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Differential Diagnosis of Pancytopenia: A Correlated Study of Peripheral Blood Smear Along With Bone Marrow Aspiration and Bone Marrow Biopsy

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Abstract ;Background*: A review of both Western and Indian literature shows that there are few comprehensive studies on pancytopenia. InIndia, thecauses ofpancytopeniaarenotwelldefined. Thisdata, would help in planning the diagnostic and therapeutic approach in patients with pancytopenia.* ***Aim****To evaluate the cases of pancytopenia by peripheral smear and/ orbonemarrowaspirationandbonemarrowbiopsytofindoutthe etiology, clinical, haematological, and histomorphologic features of pancytopenia.* ***Materials and Methods****:Total 86 cases were selected anddetailed haematological investigations including complete hemogram, peripheral smear examination, bone marrow aspiration, and biopsy examination were done.****Results****: The most common cause of pancytopenia in our study was megaloblastic anaemia (50%), followed by nutritional anaemia (16.3%) and hypoplastic/aplastic anaemia (10.5 %), myelofibrosis (8.1%) and malaria (4.3%).****Conclusion****: Megaloblastic anaemia duetovitaminB12/folatedeficiency seems toreflectthe higher prevalence of pancytopenia in Indian subjects, putting nutritional anaemia and hypoplastic/aplastic anaemia in the second and third position respectively. So, a simultaneous evaluation of BMA and BMB along with complete hemogram and peripheral blood smear is essential to give a definitive diagnosis which would help in the early treatment of pancytopenia.*

Keywords: Aplastic anaemia, megaloblastic anaemia, pancytopenia

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I. Introduction

Pancytopenia is the simultaneous presence of anemia, leukopenia and thrombocytopenia. Pancytopenia was not a discrete haematological entity till as late as 1919. The term was used almost synonymously for aplastic anemia, it being the major cause of pancytopenia in Western countries. [1,2]. It is not a disease entity but a triad of findings that may result through different mechanisms like destruction of marrow tissue by toxins, radiation (aplastic or hypoplastic marrow), replacement by abnormal or malignant tissue like Hodgkin's and non-Hodgkin's lymphoma, multiple myeloma, myelofibrosis, or suppression of normal marrow growth and differentiation like megaloblastic anemia and systemic lupus erythematosus. [1,3].Pancytopenia is an important clinico-hematological entity encountered in our day-to-day clinical practice. Pancytopenia therefore exists in the adult when the haemoglobin level is less than 13.5 g/dl in males or 11.5g/dl in females; the leucocyte count is less than 4×10^9/L; and platelet count is less than 150×10^9/L.

It is not a disease entity but a triad of findings that may result from a number of disease processes — primarily or secondarily involving the bone marrow.[3] The severity of pancytopenia and the underlying pathology determine the management and prognosis of the patients.[4].The presenting symptoms are usually attributable to the anemia or the thrombocytopenia. Leukopenia is an uncommon cause of the initial presentation of the patient but can become the most serious threat to life during the subsequent course of the disorder. Sometimes pancytopenia is detected as an incidental feature in a patient who has presented with symptoms of disorder that is capable of depressing the levels of all cellular elements in the blood. [de gr]

In India, the causes of pancytopenia are not well defined, so the present study has been undertaken to evaluate the various causes and to correlate the peripheral blood findings with bone marrow aspirate.[4,5] Thereby, this data would help in planning the diagnostic and therapeutic approach in patients with pancytopenia.

II. Materials And Methods

The present prospective study was undertaken for a period of 2 years, from September 2015 to September 2017, at the Department of Pathology, Hitech Medical College and Hospital, Bhubaneswar. Patients of all age groups and both sexes were included. Case selection was based on clinical features and supported by laboratory evidence, which included peripheral blood counts for haemoglobin, leukocytes and platelets.

Inclusion criteria were presence of all 3 of the following: haemoglobin, <9 g/dL; total leukocyte count (TLC), <4,000 / µL; platelet count, <100,000/ µL. [5]. A total of 86 cases were selected and evaluated.
Complete medical history and clinical details were obtained for each patient. Detailed haematological investigations were done in the blood samples. Measurement of Hb, mean corpuscular volume (MCV), TLC, differential leukocyte count (DC), and platelet count was done on Sysmex KX-21 and also cross-checked by peripheral blood smear examination for which Leishman stain was used. Peripheral smear examination was done systematically under low, high, and oil immersion for RBC morphology – i.e. anisocytosis, poikilocytosis, polychromasia, nucleated red blood cells, and presence of inclusions. Rouleaux formation, if present, was noted. Anaemias were then classified morphologically into four groups – normocytic normochromic, microcytic hypochromic, macrocytic, and dimorphic. Differential WBC count was done with a special note of defective granulation in neutrophils and hypo/hyper segmentation in neutrophils, and platelet count and morphology were analysed. Also, presence of any parasites was tested for.

