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Original Research Article

BONE MARROW EVALUATION TECHNIQUES IN PANCYTOPENIA

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Abstract
Background: Pancytopenia is not a distinct disease entity; rather a triad of symptoms that may arise from various disease processes, primarily or secondarily affecting the bone marrow. A comprehensive hematological workup with good clinical correlation is critical in evaluating the underlying cause of pancytopenia. **Aim:** To correlate bone marrow aspiration, imprint and biopsy findings in patients with pancytopenia. **Materials and Methods:** 64 patients of all age groups diagnosed with pancytopenia, requiring bone marrow aspiration and biopsy, were enrolled in this study over two years at a tertiary care centre. Complete clinical data, including complete blood count and other relevant investigations, were recorded for each patient. **Result:** This study analyzed 64 cases of pancytopenia, ranging from 5 to 82 years of age. The most common age group affected was between 23 and 32, with an overall male preponderance. Megaloblastic anemia was identified as the leading cause of pancytopenia, followed by combined nutritional anemia. Less common causes include myelofibrosis, multiple myeloma, MDS, and filariasis. When diagnosing pancytopenia, bone marrow biopsy demonstrated the highest specificity (100%), followed by bone marrow imprint (85.9%) and bone marrow aspiration (84.4%). **Conclusion:** It is crucial to compare findings of Bone Marrow Aspiration, Bone Marrow Imprint, and Bone Marrow Biopsy to establish a faster and more efficient diagnostic method for different hematological disorders. Such assessment will provide valuable insights into each technique's strengths and limitations and help identify the most suitable approach for accurate diagnosis.

INTRODUCTION

Pancytopenia is a condition where all three blood cell lineages decline.[1] It is characterized by low hemoglobin levels, platelet counts, and leukocyte counts. The causes of pancytopenia vary, including systemic lupus erythematosus, megaloblastic anemia, and leukemia.[2] Diagnosis requires several tests, including complete blood count, peripheral smear, and bone marrow examination. Bone marrow examination is crucial in giving a specific diagnosis.[3] Evaluation of pancytopenia involves comparative analysis of peripheral smear, bone marrow aspiration, imprint smear, and bone marrow biopsy.

MATERIALS AND METHODS

This study focuses on patients diagnosed with pancytopenia who required a bone marrow aspiration and were referred to the hematology laboratory of the pathology department at a tertiary care centre. The study period was from January, 2021, to July, 2022. The inclusion criteria compromised of patients with pancytopenia, with hemoglobin levels less than 9 g/dL, total leukocyte count less than 4×10^9/L, and platelet count less than 140×10^9/L. Biopsies less than 0.5 cm were excluded from the study. An institutional ethical clearance certificate (IEC/No-09/2021) was obtained on 11-01-2021.
The complete clinical data of the patients, including physical examination, complete blood count using Sysmex XN 1000, and other relevant investigations, were recorded on a proforma. Patients requiring

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bone marrow examination were chosen for the study. The bone marrow aspiration, imprint, and biopsy were performed according to a specified procedure[3], which included obtaining written informed consent from the patient.

The posterior superior iliac spine was the most common site chosen for the procedure. After explaining the procedure to the patient, they were made to lie in the left or right lateral position with their knees drawn up. The skin over the area was cleaned with 70% ethanol and betadine and infiltrated with 5 ml of 2% Xylocaine (lignocaine) as a local anesthetic. The Jamshidi trephine biopsy needle was passed perpendicularly into the cavity of the bone with a rotary motion. After removing the stilette, 0.2-0.3ml of marrow contents were aspirated with the help of a 10ml syringe and delivered into a bottle containing EDTA. This sample was made into smears, fixed with ethanol, and stained with Leishman's stain.

The biopsy needle was inserted into the bone using a rotary motion to obtain 1 - 2 cm of core tissue. The biopsy core was gently touched and rolled on the slide to prepare at least five touch impression smears before fixing the biopsy. A benzoin seal was applied at the puncture site. The biopsy core specimen was fixed in Bouin's solution and decalcified with 9.5% nitric acid in 1% EDTA for 24 hrs. After routine processing and paraffin embedding, hematoxylin and eosin staining were done. Special stains like Perl's Prussian Blue stain, Reticulin, and Myeloperoxidase (MPO) were done as and when necessary.

