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Original Research Article

Clinico-hematological evaluation in cases of pancytopenia at tertiary care centre: largest cross-sectional study

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ABSTRACT

Background: Pancytopenia is characterised by a reduction in all the three cellular elements of blood (erythrocytes, leukocytes and platelets) below the normal reference range leading to anaemia, leucopenia and thrombocytopenia. It is a feature of many serious conditions. The present study was conducted to assess aetiology, clinical profile and bone marrow morphology of conditions presenting with pancytopenia.
Methods: A two years cross-sectional study from July 2017 to June 2019 was conducted in the Department of Pathology. Total of 300 pancytopenia patient were studied and their clinical features, peripheral smear finding and bone marrow morphology were studied by using marrow aspiration and biopsy.
Results: Among 300 cases studied, maximum patients were in the age group 11-20 years (19.66%) with male (50.66%) predominance. Most of the patients presented with weakness (91.66%) and fever (56.66%) as chief complains. The commonest physical finding was pallor (94%) followed by splenomegaly (27.33%). Macrocytic anaemia (43.66%) was commonest peripheral finding. The commonest cause of pancytopenia was megaloblastic anaemia (32.66%) followed by dimorphic anaemia (21%), aplastic anaemia (16%) and acute leukaemia (13.33%).
Conclusions: The present study concludes that detailed clinical history, primary haematological investigations along with bone marrow examinations is essential to determine the cause of pancytopenia.

Keywords: Bone marrow, Megaloblastic anaemia, Pancytopenia

INTRODUCTION

Term 'pancytopenia' is used in medical practice when all the cellular components in the blood are reduced below a critical level. It is a common clinico-hematological condition encountered in clinical practice. It is characterized by a reduction of red blood cells, white blood cells and platelets leading to anaemia, leukopenia, and thrombocytopenia respectively.[1]

It can result from a variety of disease processes that primarily or secondarily affect the bone marrow.[2] The etiology of pancytopenia depends upon genetic and geographical factors.[3] The spectrum of diseases leading to pancytopenia may vary in different population groups with their differences in age, nutritional status and prevalence of infection.[4]

The etiology of pancytopenia can vary from treatable disorders such as megaloblastic anaemia to more serious conditions such as myelodysplastic syndromes which increase the likelihood of developing haematological malignancies in the future.[5] Major causes of pancytopenia in developing countries are megaloblastic anaemia, aplastic anaemia, splenomegaly, sepsis, leukaemia, lymphoma, multiple myeloma, myelodysplastic syndrome, autoimmune diseases, endocrine diseases and bone marrow infiltrating diseases.[6]

The complete haematological workup including complete blood count, peripheral blood smear and bone marrow examination with good clinical correlation is of utmost importance to evaluate the causes of pancytopenia and planning further investigations.[7]

In India, causes of pancytopenia are not well defined and hence the present study was carried out with primary objective as to evaluate the spectrum of diseases causing pancytopenia and secondary objective to study the clinical festures, haematological and bone marrow finding in the patient presenting with pancytopenia admitted in the tertiary care hospital.

METHODS

A prospective cross-sectional study was carried out in the department of pathology Government Medical College and Hospital Nagpur, Maharashtra, India over a period of two years from July 2017 to June 2019. Institutional ethical committee approval was obtained prior to the commencement of the study.

Patients of all age groups admitted in tertiary care hospital with haematological parameters suggestive of pancytopenia were included in this study. Criteria for diagnosis of pancytopenia was haemoglobin less than 10 g/dl, TLC less than 4.0 x 103/uL and platelet count less than 1.0 x 103/uL.[8-10] Patients who were receiving chemotherapy or therapeutic radiation, were excluded from this study.

A proforma was used to document demographic data, clinical presentation, dietary history and to record investigation findings. All the patients were subjected to examination of their complete blood count, peripheral blood smear and bone marrow examination. complete blood count was carried out by automated blood cell counter (PE 6000). Peripheral blood smears were stained using Leishman stain. Bone marrow (BM) aspiration was performed from the posterior superior iliac spine under aseptic condition. Slides were stained with routine Leishman stain and examined. Other special stains like Myeloperoxidase, Periodic acid and Schiff reagent were performed where necessary. Immunophenotyping was carried out by using flow cytometry in all cases of acute leukaemia.

