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Review Article

# Clinicopathological Study of Adult Pancytopenia with Special Reference to Bone Marrow Biopsy

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## ABSTRACT

**Background:** Pancytopenia in the peripheral blood is an indication for further haematological investigations. Bone marrow aspiration and biopsy evaluation along with good clinical correlation is of utmost importance to evaluate the causes of pancytopenia that can help in planning further investigations and treatment. The bone marrow trephine biopsy is indicated when there is a dry tap on marrow aspiration.

**Aims:** The present study was a prospective Clinico-haematological study undertaken to analyse the various causes of pancytopenia by evaluating bone marrow aspiration and biopsy and to compare the role of bone marrow aspirate cytology, touch imprint cytology and trephine biopsy for diagnosing wide spectrum of haematological diseases.

**Material and Methods:** This was a prospective study carried out to identify the causes of pancytopenia based on bone marrow examination. Bone marrow aspiration was done in 166 cases and trephine biopsy in 87 cases in the Department of Pathology, JIPMER Pondicherry. Both the aspiration and trephine biopsies were done in single prick utilizing Jamshidi needle. The information on bone marrow aspiration, imprint and biopsy techniques were compared.

**Results:** Total 166 cases of Pancytopenia were examined during period of two years. The commonest cause of pancytopenia was hypersplenism (33.7% cases) followed by aplastic anaemia (13.9%). Other causes included megaloblastic anaemia, myelodysplastic syndrome, infection induced pancytopenia, multiple myeloma and leukaemia. The study also revealed the importance of bone marrow biopsy in differentiating the causes of pancytopenia

**Conclusion:** Bone marrow aspiration coupled with trephine biopsy diagnosed majority cases of pancytopenia. The advantages of bone marrow aspiration and biopsy may vary but both are complimentary to each other and should be done simultaneously for a complete bone marrow work up and evaluation.


**Keywords:** Pancytopenia; Bone marrow aspiration; Bone marrow biopsy; Jamshidi needle

## INTRODUCTION

Pancytopenia is not a disease entity, but a triad of findings, resulting from various disease processes. The criteria for diagnosis of pancytopenia includes haemoglobin level less than 13.5g/dl in males or 11.5g/dl in females, leukocyte count less than $4x10^9$/l and platelet count less than $150x10^9$/l [1]. The causes of pancytopenia can vary from simple deficiency states like megaloblastic anaemia and infectious diseases to malignant conditions such as leukaemia, lymphoma and myeloma.

The major diagnostic problems occur when there are no specific features in the peripheral smear to point to the cause. Patients having laboratory values suggestive of pancytopenia require sequential examination of reticulocyte count, bone marrow aspiration and biopsy to detect the dreaded causes such as aplastic anaemia and subleukemic leukemia.

Bone marrow examination is extremely helpful in evaluation of pancytopenia. Bone Marrow Aspirate Cytology (BMA), Touch Imprint Cytology (BMI) and Trephine Biopsy (BMB) are the three main basic preparations for bone marrow evaluation. Marrow aspiration is assessed for cytology and trephine biopsy provides overall cellularity, detection of focal lesion and infiltration. The severity of pancytopenia and underlying pathology determines the management and prognosis of patients [2].

There were many studies highlighting pancytopenia in different study groups. In those studies, commonest causes for pancytopenia were hypersplenism [3,4], aplastic anaemia [5-8] and megaloblastic anaemia [2,9]. Other less frequent causes included in the studies were infections, leukemia, myelodysplastic- syndrome and non-Hodgkin lymphoma.

There is paucity of literature on comparison of bone marrow biopsy to bone marrow aspiration in pancytopenia. Both the procedures are complimentary to each other and should be done simultaneously along with an imprint smear for a complete bone marrow workup and evaluation.

The aim of this study was to find out the frequency distribution of causes of pancytopenia and to analyze the spectrum of bone marrow pathology and compare the role of bone marrow aspirate cytology, touch imprint cytology and trephine biopsy for diagnosing wide spectrum of haematological diseases.

## MATERIALS AND METHODS

In this prospective study of two years, 166 adult cases of pancytopenia were included from the department of pathology JIPMER, Pondicherry in collaboration with the department of medicine.

