Original Article

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Pathology Section

Clinico – Hematological Analysis of Pancytopenia: A Bone Marrow Study

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ABSTRACT

Background: Pancytopenia is characterized by anemia, leucopenia and thrombocytopenia, a common hematological problem. It is a striking feature of many serious and life threatening illnesses. The disease pattern varies in different population groups, in age pattern, nutritional status and prevalence of infective disorder. Present study was conducted to assess the etiology, clinical profile and bone marrow morphology of pancytopenia.

Objective: To know various patterns of clinical presentation and co-relate hematological parameters and bone marrow study with clinical findings, in differentiating causes of pancytopenia.

Methods: A two-years study from January 2009 to December 2010 was conducted in Department of Pathology. Total 106 pancytopenia patients aged 15-65 years were studied to determine their clinical features, peripheral smear study and bone marrow morphology. The etiological pattern was assessed through relevant investigations done on the respective patients.

Results: Bone marrow study showed 68.8% hypercellular marrow, 15% normocellular and 16.2 % hypocellular marrow. Males(57.5%) were affected more than females(42.5%) and the commonest cause for pancytopenia was megaloblastic anemia (73.5%), followed by iron deficiency anemia (12.2%), malaria(3.7%), leukemia (2.8%), SLE (1.8%), aplastic anemia (1.8%), multiple myeloma (0.9%), myelofibrosis(0.9%), MDS(0.9%) and hyperspleenism (0.9%).

Conclusion: Large number of patients had reversible etiology. Hence complete work up including clinical details with hematological examination along with bone marrow study will lead to early and proper diagnosis of case followed by proper treatment.

Keywords: Pancytopenia, Megaloblastic anemia, Iron deficiency, SLE, Malaria

INTRODUCTION

Pancytopenia is an important clinico-hematological entity encountered in our day-to-day clinical practice. The clinical pattern varies and thus treatment modalities and outcome III also varies [1].

Pancytopenia is not a disease entity but a triad of findings that may result from number of disease processes [2].

It is the simultaneous presence of anemia, leucopenia and thrombocytopenia, therefore it exists when hemoglobin is less than 13.5g/dl in males, 11.5g/dl in females, the WBC count $<4 \times 10^9$/L and platelet count $<150 \times 10^9$/L [3].

Presenting symptoms are usually attributable to anemia, leucopenia or thrombocytopenia. Anemia leads to fatigue,dyspnea and cardiac symptoms. Thrombocytopenia leads to bruising, mucosal bleeding and neutropenia to sharply increased susceptibility to infection [3].

The spectrum of disorders primarily or secondarily affecting the bone marrow may manifest with peripheral pancytopenia [4]. It is recommended that Bone Marrow Aspiration and Biopsy be done simultaneously in cases of pancytopenia. Aspiration smears are superior for morphological details while biopsy provides a more reliable index of cellularity and often reveals bone marrow infiltration, fibrosis and granulomas [5].

Pancytopenia is usually caused by bone marrow replacement or failure but is sometimes consequent on splenic pooling or peripheral destruction of mature cells. In hospital practice, pancytopenia is most often consequent on cytotoxic or in immuno-suppressive drug therapy [6].

Pancytopenia is a feature of many serious and life threatening diseases. The pattern of diseases leading to Pancytopenia may vary in different population groups with their differences in age pattern, nutritional status and the prevalence of infection [7]. The severity of Pancytopenia and the underlying pathology determine the management and prognosis of these patients [8].

Present study has been undertaken to evaluate the etiology, clinical profile and bone marrow morphology of pancytopenia. There by, this data would help in planning the diagnostic and therapeutic approach in patients with pancytopenia.

AIM

Aim is to study the spectrum of clinical presentation and co-relate hematological parameters, bone marrow study with clinical findings in diferentiating causes of pancytopenia.

METHODOLOGY

A present study was carried out from January 2009 to December 2010, in the department of pathology.

Inclusion criteria: Adults between 15-65 yrs age group and hematological parameters such as Hb <10g /dl, WBC count <4X10^9/L and Platelet < 150X10^9/L.

Exclusion Criteria: Patients ≤15 years and ≥ 65 years.

Relevant history, detail clinical examination and complete hematological analysis was done using sysmex Kx-21 analyser. The prarmeters included Hb%, RBC count, white count, platelet count, red cell indices and RDW. Bleeding time, clotting time, reticulocyte count, Peripheral smear study and Bone marrow study was done.

Bone marrow aspiration was performed in all the patients using Salah needle after obtaining written consent for the procedure either from the patient or the guardian. In the failure of aspiration, biopsy was performed and the aspirate/biopsy was processed as routine and standard staining techniques used for staining slides. All cases were stained for iron and grading for iron stores was done.

Special stain like Prussian blue stain, Reticulin stain, Myeloperoxidase, Sudan black and Masson's trichrome stain were done.

STATISTICAL ANALYSIS

Data was analyzed by using following statistical methods

1. Mean ± standard deviation
2. Diagramatic representation.
3. 't' test, Z test and x2 test.

RESULTS

Total 106 adult patients who presented with pancytopenia were studied during the period from Jan 2009 to Dec 2010.

The commonest age group affected was 15-25 year age group (39.62%) and least age group affected was 36-45 year age group (11.32%) [Table/Fig-1], with male preponderance and male: female ratio being 1.35:1.