Sample size

Sample size was calculated by using open-source statistics for public health for cross sectional study by considering expected frequency of pancytopenia 50% our minimum sample within 95 % confidence interval was 97 subjects.

Statistical analysis

Statistical analysis was done by applying descriptive stats by using Microsoft excel 2016 (mean, SD, frequency distribution, percentages) etc.

RESULTS

Total of 300 patients presented with pancytopenia were studied. In the present study, pancytopenia showed its highest incidence in the age group of 21-30 years (23%) followed by 11-20 years (19.6%). In this study 152 (50.66%) were males and 148 (49.33%) were females pancytopenia patients with a male to female ratio was 1.03:1 (Table 1).

Table 1: Age and sex wise distribution of pancytopenic patients in present study (n=300).

Age group (Years)	Males	Female	Total	Percentage
Below 10	11	12	23	7.6
11-20	28	31	59	19.6
21-30	32	37	69	23
31-40	23	24	47	15.6
41-50	13	22	35	11.6
51-60	19	6	25	8.3
61-70	18	14	32	10.6
Above 71	8	2	10	3.3
Total	152	148	300	100

Table 2: Presenting complaints in pancytopenia patients (n=300).

Presenting complaints	No. of patients	Percentage
Fever	178	59.33
Weakness	275	91.66
Bleeding manifestation's	82	27.33
Weight loss	21	7
Abdominal pain	5	1.66
Breathlessness	32	10.66
Ascites	7	2.3
Cough	9	3
Vomiting	4	1.33
Chills	3	1
Burning micturition	2	0.66

Among presenting complains generalized weakness was commonest complain present in 91.66 % followed by fever in 59.66 %, bleeding manifestation in 27.33% and other complaints in 26% of cases (Table 2).

In our study, 46.33 % were pure vegetarians and 53.66 % were having a mixed diet. In our Study pallor (94%) of commonest physical finding followed by splenomegaly (27.33%), hepatomegaly (17.33 %) and icterus in 15.66% cases (Table 3).

Finding on complete blood count (CBC) showed the haemoglobin percentage varied from 1.2 gm/dl to 9.6 gm/dl with the majority of patients (33%) having haemoglobin ranging from 2.1- 4 gm/dl. The lowest value of haemoglobin was seen in aplastic anaemia. The total

leukocytes count (TLC) varied from 300-3900 cells/cumm. Majority of patients (35.33%) had TLC from 1100-2000 cells/cumm. The platelets count varied from 10000- 99000 cells/cumm with the majority of patients (34.33 %) were having platelet count ranging from 41000-60000 cells/cumm. Reticulocyte count ranged from 0.1-4.5 %. Most of the patients had reticulocyte count in between 0.6-2.5%.

Table 3: Presenting physical findings in patients with pancytopenia (n=300).

Presenting physical findings	No. of patients	Percentage
Pallor	282	94
Icterus	47	15.66
Petechiae and purpura	31	10.33
Bony tenderness	23	7.66
Hepatomegaly	52	17.33
Splenomegaly	82	27.33
Lymphadenopathy	21	7
Clubbing	3	1
Raised JVP	2	0.66

Table 4: Final diagnosis of pancytopenic patients (n=300).

Final diagnosis		No. of cases	Percentage (n=300)
Megaloblastic anaemia		98	32.66
Dimorphic anaemia		63	21
Aplastic A		48	16
Acute leukaemia		40	13.33
ALL	22		
AML	9		
Acute leukaemia - not classified	9		
Infection related changes		14	4.66
Micronormoblastic anaemia		11	3.6
Hypersplenism		6	2
MDS		5	1.66
Myelofibrosis		5	1.66
NHL		4	1.33
Multiple myeloma		4	1.33
ITP		2	0.66
Total		300	100

In the present study, peripheral smear findings showed a higher incidence of macrocytic (43.66%) followed by dimorphic (39.33 %), normocytic normochromic in (7.3 %) and normocytic hypochromic (9.66%) blood picture in pancytopenia patients (Figure 1).