The cases those fulfilled the criteria of pancytopenia were included in the study. Children less than 13 years, patients with Idiopathic Thrombocytopenic Purpura (ITP) and patients on chemotherapy were excluded from the study.

Detailed clinical history and physical examination was done and special investigations including biochemical, microbiological and radiological investigations were done whenever necessary.

All the patients with pancytopenia were subjected to complete blood count and peripheral blood smear examination and checked for having any major coagulation disorder before undergoing further procedure. After taking informed consent, Bone Marrow Aspiration (BMA) was carried out from posterior superior iliac spine of the patients. Then the bone marrow trephine biopsy was performed using Jamshidi needle. The biopsy was fixed in 10% buffered formalin and then decalcified. Before fixation of the biopsy, touch imprint smears were prepared by using the procedure of gentle touch and rolling of the biopsy core on the slide, and then trephine biopsy specimens were processed in tissue processor and embedded in paraffin blocks, 2-3um thin sections were taken. The slides of BMA and BMI smears were stained with Leishman and Wright-Giemsa methods and special stains such as pearl's stain, periodic acid-Schiff and myeloperoxidase stain were done wherever necessary, while the bone marrow biopsy slides were stained by haematoxylin-eosin, reticulin and pearl's methods.

## RESULTS

A total of 166 adult patients who presented with pancytopenia were studied. In the present study, hypersplenism was found to be the most common aetiology of pancytopenia followed by aplastic anaemia. Other common causes included infection induced pancytopenia, megaloblastic anaemia, subleukemic leukemia and pancytopenia with normal active marrow.

Other causes of pancytopenia were multiple myeloma, metastatic carcinoma, and myelodysplastic syndrome, tropical splenomegaly syndrome with demonstrable malarial parasite, lymphoreticular malignancy, and connective tissue disorders (Table 1).

Hyersplenism was the commonest cause of pancytopenia in the present study which constituted 56 cases out of total 166 cases. Age of the patients ranged from 14-80 years and 46.19% cases were below 30 years. Predominant symptoms were symptoms of anemia, mass in abdomen, menorrhagia and haematemesis. Peripheral smear examination showed normocytic, normochromic RBCs. Bone marrow aspiration was done in all cases. 64.3% cases showed hypercellular marrow, 32.1% showed normocellular marrow. Bone marrow biopsy was done in 27 cases, 44% showed hypercellularity and 56 % showed normocellularity.

Aplastic anaemia was the second common cause for pancytopenia in the present study, which included 23 out of 166 cases. Patients predominately showed haemorrhagic manifestations like epistaxis, bleeding gums and purpura, and infective manifestations like fever and sore throat.

Out of 23 cases, 15 cases were of primary aplastic anaemia and in remaining 8 cases, underlying cause was correlated and was grouped as secondary aplastic anaemia. Peripheral smear showed normocytic, normochromic RBC morphology, leucopenia with lymphocytic preponderance, prominent thrombocytopenia & reticulocytopenia. Bone marrow aspiration was done in all cases. Sixty-one (61%) showed hypocellularity, 13% showed normocellular bone marrow. These cases on subsequent trephine biopsy showed <25% cellularity.

**Table1:** Distribution of cases presenting with pancytopenia.

| Cases of pancytopenia | Percentage of cases | Number of cases |
|---|---|---|
| Hypersplenism | 33.17% | 56 |
| Aplastic anaemia | 13.9% | 23 |
| Infection induced pancytopenia | 10.8% | 18 |
| Pancytopenia with normal active marrow | 9.6% | 16 |
| Megaloblastic anaemia | 9% | 15 |
| Sub leukemic leukemia | 9% | 15 |
| NHL | 2.4% | 4 |
| Tropical splenomegaly | 2.4% | 4 |
| Multiple myeloma | 2.4% | 4 |
| MDS | 2.4% | 4 |
| Haemophagocytic syndrome | 1.2% | 2 |
| SLE | 0.6% | 1 |
| Metastasis | 0.6% | 1 |
| Inconclusive | 1.8% | 3 |

Dry tap was seen in 26.1% bone marrow aspirations. Bone marrow trephine biopsy was done in 15 cases. All showed hypocellularity with intertrabecular marrow space occupied predominantly by adipose tissue, with scattered lymphocytes and plasma cells, thus giving cellularity ranging from 10% - 35%.

