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# BONE MARROW EXAMINATION IN 100 CASES OF PANCYTOPENIA – A PROSPECTIVE STUDY

Pathology

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## ABSTRACT

New-onset pancytopenia proves to be a diagnostic dilemma, in both children and adults. The workup of new-onset pancytopenia is extensive and should include a detailed clinical history and hematological investigations. One hundred patients of pancytopenia were included in this study. Relevant history, clinical and hematological examination was done. Peripheral smear and bone marrow examination was performed in all cases. Special stains like Perl`s, reticulin were also done wherever necessary. Aplastic anemia was found to be the most common cause of pancytopenia followed by megaloblastic anemia. Other causes were acute myeloid leukemia, myelofibrosis, myelodysplastic syndrome, malarial parasite, multiple myeloma, miliary tuberculosis and osteopetrosis. Though peripheral smear and bone marrow evaluation gives a clue in identifying the various etiologies of pancytopenia, it is only through the correlation of clinical and hematological findings, a precise and accurate evaluation and management of patients of pancytopenia can be made.

## KEYWORDS

Aplastic Anemia, Megaloblastic Anemia, Bone Marrow Examination, Pancytopenia

## INTRODUCTION

Sir William Harvey, an English physician, rightly described blood as "the fountain of life and the primary seat of the soul. Examination of the peripheral smear and bone marrow is essentially important in the diagnosis and management of wide variety of hematological disorders. Bone marrow examination although reveals an underlying pathology causing pancytopenia, is not always conclusive. Understanding the various disorders that may cause pancytopenia is essential so that additional testing and clinical evaluation can be recommended when the marrow studies are not conclusive for a single etiology.

## METHODS

This is a prospective study undertaken in the Institute of pathology and haematology, Madras medical college and Rajiv Gandhi Government General Hospital, Chennai from August 2015 to August 2016 on hundred patients of pancytopenia with the following criteria.

## INCLUSION CRITERIA:

- Hemoglobin < 9 gm/dl
- Total Leucocyte count < 4000/ µL
- Platelet Count <100000/ µL[1]

## EXCLUSION CRITERIA:

Patients on myelotoxic chemotherapy were excluded

## RESULTS

In this study there was a wide age range with the youngest patient being 13 years of age and the maximum being 80 years of age. The peak incidence was found in the age group of 51 – 60 years. (TABLE 1) Among the total 100 cases of pancytopenia there were 58 male patients and 42 female patients and the male to female ratio was 1.38 :1. In this study the most common cause of pancytopenia was aplastic anemia seen in 44 % of cases. The second most common cause was megaloblastic anemia which was observed in 37 % of cases. Other causes were acute myeloid leukemia, myelodysplastic syndrome, myelofibrosis, malarial parasite, miliary tuberculosis, multiple myeloma and osteopetrosis. (TABLE 2)

**TABLE 1: AGEWISE DISTRIBUTION OF PANCYTOPENIA**

| AGE GROUP | FREQUENCY | PERCENT |
|---|---|---|
| 11 -20 | 18 | 18 |
| 21 -30 | 15 | 15 |
| 31 -40 | 18 | 18 |
| 41 -50 | 19 | 19 |
| 51 -60 | 22 | 22 |
| 61 -70 | 4 | 4 |
| 71 -80 | 4 | 4 |
| TOTAL | 100 | 100.0 |

**TABLE 2: DISTRIBUTION OF CAUSES OF PANCYTOPENIA**

| CAUSES | FREQUENCY | PERCENT |
|---|---|---|
| APLASTIC ANEMIA | 44 | 44 |
| MEGALOBLASTIC ANEMIA | 37 | 37 |
| ACUTE MYELOID LEUKEMIA | 8 | 8 |
| MYELODYSPLASTIC SYNDROME | 3 | 3 |
| MYELOFIBROSIS | 3 | 3 |
| MALARIAL PARASITE | 2 | 2 |
| MILIARY TUBERCULOSIS | 1 | 1 |
| MULTIPLE MYELOMA | 1 | 1 |
| OSTEOPETROSIS | 1 | 1 |
| TOTAL | 100 | 100.0 |

Bone marrow aspiration and biopsy was done in all cases. Bone marrow aspiration was hypercellular in 46% cases, which included 37 cases of megaloblastic anemia. Hypercellular marrow was also seen in acute myeloid leukemia and multiple myeloma. Marrow was hypocellular in 48 % cases, which included 44 cases of aplastic anemia and 3 cases of myelofibrosis and one case of osteopetrosis. Normal marrow cellularity was seen in 4% cases which was observed in myelodysplastic syndrome and miliary tuberculosis. Megaloblastic maturation, presence of myeloblasts, dysplastic features in all three lineages, abnormal plasma cells were salient diagnostic features in bone marrow examination.

