



## Original Article

# The role of bone marrow aspiration cytology in the evaluation of the etiology of pancytopenia in patients in a tertiary hospital in Telangana, India

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## Abstract

**Background:** Pancytopenia is characterized by Anemia, Leukopenia & Thrombocytopenia. It is very often seen in day to day practice and requires thorough investigation to know the cause of Pancytopenia for timely and early management to improve the prognosis.

**Material and Methods:** This study was conducted in the Department of Pathology Malla Reddy Institute of Medical Sciences over a period of 2 years and six months from January 2017 to June 2019. Patients were in the age group of 18 months to 65 years. The ratio of male to female patients was 1:1.4. 109 patients were evaluated by Bone Marrow Aspiration cytology, complete blood picture by automated counter, which was confirmed by manual blood count along with peripheral blood examination, to establish the etiology. 43 patients who fulfilled the criteria for a diagnosis of pancytopenia were included in the study. The as patients underwent several diagnostic tests hemogram, biochemical test like serum B12, serum folic acid, serum ferritin, total iron binding capacity. Bone Marrow Aspiration cytology and Bone marrow biopsy were done. Routine Giemsa stain as well as cytochemical stains like Pearls stain, Reticulin stain and Periodic Acid Schiff test, were carried out on the bone marrow material.

**Results:** In the present study, the commonest cause of pancytopenia was found to be Megaloblastic Anemia (67.4%) followed by Hypoplastic Anemia (24.4%); the least common causes being infections (Malaria 2.3%), Aleukemic Leukemia (2.3%), Myelofibrosis (2.3%) and Myelodysplastic Syndrome (2.3%).

**Conclusion:** The majority of patients with pancytopenia are diagnosed by Bone Marrow Aspiration as an adjunct to clinical history, clinical examination, peripheral blood examination, hemogram and biochemical examination. Bone Marrow Aspiration cytology is an important diagnostic tool to establish unequivocally the cause of pancytopenia.

**Keywords:** Pancytopenia, Bone Marrow Aspiration cytology, Megaloblastic Anemia, Myelofibrosis, Lymphoproliferative Disorders.

## Introduction

Pancytopenia is commonest hematological entity encountered in day to day hematology practice. It

is manifested by Anemia, leucopenia and Thrombocytopenia (hemoglobin < 10 grams%, Leucocytes < 4000/ $\mu$ l and platelet count < 1.5 lakhs

/µl). It is evaluated by automated counter, peripheral blood smear examination, manual platelet count and finally diagnosed by Bone Marrow Aspiration cytology in majority of the cases<sup>1</sup>. Only few cases require specific tests i.e. biochemical examination, like serum B12, serum folic acid, serum iron levels, serum ferritin levels, special stains, cytochemistry, immunohistochemistry, Imageology, karyotyping, fluorescencet in situ hybridization [FISH] and flowcytometry. Hence Bone Marrow Aspiration cytology evaluation is essential in all cases of pancytopenia to know the underlying causes of pancytopenia for accurate diagnosis and early treatment to improve the prognosis<sup>2</sup>. The causes of pancytopenia vary with geographical regions and genetic variation<sup>3,4</sup>.

Causes of pancytopenia are mentioned below<sup>5</sup>

#### **Non Malignant hematological causes**

Megaloblastic anemia, Aplastic Anemia, Pure red cell Aplasia and Mylofibrosis.

**Infective Causes:** Malaria, leishmaniasis, Dengue fever.

**Storage disorders:** Gaucher disease, Neiman Picksdisease.

**Hematological Malignancy:** Non-Hodgkins lymphoma, Myelodysplastic syndrome, Multiple Myeloma,Aleukemic leukemia.

**Metastatic Deposits:** Neuroblastoma in children's, Secondary Deposits from different organs in bone.

Megaloblastic Anemia is still the commonest cause of Pancytopenia<sup>6,7</sup>.