Informed consent was taken for bone marrow studies. Bone marrow aspiration was done from posterior superior iliac spine using Salah bone marrow aspiration needle. Simultaneously, from the same puncture site but from a different plane, bone marrow biopsy was done using Jamshidi trephine biopsy needle, taking all aseptic precautions under local anaesthesia.

The Bone marrow aspirate was evaluated for cellularity, erythropoiesis, myelopoiesis, megakaryopoiesis, M: E ratio, lymphocytes, plasma cells, parasites/atypical cells/granuloma. The cellularity was assessed by estimating the percentage of hematopoietic cells compared to fat spaces in the bone marrow.The microscopic examination of trephine biopsy was evaluated for overall cellularity and presence of infiltrates.

III. Results

A total of 86 patients who presented with pancytopenia were studied. They consisted of 47 males and 39 females with a male-to-female ratio of 1.2:1. The age of patients ranged from 2 to 75 years (mean age, 40years). Out of 86 cases, pancytopenia was observed in 12paediatric patients (2-18 years); they consisted of 8 males and 5 females. No familial disease was observed in association with pancytopenia. Presenting complaints and physical findings shown in Table 1.

Table 1: Distribution of cases as per clinical symptoms

Symptoms	No. of cases	%
Fever	74	86%
Generalised weakness	65	75.5%
Fatigue	50	58.2%
Pallor	49	57%
Hepatomegaly	15	17.4%
Lymphadenopathy	7	8.1%
Bleeding	5	5.8%

The commonest mode of presentation was fever of unknown origin(PUO), generalized weakness; other main symptoms were fatigability, weight loss, bleeding etc. Pallor was noted in many cases.
Splenomegaly and hepatomegaly were seen in cases of megaloblastic anaemia and malaria. Bony tenderness was seen in multiple myeloma. Lymphadenopathy was noted in sub leukemic leukaemia.

The most common cause of pancytopenia in our study was megaloblastic anaemia (50%) [Figures 1 and 2], followed by hypoplastic/aplastic anaemia (16.3%) [Figure 3]. Other causes included aplastic anaemia (10.5%), post viral illness(dengue) (2.3%), malaria (4.6%), myelofibrosis (8.1%), ALL (3.5%), and metastasis (1.1%) **[Table 2].**

Table-2:

Diagnosis	No of cases	%
Megaloblastic anaemia	43	50%
Nutritional anaemia	14	16.3%
Aplastic anaemia	9	10.5%
Myelofibrosis	7	8.1%
Malaria	4	4.6%
Acute lymphoblastic lymphoma	3	3.5%
Multiple myeloma	2	2.3%
Dengue	2	2.3%
Hodgkin lymphoma	1	1.1%
Metastasis	1	1.1%

Three cases of megaloblastic anaemia had concurrent iron deficiency as well. Post viral illness cases comprised two cases of dengue. The age range of patients in our study was from 2 to75 years.

The commonest age group for presentation of pancytopenia was between 31 and 40 years, with a total of 34 (40%) cases belonging to this group. This was followed by 21–30 years (22%), 41–50 years (12.8%),11-20 years (10.5%),51–60 years (5.8%), 2-10yrs (3.5%),61-70 years (2.3%) and more than 70years (3.4%) [**Table 3].**

Table -3: Distribution of cases as per age group

Age group(years)	No of cases	%
2-10	3	3.5%
11-20	9	10.5%
21-30	19	22%
31-40	34	40%
41-50	11	12.8%
51-60	5	5.8%
61-70	2	2.3%
>70	3	3.4%