Fifteen (15) cases of subleukemic leukemia and aleukemic leukemia presenting with pancytopenia were studied which included 9 cases of acute lymphoid leukemia and 6 cases of myeloid leukemia. Peripheral smear examination showed blasts ranging from 3% -20% in 8 out of 15 cases. Remaining 7 cases were of aleukemic leukemia. Bone marrow aspiration was done in all cases. 3 cases resulted in dry tap and 12 cases showed hypercellularity with sheets of leukemic cells and suppression of normal haematopoiesis. In l1 out of 15 cases, bone marrow biopsy was done, which showed marked hypercellularity due to diffuse proliferation of blast cells.

Megaloblastic anaemia cases were 15 in number presented with pancytopenia features. Peripheral smear showed macro-ovalocytes with hypersegmented neutrophils. Bone marrow aspiration showed hypercellularity in 93.3% cases with characteristic megaloblastic erythropoiesis. Bone marrow biopsy was done in 5 cases. Four cases (4) showed hypercellularity with cellularity ranging from 55% to 90%.

Eighteen (18) cases of infection-induced pancytopenia were studied. Underlying confirmed infectious aetiologies were Hepatitis B, HIV, tuberculosis and enteric fever.

Four (4) cases of Non-Hodgkin's Lymphoma (NHL) cases presenting as pancytopenia were included. Bone marrow biopsy was done in 3 cases which showed hyper cellular marrow with infiltration by NHL cells varying from focal aggregates to diffuse infiltration.

Four (4) cases of tropical splenomegaly syndrome presenting as pancytopenia were included. Bone marrow aspiration showed hyper cellular marrow, with demonstrable plasmodium vivax parasite

Multiple myeloma was diagnosed in 4 cases of pancytopenia. Bone marrow aspiration showed myeloma cells and bone marrow biopsy was done in 3 cases.

Present study included two interesting cases of haemophagocytic syndrome presented as pancytopenia. Bone marrow aspiration showed increased histiocytes and haemophgocytosis.

There were 16 cases of pancytopenia with normal active marrow, where no underlying cause for pancytopenia was detected.

Four cases of MDS and one case of SLE presenting as pancytopenia were included in the study.

An interesting finding of bone marrow metastasis with advanced case of adenocarcinoma presenting as pancytopenia showed desmoplastic reaction in marrow and an osseous metaplasia secondary to bone marrow metastasis.

Based on the haematological findings and other relevant investigations, the cases diagnosed based on BMA, BMI and BMB were as per table 2.

Failed bone marrow aspiration established diagnosis by trephine biopsy in aplastic anaemia, leukemia and multiple myeloma

From these comparisons, it is obvious that in general, bone marrow biopsies gave better amount of material, followed by bone marrow aspirations, followed by bone marrow imprint. (BMB > BMA > BMI).

**Table 2:** Cases diagnosed on bone marrow aspirate, imprint cytology and trephine biopsy.

| Diagnosis | BMA | | | BMI | | BMB | | |
|---|---|---|---|---|---|---|---|---|
| | Adequate | Inadequate | Failed | Adequate | Inadequate | Adequate | Inadequate | Failed |
| Hypersplenism (27) | 25 (92.5%) | 2 | - | 24 (88.8%) | 3 | 24 (88.8%) | 1 | 2 |
| Aplastic anaemia (17) | 11 (64.7%) | 1 | 5 | 11 (64.7%) | 6 | 15 (88.2%) | 0 | 2 |
| Leukemia(11) | 7 (63.6%) | 1 | 3 | 8 (72.7%) | 3 | 9 (81.8%) | 2 | - |
| Megaloblastic anaemia (5) | 5 (100%) | 0 | 0 | 0 (100%) | - | 9 (81.8%) | 2 | - |
| Others (28) | 19 (67.8%) | 2 | 5 | 27 (96.4%) | - | 1 | 26 (92.8%) | - |

Intercellular relations were better appreciated in bone marrow biopsy followed by bone marrow imprint followed by bone marrow aspiration (BMB >BMI > BMA).