In the present study 44 cases were aplastic anemia. with the peak incidence in 51 -60 years. Peripheral smear showed normocytic normochromic red blood cells with marked neutropenia and thrombocytopenia. Bone marrow aspiration showed marrow fragments with marked increase in fat spaces. Trephine biopsy showed increased fat spaces with focal areas of cellularity. (Figure 1) Grading of iron stores were done using Perl's stain in bone marrow aspirates which showed grade 3 in 23% of cases, grade 4 in 49% and grade 5 in 28% of cases. (Figure 2)

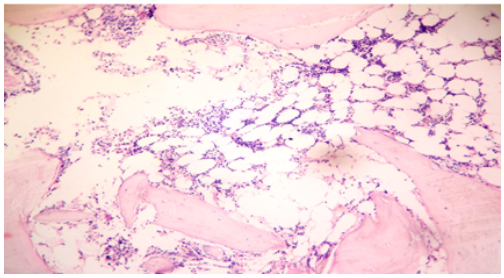

**Figure 1: Aplastic anemia: Bone marrow trephine biopsy showing increased fat spaces with focal areas of cellularity. (40 x)**

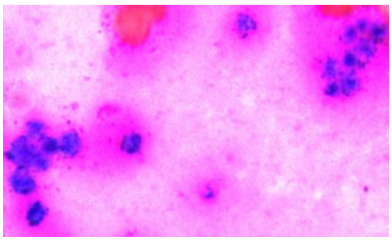

**Figure 2: Aplastic anemia: Bone marrow aspirate shows clumps of dense granules –perl`s stain (40 x)**

Out of 100 cases 37 were diagnosed as megaloblastic anemia. Most of the cases were between 51 to 60 years. Peripheral smear showed macro- ovalocytes with hypersegmented neutrophils, cabot`s ring, basophilic stippling. Bone marrow was hypercellular with erythroid hyperplasia exhibiting megaloblastic maturation. (Figure 3) Giant metamyelocytes and band forms were seen. Bone marrow trephine biopsy showed megaloblasts with uniform size and shape having open nuclear chromatin with 1 to 4 linear nucleoli. Increased marrow iron stores were demonstrated by Perl's stain. Increased iron stores could be due to malabsorption.

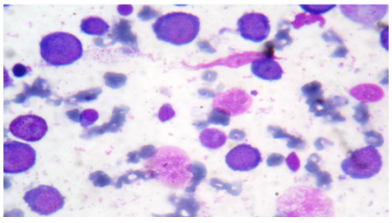

**Figure 3: Megaloblastic anemia: Bone marrow aspirate shows erythroid hyperplasia with megaloblasts (100 x)**

A total of 8 cases was diagnosed as subleukemic leukemia. In Peripheral smear 1 to 2% myeloblasts with Auer rods were seen. Bone marrow aspiration was hypercellular with 80 to 90 % myeloblasts. Trephine biopsy showed replacement of marrow by sheets of blasts with vesicular nuclei and prominent nucleoli. (Figure 4) Blasts were myeloperoxidase (MPO) positive. (Figure5). Marrow fibrosis was demonstrated by reticulin stain and the grade varied from 2 to 4.

