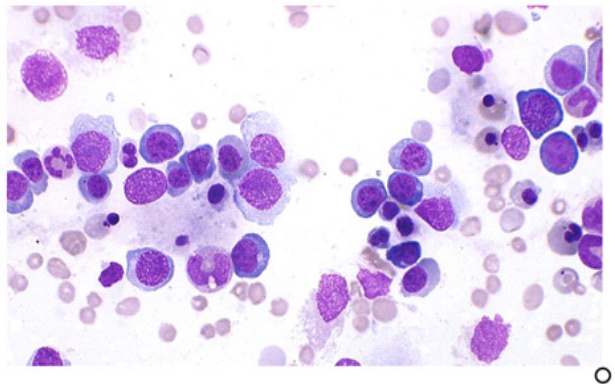

Figure 1: Photomicrograph of bone marrow aspirate showing increased number of megaloblasts with sieve like chromatin in megaloblasticanemia (Giemsa stain, 400X)

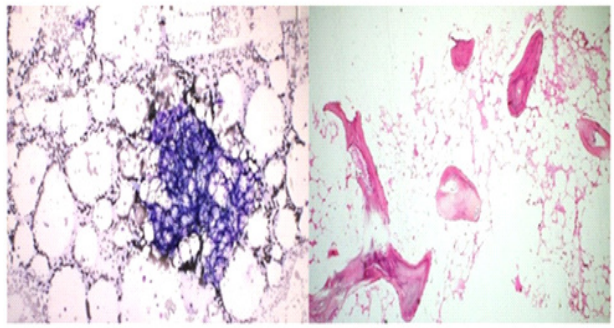

Figure 2: Photomicrograph of bone marrow aspirate in hypoplastic marrow showing mainly fat cells with decreased haematopoetic elements (Giemsa stain 100 X) (Left side). Bone marrow biopsy showing hypoplastic marrow with absence of haematopoietic cells and its replacement with fat cells (H &E stain, 100X) (Right side)

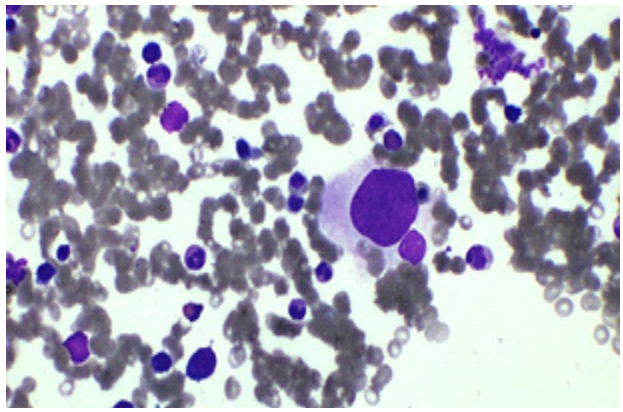

Figure 3: Photomicrograph of bone marrow aspirate showing hypolobated megakaryocyte in MDS (Giemsa stain, 400X)

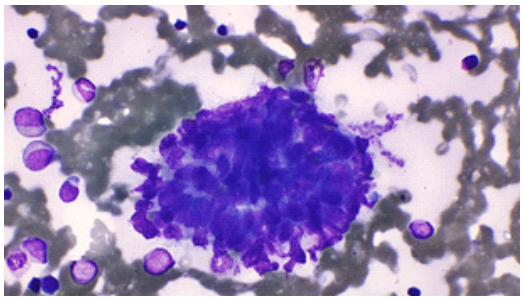

Figure 4: Photomicrograph of bone marrow aspirate showing cluster of non hematopoietic large atypical epithelial cells showing marked pleomorphism, high nuclear : cytoplasmic ratio and indistinct nuclei. (Metastasis, Prostatic Adenocarcinoma, Giemsa stain, 400 X)

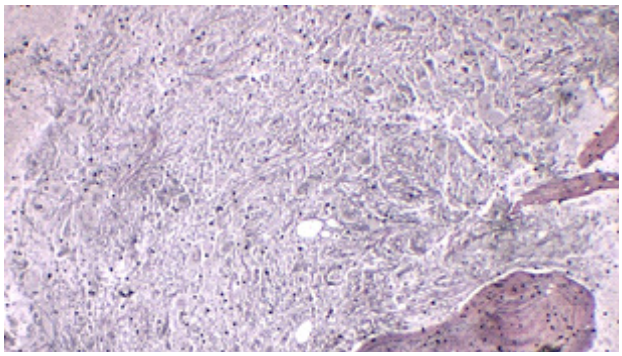

Figure 5: Photomicrograph showing Grade III fibrosis in myelofibrosis with diffuse fibre network with scattered thick coarse fibres but no mature collagen (Reticulin Stain, 400 X) discussed in result section

Discussion

Due to the diverse causes and prognosis of pancytopenia, it is crucial to conduct a thorough evaluation of laboratory parameters, peripheral blood, bone marrow findings, and their correlation with the clinical profile. This approach is essential for accurately diagnosing the condition and implementing appropriate management strategies.