Bone marrow biopsy was complimentary in infection induced pancytopenia and hypersplenism. Bone marrow trephine biopsy provides additional information in diagnosing the cases with pancytopenia (Table 3).

## DISCUSSION

Pancytopenia is a common haematological finding with variable clinical presentations. It often creates diagnostic challenge to physician and the knowledge of accurate causes of this condition is crucial in the management of the patient [10]. Bone marrow examination is a useful test in reaching the final diagnosis.

In the present study of 166 cases of pancytopenia, the most common causes were hypersplenism, aplastic Anaemia, infection induced pancytopenia, subleukemic leukemia and megaloblastic Anaemia.

In this study, most common cause of pancytopenia was hypersplenism. This constituted 33.17% of total cases of pancytopenia. Findings are similar to other study Jain a et al in which hypersplenism was a commonest cause of pancytopenia [3]. In hypersplenism there is peripheral pooling and destruction of cells in an enlarged spleen resulting in cytopenias. Increase in incidence of hypersplenism is due to chronic liver disease and infectious diseases in India. Malaria, especially Plasmodium falciparum, may cause pancytopenia as a result of hypersplenism, immune haemolysis, DIC, bone marrow necrosis, haemophgocytosis, and impairment of marrow function or direct bone marrow invasion by the parasite [3].

The second major cause of pancytopenia was aplastic anaemia in present study (13.9%) which correlated with the study done by Tilak, et al and khodke, ET al [11,12]. Aplastic anaemia may be due to environmental factors or exposure to pesticides/drugs/toxic chemicals. Causes for secondary aplastic anemia in our study were chloromphenicol, ciprofloxacin, ibuprofen, hepatitis B.

Infection induced pancytopenia was third most common cause in our study. Underlying infections causes were hepatitis B, HIV, tuberculosis and enteric fever. Infections causing pancytopenia was the 2nd commonest cause of pancytopenia in the study done by Jain a et al, accounting for 25.6% cases [3]. HIV has been shown to cause bone marrow failure and subsequent pancytopenia. The degree of haematologic findings in the course of HIV infection varies widely. However, after a period of clinical latency, the bone marrow becomes hypo- cellular with a resulting pancytopenia [13].

**Table 3:** Added information gained by trephine biopsy over aspiration in pancytopenia.

| Pancytopenia causes | Bone marrow biopsy findings |
|---|---|
| Hypersplenism | Decreased marrow iron |
| Aplastic anaemia | Hypo plastic bone marrow documentation |
| Acute leukemia | Increased intertrabecular cell density<br>Diffuse replacement by blasts<br>Variable increase in recticulin<br>Morphologically differentiation between ALL & AML was possible. |
| Megaloblastic anaemia | Pseudoleukemia due to abnormally large dyspoietic erythroid nuclei |
| Metastatic adenocarcinoma | Desmoplasia with neoosteogenesis |
| MDS | Clustering of myeloblasts and promyelocytes in the centre of marrow spaces<br>Clustering of dysplastic megakaryocytes and micro- megakaryocytes. |
| Lymphoma | Focal non-paratrabecular infiltrate with lymphoma cells<br>Diffuse infiltration with lymphoma cells |
| Tuberculosis | Epithelioid granuloma |
| HIV | Hypocellularity, edema<br>Histiocytic and epithelioid granuloma<br>Bone marrow haemosiderosis |

Development of pancytopenia in enteric fever has been attributed to bone marrow suppression, necrosis, infection associated haemophagocytic syndrome, disseminated intravascular coagulation and development of septicemic complications [14].

Tuberculosis is a common disease in India and in many other countries. Miliary tuberculosis is known to cause pancytopenia and there are few reports of pulmonary tuberculosis too causing pancytopenia. It is advised to consider tuberculosis as differential diagnosis in patients presenting with pancytopenia, unexplained fever and weight loss. Degree of pancytopenia is affected more by duration of infection than by its severity [15,16].

Megaloblastic anaemia in our study constituted 9% of total cases of pancytopenia. Megaloblastic anaemia was found to be predominant cause in various studies. There is a high correlation for aspiration and biopsy especially in the case of megaloblastic anaemia with erythroid hyperplasia.