RESULTS

A total of 64 patients with pancytopenia were evaluated. The most common age group affected was between 23 and 32 years (23.4%). A male predominance of 57.8% was noted, whereas females accounted for 42.2%. Dimorphic anemia was seen in 31.3% of cases, followed by normocytic normochromic anemia (29.7%), macrocytic anemia (26.6%) and microcytic hypochromic anemia (12.50%).

We observed that 84.38 % of cases had hypercellular marrow. Megaloblastic anemia was the most common cause of hypercellularity [Figure 1]. Hypocellular and normocellular constituted 10.94% and 4.69% of cases, respectively.

Out of 64 cases, 31 were identified as megaloblastic anemia, the most common cause of pancytopenia. The second most common cause was erythroid hyperplasia with both micro normoblastic and megaloblastic maturation, which was identified in 18.2% of cases and was suggestive of combined deficiency. Less common etiologies include myelofibrosis, multiple myeloma, myelodysplastic syndrome, and filariasis. [Figure 2].

Among 31 cases of megaloblastic anemia, the most common age group affected was 12-23 years, with males being more affected (62.33%) than females (37.66%). Peripheral smear examination revealed features of dimorphic anemia in 72.72% of cases, while bone marrow analysis demonstrated hypercellularity in 89.16% of cases.

In addition, six cases of iron deficiency anemia were identified, with higher prevalence in females aged 15-25 years. Bone marrow studies in most cases showed hypercellularity with an altered M: E ratio, increased erythropoiesis with micro normoblasts, and normal myelopoiesis with minimal increase in megakaryocytes. The bone marrow iron stores were within grade 0-2, indicating absent to reduced iron stores.

Four patients had hypercellular marrow due to leukemia, of which 3 cases were chronic lymphoid leukemia (CLL), and one was acute myeloid leukemia (AML).

In all cases of CLL, peripheral smear showed features of microcytic hypochromic anemia with neutropenia and thrombocytopenia. 90% were mature lymphocytes with the presence of numerous smudge cells. Bone marrow was hypercellular, with 80% mature lymphocytes with smudge cells. Erythroid, myeloid and megakaryocytic series were reduced.

In the case of AML, the peripheral smear showed microcytic hypochromic anemia with neutropenia and thrombocytopenia. Myeloblasts constituted 20% of the differential count, with few showing Auer rods. Bone marrow was hypercellular. Erythroid and megakaryocytic series were reduced. Marrow constituted 40% of myeloblasts. Myeloperoxidase stain on bone marrow aspirate smears showed 30% blast positivity.

Three cases of aplastic anemia were diagnosed. Peripheral smear was normocytic normochromic anemia with neutropenia and thrombocytopenia. Bone marrow aspirate was hypocellular with increased fat cells. Imprint smears also helped access the cellularity when aspiration smears were inadequate for opinion. Bone marrow biopsy showed an increase in adipose tissues in intertrabecular spaces with a relative increase in plasma cells and lymphocytes. All three lineages were reduced in number [Figure 3].

Three cases of multiple myeloma were observed. Peripheral smear findings were predominantly normocytic normochromic anemia with rouleaux formations. Bone marrow was hypercellular. Erythropoiesis, leucopoiesis and megakaryopoiesis were suppressed. There was an abnormal proliferation of plasma cells constituting >40% of differential count with few binucleate and abnormal forms. [Figure 4] In all three cases, hypercalcemia with renal failure was noted, and the presence of 'M' band was seen on immunoelectrophoresis.

One case of myelofibrosis was also detected. Peripheral smear showed microcytic hypochromic anemia. Bone marrow aspirate was dry tap. Biopsy was performed, and imprint smears were prepared and stained with Giemsa stain. A diagnosis of

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microfilaria was given on imprint smears, which completely transformed the patient management [Figure 5]. Clot preparation showed dancing nematodes (Video 1). Bone marrow biopsy showed increased fibrosis, and no parasite was noted. Reticulin stain revealed increased fibrosis.