Figure1: PBS of megaloblastic anaemia

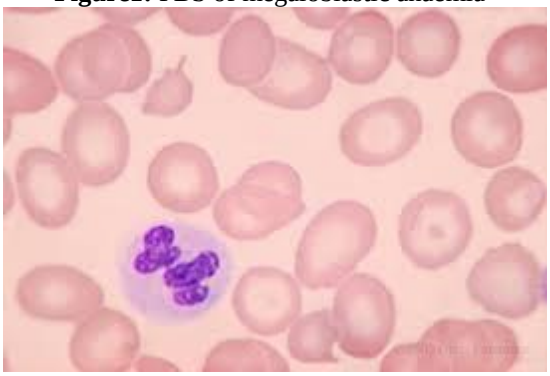
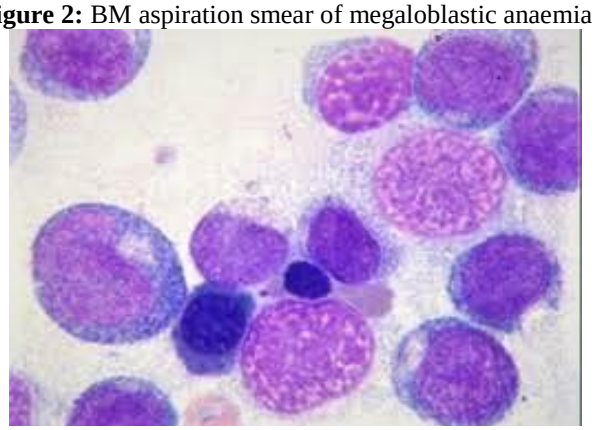


Figure 2: BM aspiration smear of megaloblastic anaemia



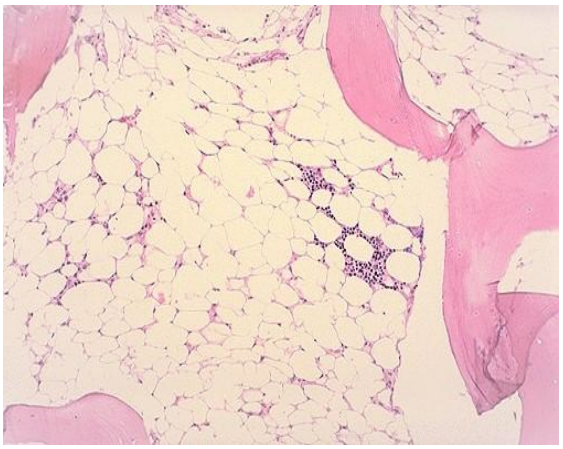


Figure3: BMB of aplastic anaemia showing hypocellular marrow

Figure 4: PBS showing malaria parasite (P. falciparum)

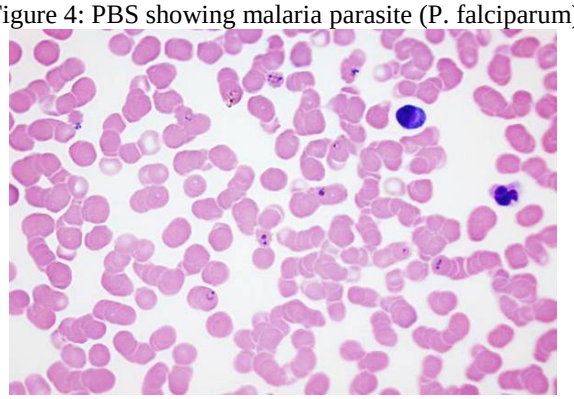


Figure 5: BMB showing myelofibrosis

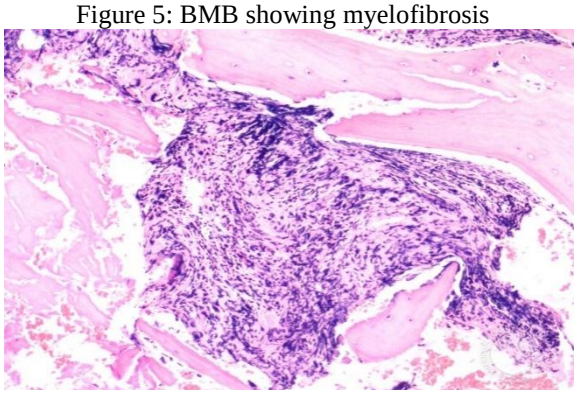
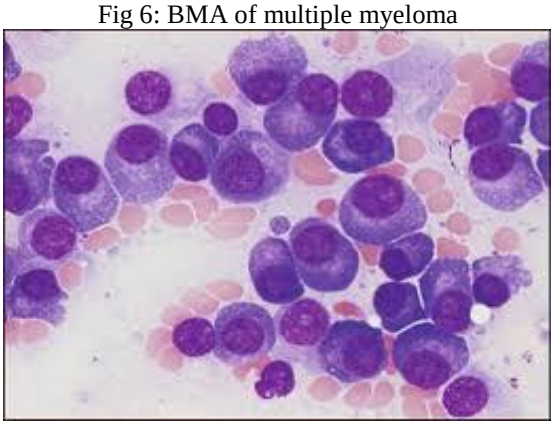


Fig 6: BMA of multiple myeloma



Out of the 86 cases, 47 were males and 39 were females. Slight male preponderance was seen. The overall male to female ratio was 1.2:1.