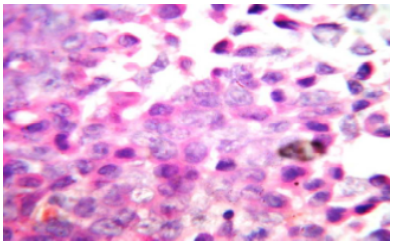

**Figure 4: AML: Bone marrow trephine biopsy shows a cellular marrow with large blasts with round to oval nuclei and prominent nucleoli (40 x)**

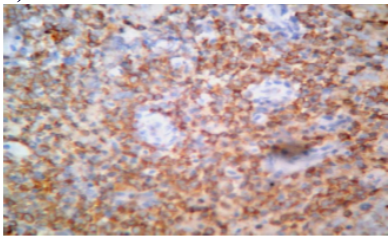

**Figure 5: AML: Bone marrow trephine biopsy demonstrates myeloblasts with MPO positivity (40 x)**

A total of 3 cases were diagnosed as myelofibrosis. Out of the 3 cases ,2 cases were reported in 51-60 years of age and one case was reported in 65 years of age. Peripheral smear showed a leukoerythroblastic blood picture. Marrow aspiration yielded a dry tap due to fibrosis. Trephine biopsy revealed sinusoidal dilation with clustering of megakaryocytes exhibiting hypolobation with hyperchromatic and bulbous nuclei. (Figure 6) Evidence of marrow fibrosis was seen and reticulin stain showed grade 3 to 4. (Figure 7)

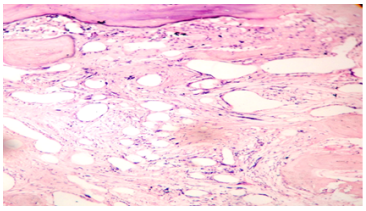

**Figure 6: Myelofibrosis. Bone marrow trephine biopsy showing dilated marrow sinusoids with marked fibrosis (40 x)**

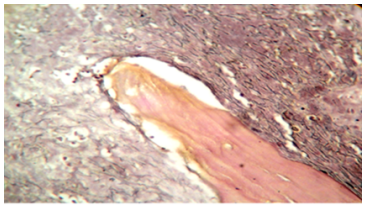

**Figure 7: Myelofibrosis: Reticulin stain demonstrates thick reticulin fibrils throughout marrow (40 x)**

In the present study 3% of cases were diagnosed to have myelodysplastic syndrome. All the three cases were seen in males. It was diagnosed as multilineage dysplasia with refractory cytopenia. Peripheral smear showed dimorphic blood picture with marked anisopoikilocytosis. Bone marrow aspiration showed dysplasia in all the three cell lineages. Erythroid series showed megaloblastoid maturation with nuclear budding, internuclear bridging and multinucleation. Defective granulation, nuclear hypolobation and maturation arrest at myelocyte stage were seen in myeloid series. Nuclear hypolobation and hypogranulation were evident in megakaryocytes. Bone marrow trephine biopsy shows megakaryocytes with features of dysmegakaryopoiesis. (Figure 8) Increased marrow iron stores were seen and grading varied from 3 to 5.

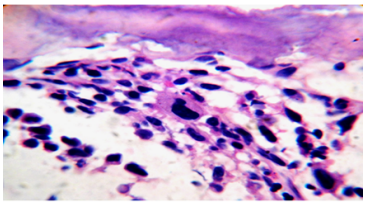

**Figure 8: Myelodysplastic syndrome: Bone marrow trephine biopsy shows megakaryocytes with features of dysmegakary opoiesis (40 x)**

In this study, one case was diagnosed as multiple myeloma with pancytopenia. The age of patient with multiple myeloma was 69 years and the patient were a female. X-ray skull showed punched out lytic lesions. The serum electrophoresis showed presence of M-band. Increased rouleaux formation was observed in peripheral smear. There were 38% of plasma cells in bone marrow with binucleate forms. (Figure 9) Trephine biopsy showed plasma cells with CD 138 positive. (Figure 10)

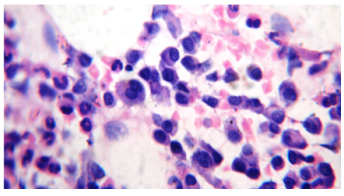

**Figure 9: Multiple myeloma: Bone marrow trephine biopsy shows sheets of plasma cells having eccentric nuclei (40 x)**

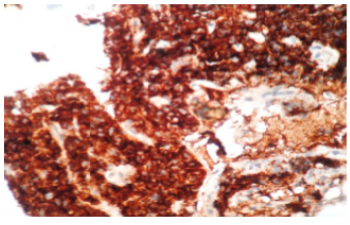

**Figure 10: Multiple myeloma: Plasma cells showing strong positivity for CD 138 (40 x)**

One case was reported as miliary tuberculosis causing pancytopenia. Patient was a 31-year-old female. Leukoerythroblastic blood picture was seen in peripheral smear. In this case numerous epithelioid granulomas were seen in the marrow. (Figure 11) But this patient died despite treatment given.