Two cases were diagnosed as myelodysplastic syndrome (MDS). The peripheral smear of one case showed macrocytic anemia with 10% blast/atypical lymphocyte. Bone marrow aspiration was normocellular with erythroid hyperplasia and megaloblastic maturation. 10% dyserythropoietic cells were noted in aspiration showing karyorrhexis and cytoplasmic irregularity with basophilic granules. Bone marrow biopsy showed hypercellular marrow with erythroid hyperplasia and megaloblastic maturation.

Abnormal localization of myeloid precursors was noted in the intertrabecular spaces of the marrow. Few hypolobated and micro megakaryocytes were also noted. It was reported to have features suggestive of MDS - Single lineage dysplasia, and cytogenetic study was advised.

The peripheral smear finding of the other case of MDS was dimorphic anemia. Bone marrow aspirate showed hypercellular marrow with increased erythropoiesis. Dyserythopoiesis was noted in more than 10% of erythroid series. Myeloid series showed hypogranular myelocytes, metamyelocytes, band forms and neutrophils with few ring neutrophils. Few hypolobated and micro megakaryocytes were noted. It was reported to have features suggestive of MDS with multilineage dysplasia, and cytogenetic study was advised.

Our study demonstrated that the diagnostic accuracy of bone marrow imprint (BMI) was higher (85.9%) than that of bone marrow aspiration (BMA) (84.4%) for diagnosing cases of pancytopenia. However, the specificity was highest for bone marrow biopsy (BMB) at 100% (as shown in [Table 1]). The results of our study indicated a positive correlation with a P value of 0.03.

**Table 1: Diagnostic Accuracy of Bone Marrow Aspiration, Imprint and Biopsy.**

| IMPRESSION	BMA	BMI	BMB
Acute leukemia	6.3	6.3	6.3
Aplastic anemia	40.6	4.7	4.7
Erythroid hyperplasia with megaloblastic maturation	12.5	45.3	48.4
Erythroid hyperplasia with micronormoblastic and megaloblastic maturation	7.8	9.4	12.5
Erythroid hyperplasia with micronormoblastic maturation	3.1	7.8	7.8
Myelodysplastic syndrome(mds)	3.1	1.6	3.1
Normocellular marrow with mild erythroid hyperplasia	7.8	3.1	10.9
Plasma cell neoplasm s/o multiple myeloma	3.1	3.1	4.7
Myelofibrosis	0	4.7	1.6
Total	84.4	85.9	100.0
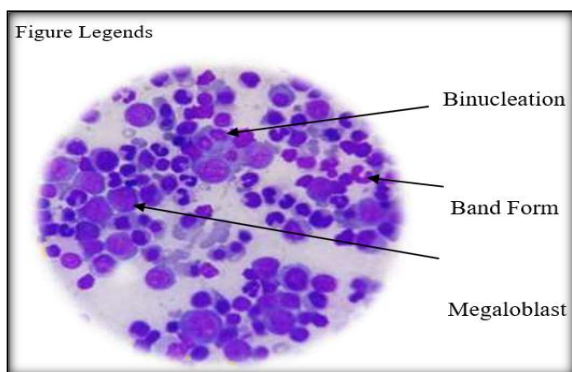
Figure 1: Microphotograph of bone marrow aspiration showing features of megaloblastic anemia.
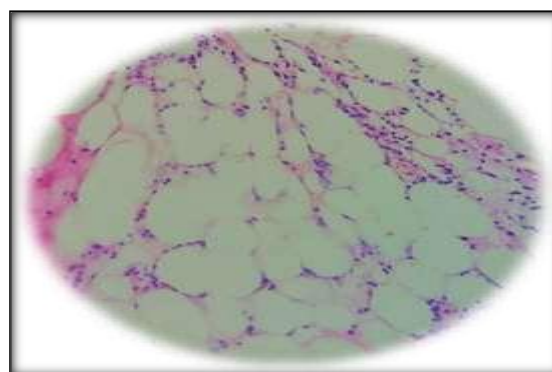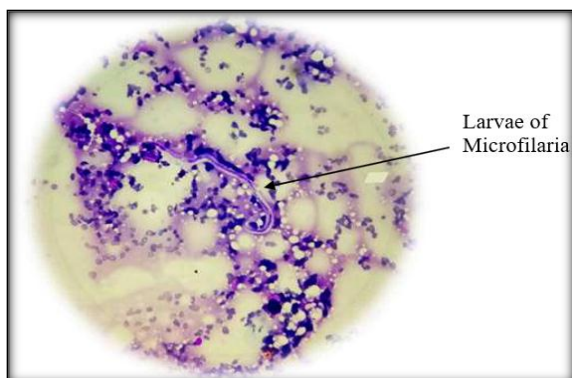