Peripheral blood findings

Anisocytosis of varying severity was the commonest morphologic type (58%) followed by normocytic normochromic (34%) and dimorphic picture (8%) in the decreasing order of frequency. Normocytic normochromic anaemia was the common finding in all other causes of pancytopenia except for megaloblastic anaemia and myelofibrosis where anisocytosis was common. In myelofibrosis, peripheral blood smear was showing characteristic tear drop red cells. Hypersegmented polymorphs were the commonest peripheral blood finding in megaloblastic anaemia.

Hb level distribution in various causes of pancytopenia

The Hb levels of patients in our study ranged from 2 to 9 g/ dl. Of the 86 cases, 44 cases (51%) had Hb levels between 3 and 5 g/dl, suggesting severe degree of anaemia at the time of presentation as pancytopenia. Hb levels were more severely reduced in the hypoplastic/aplastic anaemia group.

Platelet count distribution in various cases of pancytopenia

The platelet counts of patients in our study ranged from 10,000/ mm3 to 1.2 lakh/mm3. Majority, i.e. 58 cases (67.4%), had platelet counts between 50,000/mm3 and 1 lakh/mm3. Also, 9% of the patients had platelet counts below 20,000/mm3 and all these patients were from hypoplastic/aplastic anaemia group.

Distribution of megaloblastosis

Out of 43 cases, 35 cases (81.4%) showed pure megaloblastic anaemia of varying severity whereas 8 cases (18.6%) showed dimorphic anaemia, i.e. a combination of iron deficiency and megaloblastic anaemia, in varying proportions [Table 3].

Table 4: Distribution of megaloblastosis

Causes	No of cases	%
Megaloblastic anaemia	35	81.4%
Dimorphic anaemia	8	18.6%
Total	43	100%

IV. Discussion

Though pancytopenia is not a disease by itself, it is a striking feature of many serious and life-threatening illnesses and is caused by various different disorders. [1,7] Extensive studies have been conducted for the different etiological factors of pancytopenia like megaloblastic anaemia, aplastic anaemia, leukaemia, myelodysplastic syndrome, myelofibrosis, hypoplastic bone marrow etc. However, there are a limited number of comprehensive studies on this subject. [6]

The present study of pancytopenia was carried out mainly with the aims of diagnosing the patients of pancytopenia and finding out the common disease entities responsible for it. [6,7]. A total of 86 cases of pancytopenia were studied. Age, sex, presenting complaints, peripheral blood picture, bone marrow aspiration smears and bone marrow biopsy and various causes of pancytopenia were studied in all cases, and observations were compared with those in studies published in the literature.

Comparison of the common causes of pancytopenia reported from various studies conducted in the Indian subcontinent with those of the present study is presented in **Table 5**. [4,9]

Table 5: Comparison of etiology of pancytopenia found in the present study with that in other Indian studies

	Tilak and Jain [4] (1999)	Khodke et al. [7] (2001)	Kumar et al. [5] (2001)	Niazi and Fazl-i-Raziq [15] (2004)	Present study (2018)
No of cases	77	50	166	89	86
Commonest cause	Megaloblastic anemia(68%)	Megaloblastic anemia(44%)	Aplastic anemia(29.5%)	Aplastic anemia(38.3%)	Megaloblastic anaemia (50%)
Second common cause	Aplastic anemia(7.7%)	Aplastic anemia(14%)	Megaloblastic anaemia (22.8%)	Megaloblasticanaemia (24.7%)	Nutritional anaemia (16.3%)
Third common cause	Malaria (3.8%)	Kala-azar (14%)	Aleukemic leukaemia (12%)	Hypersplenism (16%)	Aplastic anaemia (10.5%)
Fourth common cause	Kala-azar (2.5%)	Normoblastic erythroid hyperplasia (14%)	Hypersplenism (11.44%)	Acute leukemia(13.6%)	Myelofibrosis (8.1%)