Causes of Megaloblastic anemia:Nutritional Factors, Recurrent Infections and deficiencies of vitamin B12 and folate seem to be associated strongly with Megaloblastic Anemia<sup>8</sup>.Less common causes are, inherited or drug induced disorders of DNA synthesis. Causes of non – Megaloblastic Macrocytic Anemia are: Hepatic disease, Refractory anemia, Alcoholism, Hypothyroidism, Chronic obstructive pulmonary disease.

Hypoplastic Anemia result s from inadequate functioning bonemarrow, can develops into

Aplastic anemia asa result of bone marrow damage present bybirth or occur after exposueto radiation, chemotherapy toxic chemicals, some drugs and infection.

There is increased worldwide concern about the consequences of vitamin B12 and folic acid deficiencies on health, which includes Megaloblastic Anemia<sup>9, 10</sup>. Inadequate dietary vitamin B12 intake is one of the commonest cause of deficiency among vegetarians as vitamin B12 is known to occur in animal products. It also causes Hyperhomocystenemia. Elevated Methyl Malonic acid/ Total Homocysteine have been found to be sensitive marker of B12 deficiency<sup>11</sup>. Vitamin B12 deficiency is difficult to pickup clinically as it presents with vague complaints such as decreased physical and mental work capacity, Reduced attention span, Memory loss, Irritability and low mood<sup>12</sup>.

The Etiology of B12 deficiency is inadequate dietary intake, impairabsorption due to intrinsic factor deficiency (Pernicious Anemia) or generalized Malabsorption syndrome such as Crohns disease, intestinal infestation by fish tapeworm Diphyllobothrium, Ileal resection and hereditary causes such as homocystinuria and Transcobalamin deficiency.

Bone Marrow Aspiration not only helps to make specific diagnosis but should also be used as an extremely valuable, quick and economically viable procedure to exclude major hematological disorders.

#### **Material and Methods**

The present study was conducted in Department of Pathology, Malla Reddy Institute of Medical Sciences over a period of 2 years 6 months from January 2017 to June 2019. Patients were of both sexes (male and female) (in the ratio of 1:1.4), in the age group of 1year 6 months to 65 years. 109 cases were evaluated by Bone Marrow Aspiration along with complete blood pictureby automated analyzer, confirmed by manual count and peripheral blood smear examination for various etiologies. Out of which 43 patients fulfilled the

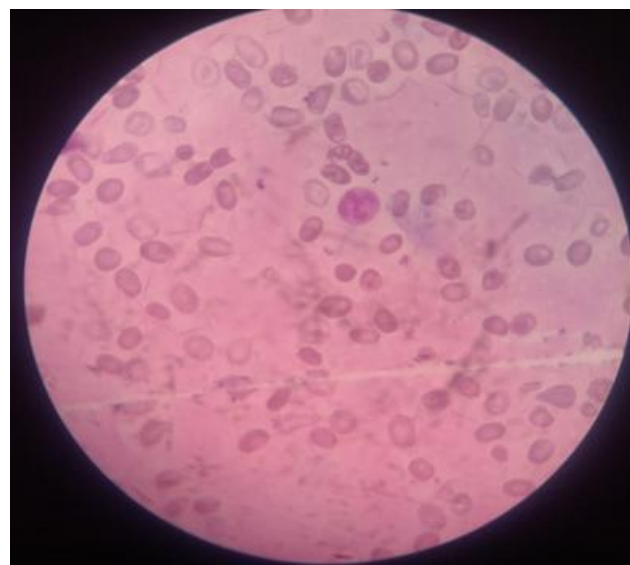
criteria of pancytopenia. Different tests were performed to study the cause of pancytopenia established by hemogram; biochemical tests like serum B12, Folic acid, serum Iron, serum ferritin, total iron binding capacity, and Imageology. Confirmatory Bone Marrow Aspiration was done to study its cytology and unequivocally establish the exact etiology of pancytopenia. The criteria of pancytopenia was hemoglobin<10grams / dL, total leukocytic count less than 4000/Cu mm and platelet count less than 140000/Cu mm.

Peripheral blood smears were stained by Leishmans stain as per standard operating procedures. The films thus prepared and stained were evaluated for the quality of film, rouleaux formation, number, distribution and staining of erythrocytes, Leucocytes, thrombocytes and hemoparasites. The smears were examined under oil immersion to further assess the Erythrocytes [size, shape and inclusions], Leucocytes [granulocytes and Agranulocytes], Thrombocytes; Morphology, number, distribution and count.