We had 9% cases of subleukemic leukemia (4.8%) and aleukemic leukemia (4.2%). In the study of Aziz, et al. acute leukemia constituted almost 10% of cases of pancytopenia [17]. Immature cells can be observed in peripheral smears or in smears made from buffy coat. Bone marrow aspiration establishes the diagnosis; however, if the tap is dry then bone biopsy is must for diagnosis.

We had 4 (2.4%) cases of NHL with evidence of bone marrow involvement. NHL is known to infiltrate bone marrow more commonly than Hodgkin's disease and thus leading to pancytopenia. The incidence of NHL in other similar studies varies from 0.9 to 10% [8,18].

Four cases (2.4%) of Myelodysplastic Syndrome (MDS) were diagnosed in our study. It was the third most common cause of pancytopenia in the study by PM Devi, et al [5]. MDS is most common in the elderly and should be included in the differential diagnosis of elderly patients presenting with pancytopenia.

We found 4 (2.4%) cases of multiple myeloma presenting as pancytopenia. Similar other studies too have reported multiple myeloma presenting as pancytopenia, the incidence varies from 0.9 to 4% [3].

Difference in the frequency of disorders causing pancytopenia has been due to variation in study design, geographic area, genetic differences, and duration of observation, diagnostic criteria and varying exposure to cytotoxic/chemical agents [2] (Table 4).

## CONCLUSION

The present study concluded that most common cause of pancytopenia is hypersplenism, followed by aplastic anaemia and infection induced pancytopenia. Bone marrow aspiration coupled with biopsy in patients with pancytopenia is helpful for understanding disease process and to diagnose or to rule out the causes of pancytopenia**.** Though the advantage of each procedure varies, both the procedures are complimentary to each other and should be done simultaneously along with an imprint smear for a complete bone marrow workup and evaluation. BMA gives better cellular details when compared to BMI and BMB. BMB is the diagnostic investigation in dry tap cases like aplastic anaemia, myelodysplastic syndrome, idiopathic myelofibrosis and metastatic tumours. BMB is also useful in granulomatous diseases affecting bone marrow and in staging of lymphoma.

Bone marrow sampling for both aspiration and biopsy is a painful experience for patients according to the authors of this paper. Hence, the novel approach of bone marrow aspiration and biopsy with one prick of Jamshidi needle eliminates this shortcoming.

**Table 4:** Common causes of pancytopenia in different study groups.

| Study | Country | No. of cases | Commonest cause | 2nd common cause | 3rd common cause | 4th common cause |
|---|---|---|---|---|---|---|
| Shah P, et al [2] | India | 40 | MA (35%) | AA (32.5%) | Normocellular marrow (5%) | Other causes (25%) |
| Jain A, et al [3] | India | 250 | HS (29.2%) | Infections (25.6%) | Myelosuppressants (16.8%) | MA (13.2%) |
| Kale P, et al [4] | India | 70 | HS (47.6%) | MA (25.4%) | AL (14.5%) | Infections (7.25%) |
| Devi PM, et al [5] | India | 50 | HA (22%) | MA (18%) | MDS (18%) | Subleukemic leukemia (14%) |
| Jha A, et al [6] | Nepal | 148 | HA (29.05%) | MA (23.64%) | EH (13.25%) | AL (9.64%) |
| Jalbani A, et al [8] | Pakistan | 40 | AA (32.5%) | HS (22.5%) | MA (15%) | NHL (10%) |
| Gayathri BN, et al [9] | India | 104 | MA (74.04%) | AA (18.3%) | Subleukemic leukemia (3.8%) | Malaria (2%) |
| Present study | India | 166 | HS (33.7%) | AA (13.9%) | Normocellular marrow (9.6%) | MA (9%) Subleukemic leukemia (9%) |

**HS: Hypersplenism; MA: Megaloblastic anemia; AA: Aplastic Anemia; AL: Acute leukemia; HA: Hypoplastic anemia; NHL: Non-Hodgkin's lymphoma; MDS: Myelodysplastic syndrome; EH: Erythroid hyperplasia.**

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