Figure 3: Microphotograph showing increase adipocytes with decrease in all 3 lineages.
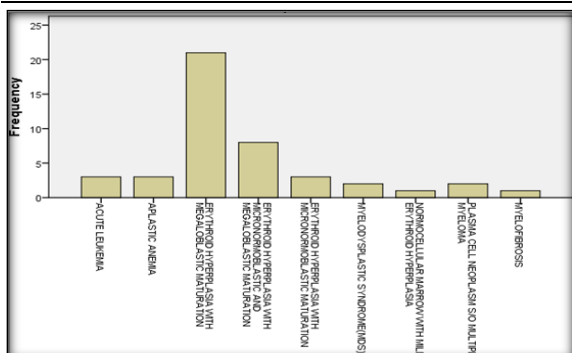
Figure 2: Distribution of Various Causes of Pancytopenia
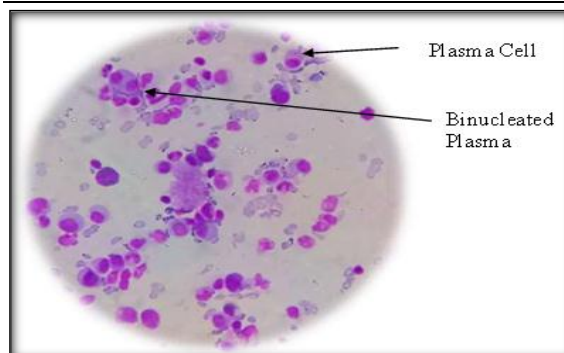
Figure 4: Microphotograph Showing Proliferation Of Plasma Cells

Figure 5: Bone Marrow Imprint Showing Microfilaria.

DISCUSSION

Pancytopenia is a frequently encountered hematological disorder in clinical practice. A comprehensive evaluation that includes clinical assessment, hematological investigations, and bone marrow examination is usually helpful in identifying the underlying cause. However, due to the diverse etiological factors, diagnosing pancytopenia remains challenging. When performed correctly, bone marrow aspiration is essential in investigating hematological disorders.

Trephine biopsy offers a significant advantage in providing information on the architecture of relatively large pieces of marrow while also allowing for the detection of morphological characteristics of individual cells through impression smears.

In our study, the most common age group presenting with pancytopenia was between 23 - 32 years, with a male predominance of 57.81%. These results were consistent with previous study by Bhuyan et al.[4]

According to Singh B et al., R.Pasam et al., Metikurke SH et al., Khunger et al., Gayathri BN et al., Taori G. et al. and Tilak et al., megaloblastic anemia was the most common cause of pancytopenia in their studies, accounting for 50% of cases. In our study, erythroid hyperplasia with megaloblastic maturation (47.7%) was the most common finding, followed by erythroid hyperplasia with micro normoblastic and megaloblastic maturation (12.5%). In the investigations conducted by Tilak et al. and Khunger et al., aplastic anemia was the second most prevalent cause. However, our study only had three cases, indicating that the condition's prevalence varies.[5-11]

In a study of 100 patients with pancytopenia, Ishtiaq O. et al. found five cases of iron deficiency anemia (5%), similar to our study, which had six cases of iron deficiency anemia presenting as pancytopenia. Even though iron deficiency anemia is often associated with reactive thrombocytosis, the severity of the condition causes platelet counts to normalize and, on rare occasions, even decline. Although the precise process is unknown, it may be attributed to the altered activity of iron-dependent enzymes during thrombopoiesis and leucopoiesis.[12]