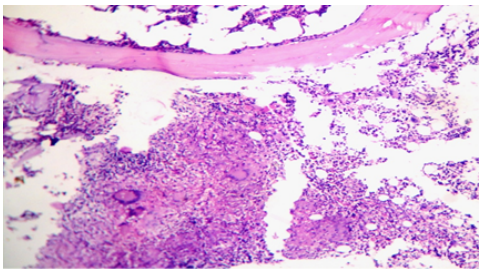

**Figure 11: Miliary tuberculosis: Bone marrow biopsy shows epithelioid granuloma with langhans giant cells (40 x)**

Osteopetrosis is a rare cause of pancytopenia. One case of osteopetrosis was reported in this study. The patient was a male and the age of the patient was 20 years. Bone marrow aspiration showed clusters of osteoblasts and biopsy showed increased thickening of the bony trabeculae with reduction in the medullary cavity. Medullary space showed increase in connective tissue. (Figure 12)

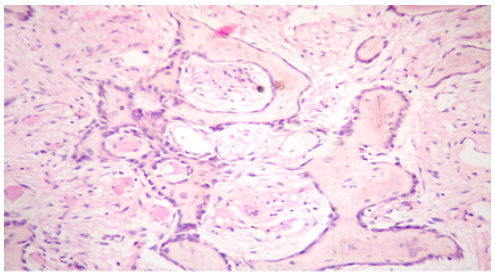

**Figure 12: Bone marrow biopsy shows thickened bony trabeculae with diminished medullary cavity (40 x)**

## DISCUSSION:

Pancytopenia is a commonly encountered hematological problem in clinical practice. However, there are only few numbers of studies and researches available from subcontinents like India, on the frequency and distribution of various etiological profile of pancytopenia.

Most of the studies done in our country revealed megaloblastic anemia as the most common cause of pancytopenia followed by aplastic anemia. However, few studies done by Kumar et al, Jha et al, Tariq et al, Santra and Das and Qamar and Aijaz, Bajracharya et al. showed aplastic anemia as the most common etiology followed by megaloblastic anemia. [2,3,4,5,6,7] This was similar to the present study.

The incidence of aplastic anemia in this study was 44% (44/100 cases) which is higher than the studies done by Khodke et al. and the Khunger et al. in which the incidence was 14 %.[8,9] Kumar et al reported a higher incidence viz 29.5%.[2] In this study all the cases of aplastic anemia had reticulocyte counts <1%, which is a criterion for diagnosis of severe aplastic anemia as mentioned by Kaufman et al. in their study.[10] Bone marrow biopsy is performed in addition to aspiration to assess cellularity qualitatively and quantitatively.

Aspiration samples alone may appear hypocellular because of technical reasons (eg, dilution with peripheral blood), or they may appear hypercellular because of areas of focal residual hematopoiesis. By comparison, core biopsy better reveals cellularity.

Megaloblastic anemia constituted 37% of cases in the present study which was the second common cause of pancytopenia. The study done by Qamar and Aijaz reported the incidence to be 36.6% which was similar to the present study.[6] Among the 37 cases, 27(73%) cases of megaloblastic anemia were vegetarian and presented with pancytopenia which was similar in the study by Khanduri and Sharma in which 71% of vegetarian suffered from megaloblastic anemia.[11] Reticulocyte counts was below 2% in all 37 patients with megaloblastic anemia. This has been observed by different studies done by Khanduri and Sharma and Aslinia et al.[11,12] Bone marrow aspiration is usually not needed to make the diagnosis of vitamin B-12 deficiency. However, it can help rule out myelodysplasia and assess iron stores. Bone marrow megaloblastic changes are reversed within 12 hours after treatment with cobalamin or folate, and bone marrow morphology appears to be normal within 2-3 days. Therefore, bone marrow aspiration, if necessary, should be performed as soon as possible and preferably before therapy.