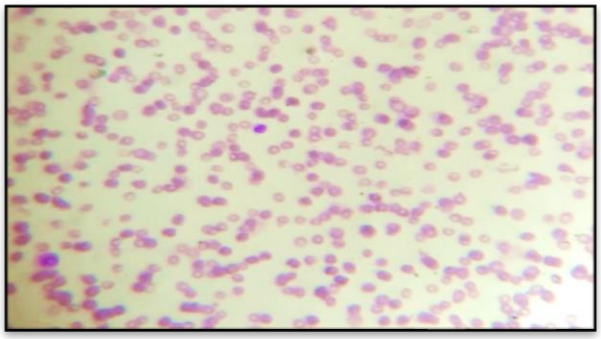

Figure 1: Peripheral blood smear pancytopenia showing dimorphic blood picture (Leishman stain 100x).

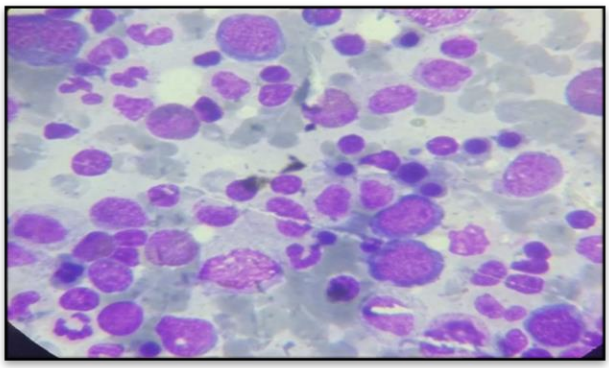

Figure 2: Bone marrow aspirate smear showing hyper cellular marrow with erythroid hyperplasia showing megaloblastic maturation (Leishman stain 1000x).

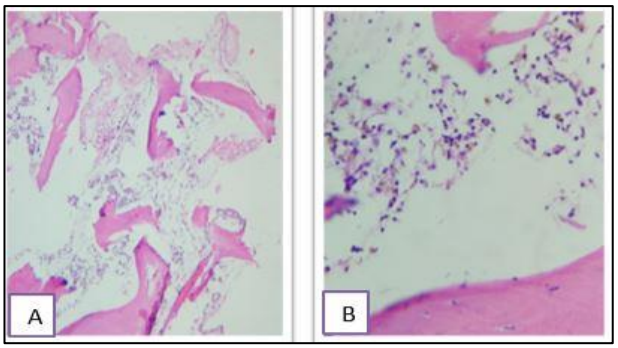

Figure 3: (A, B) Bone marrow biopsy showing bony trabeculae with reduced marrow elements seen in aplastic anemia (H and E stain 100 x and 400 x).

In the present study bone marrow smears were hypercellular in 52% of pancytopenia patients followed by hypocellular in 25% and normocellular in 9%. Bone marrow smears were diluted in 14 % of cases where bone marrow biopsy was carried out. Most common finding on bone marrow aspiration was megaloblastic anaemia (32%) followed by dimorphic anaemia (20%), acute leukaemia (13.33%) micronormoblastic anaemia (3.3%) and diluted marrow in 14 % cases (Table 4 and Figure 2).

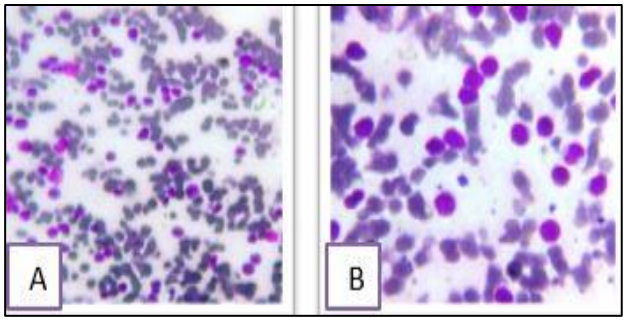

Figure 4: (A, B) BMA showing hypercellular marrow with blast >80 %. (Leishman stain 100x and 400x).