Khunger JM et al. reported 1% cases of multiple myeloma in their study, whereas we had 2 cases of multiple myeloma, with an incidence of 6.45% [9]. AML, hairy cell leukemia, multiple myeloma, myelofibrosis, hemophagocytic syndrome, tuberculosis, multiple myeloma and drug-induced pancytopenia were uncommon causes of pancytopenia in the studies by Khunger JM et al. and Tilak et al. Myelofibrosis, MDS, and multiple myeloma were rarely seen in our study.[10,11]

One case was diagnosed as myelofibrosis on biopsy, where aspiration was dry tap. One of the imprints showed an incidental finding of microfilaria. Bone marrow biopsy diagnosis of myelofibrosis was given. In a case study by Rahman K. et al., they discussed the association of microfilariae with Myeloproliferative neoplasms or myelofibrosis, which correlated with our clinical scenario.[14]

Studies by Chandra S. et al., Pant S. et al. and Taori G et al. correlated with this study. They concluded that the diagnostic accuracy of bone marrow imprint (95%, 83%, 87%, respectively) was higher than aspiration (84%, 75%, 79%, respectively), whereas biopsy was mostly confirmatory (100%, 99%, 100%, respectively).[7,14,15]

There are some limitations of the bone marrow examination procedure and instruments used, which may affect the accuracy of the diagnosis. Firstly, bone marrow aspiration is a blind procedure, and it may not always yield sufficient material for diagnosis, leading to a dry tap. In such cases, a trephine biopsy or repeat aspiration may be necessary. Additionally, using a small-bore needle during aspiration may result in inadequate sampling, which can affect the diagnostic yield.

Furthermore, the interpretation of bone marrow specimens is highly dependent on the pathologist's expertise, and incorrect interpretation may result in misdiagnosis. The use of suboptimal staining techniques, inadequate fixation, or processing of the specimens can also affect the accuracy of the diagnosis. In addition, rare cases may require specialized techniques such as flow cytometry, cytogenetics, or molecular genetic testing for a definitive diagnosis, which may not be available in all settings.

Finally, using older or suboptimal instruments, such as needles, aspiration syringes, or biopsy needles, can result in inadequate samples, which can negatively impact diagnostic accuracy. Using outdated or poorly maintained instruments can also increase the risk of complications such as bleeding, infection, or nerve damage, which can further complicate the diagnosis and treatment of the underlying hematological disorder.

CONCLUSION

Pancytopenia is a common haematological problem encountered in clinical practice and should be suspected on clinical grounds when a patient

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presents with unexplained anaemia, prolonged fever and tendency to bleed. The physical findings and peripheral blood picture provides valuable information in the work of cytopenic patients. Evaluation of peripheral blood film reveals the most probable cause of anaemia, presence of nucleated RBC's and/or immature myeloid cells may suggest marrow infiltration or primary haematologic disorder.

Bone marrow aspiration is an important diagnostic tool in haematology which helps to evaluate various cases of cytopenia. Bone marrow examination is accurate, reproducible, rapidly available information at an economical cost and with minimal discomfort to the patient. Bone marrow aspiration is sufficient to make a diagnosis in cases of nutritional anaemias and initial diagnosis of leukemia. Megaloblastic anaemia was the commonest cause which indicates the high prevalence of nutritional anaemia in our region. The other common causes were combined nutritional anemia, aplastic anemia and leukemia. However, uncommon and rare causes such as multiple myeloma, storage disease should be kept in mind while planning investigation for complete work up of cytopenic patients.

Present study concludes that numerous causes of pancytopenia which include both non neoplastic and neoplastic conditions should be evaluated by clinical findings and peripheral smear with Bone marrow examination which includes BMA, BMI and BMB. Though the advantage of each procedure differs, both the procedures are complimentary to each other and should be performed simultaneously along with an imprint smear for a complete bone marrow workup and evaluation.

BMI are unquestionably important for investigation of bone marrow pathologies. It provides quick and better information regarding marrow cellularity in comparison to BMA in cases of dry tap. Owing to less mutilation, cellular cytological details are better appreciated in BMI. Even it provides some important diagnostic clues (e.g. marked adipocytosis). All these are ultimately reflected in better diagnostic accuracy of BMI as compared to BMA. It also avoids the unnecessary delay caused by decalcification and processing of BMB sections in routine histopathology laboratories.

Acknowledgement

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