In the present study, 8 (8 %) patients with pancytopenia had acute myeloid leukemia. Bone marrow aspiration was hypercellular with cellularity ranging from 80 % to 90 %. Blasts in the study group ranged from 40 to 80%. Auer rods were seen in the blasts. Myeloperoxidase was positive in all the cases. These findings correlate well with observations of studies done by Prashanth et al.[13] Bone marrow aspiration and biopsy are complimentary in diagnosing cases of acute and chronic leukemia. Trephine biopsy is more accurate than aspiration in assessing the degree of cellularity, pattern of infiltration, marrow fibrosis and myeloperoxidase positive blasts.75% showed grade 4 fibrosis Grade 2 fibrosis was seen in 20% of cases and 5% showed grade 1 fibrosis. Normal iron stores (grade 2 to 3) were seen in 80% of cases and reduced iron stores (grade 0 to 1) was seen in 20% of cases.

In the present study 3% of cases were diagnosed to have myelodysplastic syndrome. In the studies done by Prashanth et al incidence was 5.97%.[13] Peripheral smear shows only anisopoikilocytosis therefore bone marrow examination is the gold standard in arriving at a precise diagnosis for myelodysplastic syndrome. Trilineage dysplasia and hypercellular marrow in the absence of vitamin deficiency is diagnostic of MDS. The presence of typical chromosomal abnormalities supports the diagnosis and contributes to determining the prognosis of MDS. Usually the marrow is hypercellular but sometimes marrow can be hypocellular. Proper diagnosis is crucial for appropriate treatment and management.

Among the 100 cases three cases were reported as myelofibrosis. The present study had a lesser incidence when compared to the study done by Tejeswini et al with reported incidence of myelofibrosis in 5.2% of cases.[14] Bone marrow aspiration yields only dry tap. The bone marrow trephine biopsy typically shows abnormal fibrosis of the marrow cavity. Cytogenetic and molecular analysis of blood and bone marrow cells is also carried out to help confirm the diagnosis and may help with prognosis.

In this study, one case was diagnosed as multiple myeloma with pancytopenia. The age of patient with multiple myeloma was 69 years and the patient were a female. Reported incidence of Multiple Myeloma in pancytopenia patients was 4% by Khodke et al.1.3 % by Khunger JM et al and 1.3 % by Tilak V et al.[8,9,15] Though marrow examination helps in diagnosing multiple myeloma, a confirmatory diagnosis can be arrived after skull X-ray, protein electrophoresis and Bence jones protein in urine. Immunohistochemistry using plasma cell markers can be done in marrow.

One case of miliary tuberculosis was reported as a cause of pancytopenia. This was similar to the studies done by Sweta et al and Fauzia Shafi Khan et al in which one percent cases were reported.[16,17] Peripheral smear showed a leucoerythroblastic blood picture. Bone marrow aspiration smear was diluted with peripheral blood. Trephine biopsy revealed granulomas with caseation. Besides tuberculosis a number of diseases manifest granulomas in the marrow. A confirmatory diagnosis can be established with relevant history, hematological investigations, imaging and special stains like ziehl neelsen stain for acid fast bacilli.

One case of osteopetrosis was diagnosed as a cause of pancytopenia. It refers to increase in mineralized bone mass. Peripheral smear frequently shows a leucoerythroblastic blood picture. This condition is usually diagnosed by increased radiodensity in bone on X-ray.

In the literature reviewed thus far, osteopetrosis as a cause of pancytopenia has not been reported.

## CONCLUSION:

Bone marrow aspiration and trephine biopsy is mandatory for the accurate diagnosis of etiology of pancytopenia. Though clinical history and hematological investigations give a clue to the diagnosis of

the cause of pancytopenia like megaloblastic anemia, leukemia, myelodysplastic syndrome, malarial infection, etc. a confirmatory diagnosis can be established by bone marrow examination especially in certain conditions like aplastic anemia, myelofibrosis, tuberculosis and osteopetrosis. Trephine biopsy is preferred over aspiration in conditions were aspiration would yield a dry tap due to overt fibrosis and focal marrow involvement.

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