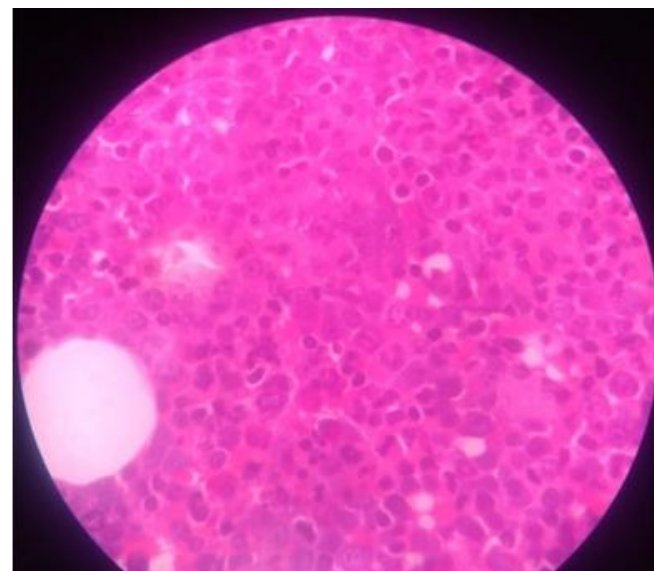
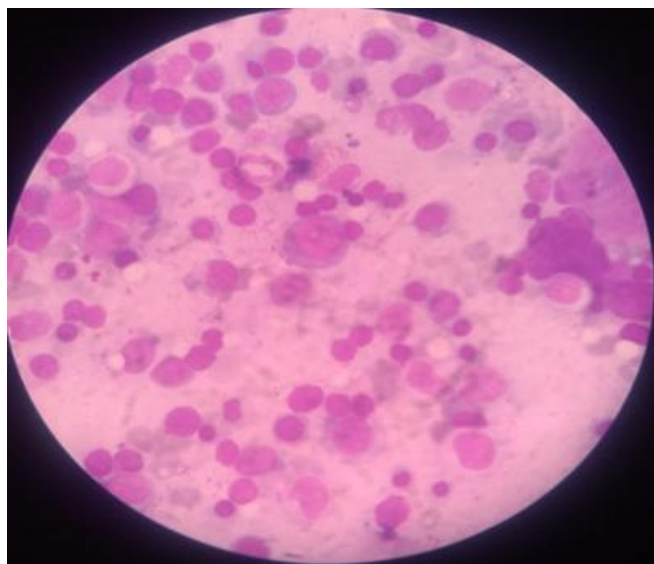
In all cases Bone Marrow Aspiration cytology was performed under Aseptic precautions by standard operative procedures after taking written consent from the patient. The skin, subcutaneous tissue and periosteum over posterior iliac crest or manubrium sternum, or the medial aspect of head of tibia in children, were infiltrated with 2% xylocaine. Using Salah's Bone Marrow aspiration needle, bone marrow aspirate was obtained using standard techniques. Immediately on completion of the aspiration, a Trepine biopsy (with Jamshedi) needle of an adjacent area of bone was performed through the same puncture site. After expulsion of 2 cm core of the bone and its marrow from the needle, the bony specimen was smeared gently across the glass slide and then placed in fixative for subsequent histopathological processing. Bone Marrow Aspiration cytology smears were examined under oil immersion (x1000 magnifications) and Bone marrow core biopsy slides stained by Hemotoxylin and eosin were examined under high power (x450), and the results interpreted. Cytochemical stains like

periodic acid Schiff's, Pearls stain and Reticulin stain, were done where ever necessary, keeping in mind the results of biochemical tests.