In our study, total 60 cases of pancytopenia were studied during the period of 12 months. The age of patients ranged from 3 to 90 years, with a mean age of 52.3 years. Similarly, the studies done by Khunger et al.,[4] Chandan RH et al.,[9] Khodke et al.,[10] Tilak et al.[6] and Gayathri et al.,[11] the age range was almost same. However, discordance was also observed from research of Yadav et al.,[12] Makheja et al.[13] in which age range was less than 30 years and 13 years respectively.

In present study, 35 were male and 25 were female, indicating a slight male predominance (male-to-female ratio of 1.4 : 1) which was similar to findings of Patel et al.,[14] Kumar et al.,[7] Khodke et al.,[10] Khunger et al.,[4] Tilak et al. [6] and Gayathri et al.[11]

Age group affected most commonly was 31-40 years. Studies by Khunger et al. [4], Makhejaet al.[13] and Agrawal et al· [15] also reported maximum number of cases in the third decade of life. Some study showed discordance like Chandan RH et al.[9] with maximum cases in second decade of life.

Macrocytic normochromic blood picture was seen in 21 cases (35.0%) followed by normocytic normochromic blood picture in 19 (31.7%) cases. This was similar to study done by Gayathri et al.[11]

Hypercellular marrow was observed in 33 (55%) cases, hypocellular in 11(18.3%) cases. Normocellularity was observed in 9 (15.0 %) cases. Dry tap was seen in 7 cases (11.7%).

Chandan et al.[9] , Marwah et al.[16] and Prakash et al.[17] also found almost similar results in their studies.

In present study, megaloblastic anaemia was the most common cause of pancytopenia in 25 cases(41.7%), followed by hypoplastic marrow in 13 (21.7%) cases which was in accordance to studies done by Chandan et al.[9], Tilak et al.,[6] Khunger et al.,[4] Gayathri et al[11].However, in studies done by Jha et al.,[18] Khodke et al.,[10] they reported hypoplastic marrow as most common cause.

Incidence of hypocellular marrow in present study was 21.7 %, which correlated with the study done by Khunger JM et al.[4] who showed hypoplastic marrow in 28% cases .

In present study, 4 cases(6.6%) of acute leukemia was diagnosed as haematological malignancy in which 2 cases (3.3%) were of acute myeloid leukemia and 2 cases (3.3%) of acute lymphoblastic leukemia. This was similar to the study done by Khunger et al.[4] (5%).However, in the study of Khare et al.,[19] they reported 2 cases of acute leukaemia; one of Acute

myeloid leukemia and 1 of Acute lymphoblastic leukemia.

Two cases (3.3%) were diagnosed as myelodysplastic syndrome in our study, which was similar to the studies of Prakash et al.[17] and Agarwal et al[15] .

Single (1.7%) case of myelofibrosis was seen in our study which showed similarity with Prakash et al.[17] study, where 1 case was detected and other studies by Khunger et al.[4] and Shah and Patel et al.[20]

Myelofibrosis showed fibrosis grade 3 on reticulin stain in our study which was in concordance with study done by Shah and Patel et al.[20]

Only 1 case (1.7%) of multiple myeloma was diagnosed in present study which was in concordance with Khunger JM et al.[4] and Prakash et al.[17]

In present study, Perl's Prussian stain was done on bone marrow aspiration smears to assess the grading of bone marrow iron stores which showed increased iron stores in 45% cases, decreased in 31% and normal iron stores in 24% cases. This was similar to the study done by Desalphine et al.[21]

We reported only single case of hairy cell leukemia (1.7%) which was diagnosed on BMB. In study by Patel at al.,[14] 1.3% cases were diagnosed as Hairy Cell Leukemia.

Conclusion

We conclude that bone marrow aspiration and biopsy are essential diagnostic tools in hematology, aiding in the identification of underlying causes of pancytopenia and guiding further patient evaluation and management. This study creates the framework for the methodical planning of the diagnostic algorithm of pancytopenia that determines the cause.

Studying the epidemiology of pancytopenia globally could assist in directing diagnostic investigations, thereby accelerating treatment and enhancing prognosis which depend on the underlying cause of pancytopenia.

Few cases may require advanced technique like flow cytometry, cytogenetic or molecular genetic testing for typing and definitive diagnosis which may not be available all setup that's why bone marrow examination is rapidly available test which is cost effectice and causes minimal discomfort to the patient. It makes the initial diagnosis of megaloblastic anemia, nutritional anemia (in conjunction with serum studies), multiple myeloma and leukemia.

Public health professionals should be educated about pancytopenia which should be suspected clinically whenever patient presents with unexplained anemia, fever and bleeding tendency. In this scenario, bone marrow examination is the primary test to evaluate the underlying cause.

However, with recent genomic analysis, researchers have been producing Bone Marrow Atlas which require development of novel confocal imaging methods. More studies are needed to identify the molecular signal produced by blood vessels.[22]

Limitations

This study was limited to a single centre with a small sample size which may not represent the full spectrum of pancytopenia causes in the entire region or other parts of India. Therefore, additional large-scale, multicentre studies are needed to accurately identify the causes of pancytopenia.

Recommendation

In India, megaloblastic anemia is often caused by nutritional deficiencies. To prevent this, the vegetarian population should practice regular supplementation of vitamin B12 and folic acid.

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Conflicts of Interest: None

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