The age of the patients ranged from 2 to 76 years, with a mean age of 40 years. Cytopenias were observed more in males (54.81%) than females (45.19%), with male-to-female (M: F) ratio of 1.2: 1. Age and sex distribution was compared with other studies as shown in**table-6**

Table -6: Age/sex distribution in different study groups compared with present study

Serial no	Authors	No of cases	Age range(years)	M: F
1	Khunger JM et.al [6]2002	200	2-70	1.2:1
2	Kumar R et.al; [5]2001	166	12-73	2.1:1
3	Khodke K et al; [7]2001	50	3-69	1.3:1
4	Tilak V et al; [4]1999	77	5-70	1.14:1
5	Present study	86	2-75	1.2:1

Comparison of haematological parameters between major subgroups of cytopenias:

Hyper segmented neutrophils were noted in 77% of cases out of 43 cases of megaloblastic anemia in our present study, compared to 84.9% in Tilak V et al. study, and Khunger JM et al. demonstrated no hyper-segmented neutrophils in megaloblastic anaemia. Also, relative lymphocytosis in aplastic anaemia was noted in

42.3% of the cases in our study compared to 50% in Tilak V et al. study and 85.71% in Khunger JM et al. study. [4,6]

Comparison of peripheral blood findings with those in other studies:

In this present study, we came across 12 pediatric pancytopenia cases; again, megaloblastic anaemia was the common cause for pancytopenia, followed by nutritional anaemia and aplastic anaemia. A study by Gupta and colleagues, 105 patients aged 1.5 to 18 years, with a mean age of 8.6years showed Aplastic anaemia was the most common cause of pancytopenia (43%), followed by acute leukaemia (25%). In some studies, by Khodke K et al [7] showed infections were the third most common cause of pancytopenia, of which kala-azar was the most common. In another study, 64 children were identified with diagnosis of pancytopenia. The most common cases were infectious in origin (64%), followed by haematological (28%) and miscellaneous (8%) etiologies. [10]

Variations in the frequency of various diagnostic entities causing pancytopenia have been attributed to difference in methodology and stringency of diagnostic criteria, geographic area, period of observation, genetic differences and varying exposure to myelotoxic agents, etc. [4]

The haematological parameters were usually nonspecific in many cases and showed considerable overlap. In all these cases a peripheral blood film was important in indicating towards a diagnosis of megaloblastic anaemia or leukaemia. Bone marrow aspiration and biopsy are important diagnostic tools in the diagnosis of various haematologicaldisorders, includingpancytopenia.Both of these procedures are complimentary to each other. Pampa Ch et al (2010) found positive correlation in 61.25% of cases when simultaneous BMA and BMB were performed.

In the present study there was 89.5% positive correlation between BMA and BMB. The highest correlation was seen in the cases of megaloblastic anaemia and with erythroid hyperplasia in cases of nutritional anaemia. Other cases which were diagnosed on BMB included acute lymphoblastic leukaemia, myelofibrosis, Hodgkin lymphoma and metastatic tumours.

Table-7: Positive correlation between BMA and BMB:

Diagnosis	No of cases	%
Megaloblastic anemia	41	53.2%
Nutritional anemia	15	19.4%
Aplastic anemia	8	10.4%
Myelofibrosis	6	7.8%
Malaria	2	2.6%
Acute leukemia	3	4 %
Multiple myeloma	1	1.3%
Dengue	1	1.3%
Total	77	100%

V. Conclusion

Pancytopenia is a common haematological entity that we come across in our routine practice. There are numerous causes of pancytopenia which include both non-neoplastic and neoplastic conditions. With the help of detailed clinical history, physical examination, and haematological investigations, pancytopenia can be diagnosed and the causes can be ascertained. Megaloblastic anaemia due to vitamin B12/folate deficiency seems to reflect the higher prevalence of pancytopenia in Indian subjects, putting nutritional anaemia and hypoplastic/aplastic anaemia in the second and third position respectively. Pancytopenia due to megaloblastic anaemia is albeit transient and easily reversible with appropriate treatment. Thus, megaloblastic anaemia should always be considered in the evaluation of pancytopenia in Indian settings. However, other important causes of pancytopenia should be kept in mind while planning investigations for the complete workup of pancytopenic patients.

Bone marrow examination which includes BMA and BMB along with peripheral blood smear study are the essential pre-requisite for its diagnosis. Both these procedures are complimentary to each other and to be performed simultaneously.

Early treatment can be planned depending upon the cause and severity of pancytopenia. In our study, majority of the cases had a treatable cause and so carried better prognosis.

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