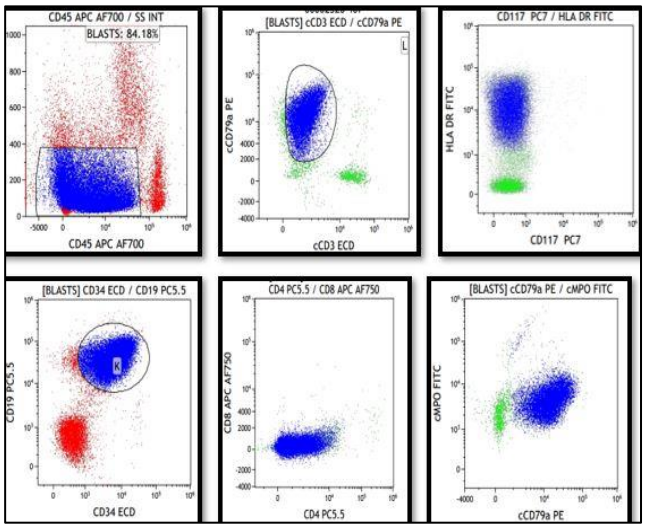

Figure 5: Immunophenotyping in case of B ALL showing CD45 positive cells with CD34, CD 19, cCD79a positivity and CD4, CD8, cMPO and cCD3 negativity.

Bone marrow biopsy was carried out in all diluted smears as well as hypocellular smears in which diagnosis was inconclusive. The most common finding in on bone marrow biopsy was aplastic anaemia (Figure 3) noted in 54% cases followed by acute leukaemia (10.22%), megaloblastic anaemia (7.95%) and dimorphic anaemia in 7.95% cases. Among diluted marrow aplastic anaemia (73.8%) was the commonest finding followed by megaloblastic anaemia (7.1%).

Immunophenotyping was carried out by using flow cytometry in 33 cases of acute leukaemia which showed acute lymphoblastic leukaemia (ALL) in 22 (66.66%) and 9 (27.27%) cases were having acute myeloid leukaemia (AML). Two cases (6.06 %) showed reactive morphology flow cytometry (Figure 4, 5). In our study various causes of pancytopenia-based examination of both marrow aspiration and biopsy we found, megaloblastic anaemia was commonest finding found in 98 (32.66 %) cases followed by dimorphic anaemia in 63 (21%) cases, aplastic anaemia in 48 (16%) cases and acute leukaemia in 40 (13.66%) cases (Table 4).

DISCUSSION

Frequency of various conditions causing pancytopenia depends upon the difference in methodology, diagnostic criteria employed, and geographic area.[11]

In a present study among 300 pancytopenia cases, it showed the highest incidence in the age group of 11-30 years (42.6%), these findings were comparable to studies carried out of by Anita et al who found most common age group of patients presenting with pancytopenia was 15-25 years comprising 39.42% of cases.[12] Also similar study carried out by Vaidya et al found the most common age group was 21-30 years comprising 33.24 % of cases.[13] Similarly our results of age and sex ratio were comparable with other studies carried out by Thakkar et al, Manzoor et al, Yadav et al and Zeeshan et al.[14-17]

The study carried out by Lakhey et al in Nepal found weakness was the predominant presenting clinical feature in 73.9% cases.[18] Khodke et al studied 50 pancytopenia cases and found commonest presenting complaint was fever (40%) followed by weakness (30%) and bleeding manifestations (20%).[19] In a present study among all clinical complains generalized weakness was commonest complain present 91.66% of pancytopenia patients followed by Fever in 59.66%, bleeding manifestation in 27.33% and other complaints in 26% of cases which were comparable to other studies.

Various studies throughout the world reported aplastic anaemia the commonest cause of pancytopenia.[20] Jha et al from Nepal studied the causes of pancytopenia in 148 patients. The commonest aetiology of pancytopenia in their study was hypoplastic bone marrow seen in 43 cases (29%) followed by megaloblastic anaemia in 35 cases (23.6%) and haematological malignancy in 32 cases (21.6%).[21] This is in sharp contrast with the results of our study where the commonest cause of pancytopenia was found to be megaloblastic anaemia. This variation in the etiology causing pancytopenia may be due to differences in a geographic area, nutritional status.

Incidence of megaloblastic anaemia in the different studies in India varied from 18% to 74.04%. Devi et al reported its incidence rate 18 % whereas, Khunger et al reported its incidence 72%.[22,23] In the present study megaloblastic anaemia was the commonest cause seen in 32.66% of causes which coincides with a study done by Azaad et al (28%) and Yadav et al (35%).[24,16] Anita et al reported commonest age of megaloblastic anaemia in pancytopenia cases was 15-25 years with male preponderance.[25] This finding coincides with our study where the majority of cases presenting as pancytopenia were in the age group of 15-25 years (33.71%) with males preponderance (Table 5).