109 patients, with different hematological presentations, underwent evaluation by bone marrow aspiration cytology studies, over a period of 2 years 6 months from January 2017 to June 2019. The patients were in the age group of 18 months to 65 years of both sexes. 43 patients fulfilled the criteria of pancytopenia as evaluated by complete blood picture by auto analyzer, manual blood count and peripheral blood smear examination. Bone Marrow aspiration cytology was done in these patients to establish the exact cause of pancytopenia. The various etiologies of pancytopenia by bone marrow aspiration in the present study were varied, the commonest being Megaloblastic Anemia 67.4% (29 cases), followed by mixed Hypoplastic anemia 23.4% (10 cases), infections (malaria) 2.3% (1 case), aleukemic leukemia 2.3% (1 case), Myelofibrosis 2.3% (1 case), Myelodysplastic syndrome 2.3% (1 case). The results are shown in Table no.1. The male & female ratio in this study conducted is 1:1.4 as shown in Table No.2.



**Figure No. 1** Peripheral Blood Smear showing Pancytopenia (Leishman stain x1000).



**Figure 2:** Bone Marrow Aspiration cytology showing Megaloblastic picture (Leishman stain x1000)

**Figure 3:** Bone Marrow Biopsy showing Megaloblastic picture (Hemotoxylin and eosin stain x1000)

**Table No.1** Frequency and Percentage of etiologies of pancytopenia in our study

S.No.	Causes	Frequency	Percentage
1	Megaloblastic Anemia	28	67.4%
2	Hypoplastic Anemia	10	23.4%
3	Infections(Malaria)	01	2.3%
4	Myelofibrosis	01	2.3%
5	Myelodysplastic Syndrome	01	2.3%
6	Aleukemic leukemia	01	2.3%

**Table No.2** Distribution of Male and female Patients of Pancytopenia in our study

S.No.	Sex	Frequency	Percentage
1	Male	18	42%
2	Female	25	58%

**Table No.3** A comparison of the two most frequent causes of pancytopenia as assessed by bone marrow aspiration evaluation in different studies

S.No.	Study	No. of cases	1 <sup>st</sup> common cause	2 <sup>nd</sup> common cause
1	Jha etal <sup>5</sup>	148	Hypoplastic Anemia [29.05%]	Megaloblastic Anemia [23.64%]
2	Khodke etal <sup>13</sup>	50	Megaloblastic Anemia [44%]	Hypoplastic Anemia [14%]
3	Tilak etal <sup>14</sup>	77	Megaloblastic Anemia [68%]	Hypoplastic Anemia [ 7.7%]
4	Verma etal <sup>15</sup>	202	Hypoplastic Anemia [40.6%]	Megaloblastic Anemia [23.23%]
5	Kumar etal <sup>16</sup>	166	Hypoplastic Anemia [29.5%]	Megaloblastic Anemia [22.3%]
6	Bhajracharya etal <sup>17</sup>	10	Hypoplastic Anemia [46.5%]	Megaloblastic Anemia [31.8%]
7	Present Study	43	Megaloblastic Anemia [67.4%]	HypoplasticAnemia [23.4%]

**Discussion**

Bone Marrow Aspiration is performed in situations where the cause cannot be determined by routine hematological investigations or when specific Bone Marrow pathology is suspected like Malignancy, congenital/acquired Dyserythropoitic

Anemia, Congenital/ acquired Bone Marrow failure effecting one or more Hematological lineage, cytopenia which remains refractive to treatment, infiltrative Marrow disorders, Myelofibrosis, Acute Leukemia, aleukemic leukemia, Aplastic Anemia and storage disorders.

The first and the second commonest cause of pancytopenia in the present study conducted is Megaloblastic Anemia and Hypoplastic Anemia which is in consonance with other studies conducted by other authors Khodke et al, Tilak et al<sup>13,14</sup>. The studies conducted by Kumar et al, Verma et al, Jha et al, Bhadracharya et al revealed Hypoplastic Anemia to be more common than Megaloblastic Anemia as a cause of pancytopenia<sup>15, 16, 17</sup>. A comparison of our results with those of other authors is shown in Table No.3.

### Conclusion

Majority of pancytopenia cases identified by hemogram in the present study were diagnosed etiologically by Bone Marrow Aspiration and biopsy as Megaloblastic Anemia, followed by Hypoplastic Anemia

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