Megaloblastic anaemia being the major cause of pancytopenia in India, since it is a rapidly correctable disorder and should be promptly notified.[26] Bone marrow aspiration studies are indicated in suspected cases of

megaloblastic anaemia, the patient requires urgent treatment and haematological assays such as folic acid and vitamin B12 levels are not routinely available in most centres in India.

Dimorphic anaemia (21%) was the second most common cause of pancytopenia in the present study reported in 63 cases. Raphael et al found 8.7% cases of dimorphic anaemia as the second most common cause and Prabhala et al found 14.53% cases of dimorphic anaemia as the third most common cause of pancytopenia.[27,28]

A dimorphic blood film is seen when iron deficiency anaemia responds to iron therapy after the transfusion of normal blood to a patient with hypochromic anaemia, sideroblastic anaemia, an unmasking of an iron deficiency following treatment of megaloblastic anaemia, delayed transfusion reactions and dual deficiency of iron and either vitamin B12 or folic acid.

Incidence of aplastic anaemia varies from 10% to 52% among pancytopenia patients.11 Present study we found of aplastic anaemia as a most common cause among non-nutritional anaemia's accounting 16% of all causes in pancytopenia patient. This finding coincides with the study done by Sweeta at (18%) and Gupta et al (14.8%).[29,30] We found aplastic anaemia as the most common cause among children less than 14 years of age in 37.83 % cases and megaloblastic anaemia in 8.1 % cases.

Kumar et al and Singh et al reported a 12 % incidence of acute leukaemia in pancytopenia patients with age group varying from 21-70 years.[31,32] A similar study by Bhatnagar et al reported 25% incidence of acute leukaemia in the children with pancytopenia.[33] We encountered a 13.66% incidence of acute leukaemia on bone marrow examination in pancytopenia patients with age varying from 3-80 years and male to female ratio 1.3:1.

The study by Subrahmanyam et al reported 10 (7.5%) cases of pancytopenia associated with chronic inflammation in the age group of 18-46 years with a male to female ratio 1.5:1.[34] In our study, we have reported 14 (5%) cases of pancytopenia associated presenting with fever and bone marrow finding were associated with infection /inflammation-related changes.

Jain et al reported single (0.4%) cases of MDS associated with pancytopenia.[1] Incidence of MDS as reported in other studies varies from 0-4.5%. These findings coincide with our study where we encountered 4 (1.33%) cases of MDS; out of which, 3 cases were above the age of 60 years. MDS are more common in the elderly and that should be included in the differential diagnosis of elderly with pancytopenia.

Hypersplenism is known to cause pancytopenia by sequestration of blood cells. In a study of 195 patients by Retief et al who found hypersplenism to be the cause of pancytopenia in 7.7% of the patients.[35] Similarly the study carried out by Memon et al in their study of 230 cases found hypersplenism in 10 patients (4.34%).36 In the present study, there were 6 (2%) cases of hypersplenism associated with pancytopenia in the age group of 22-65 years.

We have reported 4 (1.33%) cases of non-Hodgkin's lymphoma (NHL) Initially all cases presented with pancytopenia on bone marrow examinations. This was comparable to studies by Ahmad et al who reported 5 cases and Rahmani et al who reported 3 cases.[37,38] Overall NHL is one of a rare cause of pancytopenia. Evidence suggests that the neoplastic T cells cause suppression of haematopoiesis through the lymphokines.

Limitations

This is a hospital-based study of a short duration. Follow up of study population would have helped to evaluate the various causes better way.

CONCLUSION

This study showed that pancytopenia can be the presenting feature of many diseases, with megaloblastic anaemia, dimorphic anaemia and aplastic anaemia constituting the largest subgroups in the cases studied. Haematological values in these major groups showed considerable overlap making bone marrow examination mandatory to reach a diagnosis. Bone marrow aspirate specimens are superior for morphological detail over biopsy, while biopsy specimen provides a more reliable index of cellularity and often reveal marrow infiltration, fibrosis and granulomas which are not detected on aspiration. Thus both the procedures are complementary in diagnosis in the setting of pancytopenia.

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