The most common presenting feature was generalized weakness with 86.79% followed by pallor and fever, 83.96% and 53.49% respectively. The physical findings included hepatomegaly in 26 cases (24,52%), spleenomegaly seen in 19 cases (17.92%) and lymphadenopathy in 6 cases (5.66%). Hepato-spleenomegaly was commonly seen in megaloblastic anemia, leukemia and malaria [Table/Fig-2].

The hematological parameters obtained on analyzer were, Haemoglobin percentage varied from 1.8 – 9.2g%. Majority of patients had haemoglobin percentage between 5.1- 8g%. Lowest count of 1.8g%, recorded in a case of megaloblastic anaemia. Total leucocyte count ranged from 500 – 3,900 cells/mm^3. Majority had white cell count in range of 2,501 – 3,900 cells/mm^3. Lowest count of 500 cells/mm^3 was noted in a case of megaloblastic anaemia. Reticulocyte count ranged from 0.5 – 2%. In our study majority patients had reticulocyte count between 0.6 – 1%, which is reduced. Platelet count ranged from 10,000 – 1,50,000 cells/mm^3. Majority patients had platelet count between 1,00,000 – 1,50,000 cells/mm^3. Lowest platelet count of 10,000 cells/mm3 was recorded in a case of aplastic anaemia [Table/Fig-3].

In present study dimorphic anemia was seen in majority of cases, very few cases showed normocytic hypochromic anemia.

In the present study, megaloblastic anemia was most commomest cause with 72.6% followed by iron deficiency anemia 12.2%. Least common cause were myelofibrosis, multiple myeloma, MDS and hyperspleenism (0.9%) [Table/Fig-4].

Pancytopenia Associated with Megaloblastic Anemia

Out of 106 cases, 77 cases were of megaloblastic anemia, the commonest age group affected was 15-25 years. Males (62.33%) affected more than females (37.66%). Majority of

Sl. No.	Age group	No of cases (%)
1	15-25	42 (39.62%)
2	26-35	24 (22.64%)
3	36-45	12 (11.32%)
4	46-55	13 (12.26%)
5	56-65	15 (14.15%)

[Table/Fig-1]: Incidence of pancytopenia in different age groups

Sl. No.	Clinical presentation	No of cases (%)
1	Generalised weakness	92 (86.79%)
2	Fever	62 (58.49%)
3	Dyspnea	48 (45.28%)
4	Bleeding manifestation	3 (2.83 %)
5	Weight loss	24 (22.64%)
6	Pallor	89 (83.96%)
7	Lymphadenopathy	6 (5.66%)
8	Hepatomegaly	26 (24.52%)
9	Spleenomegaly	19 (17.92%)
10	Bone tenderness	5 (4.71%)

[Table/Fig-2]: Clinical presentation of cases

cases were having Hb in the range of 5-8gm%, TLC: 2,500-3,900cumm and platelet count 50000-100,000cumm. Peripheral study had dimorphic picture in most cases (72.72%). Bone marrow study had hypercellularity in 89.16% cases of MA [Table/Fig-5].

Sl. No.	Parameters	Range	No of cases (%)
1	Haemoglobin (gm%)	1.8 -5	32 (30.18%)
		5 – 8	58 (54.71%)
		8.1-9.2	16 (15.09%)
2	Total leucocyte count (cells/cumm)	500-1000	5 (4.71%)
		1001-2500	23 (21.69%)
		2501-3900	78 (73.58%)
3	Reticulocyte count (%)	0-0.5	24 (22.64%)
		0.6-1	58 (54.71%)
		1.1-2	24 (22.64%)
4	Platelet count (cells/cumm)	10000-50000	27 (25.47%)
		50001-100000	35 (33.08%)
		100001-150000	44 (41.50%)

[Table/Fig-3]: Vital haematological parameters

Pancytopenia Associated with Iron Deficiency Anemia

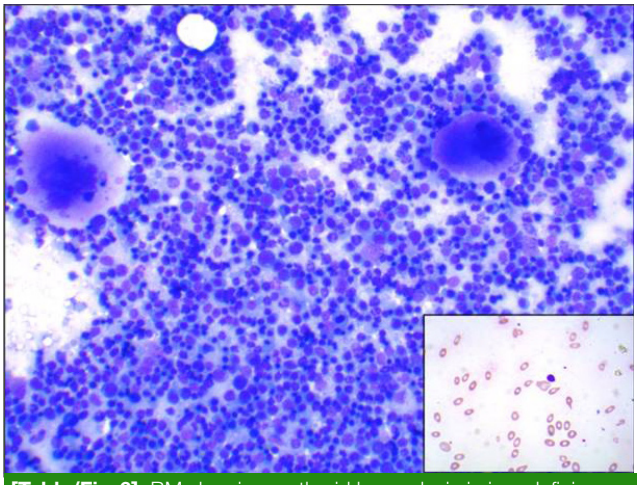
Thirteen cases of iron deficiency anemia were noted with commonest age group affecting 15-25 years and female show preponderance, with male: female 5:8. Haematological parameters in iron deficiency anemia includes 61.53% cases had haemoglobin range between 5-8 gm%, TLC 2,501-3,900cumm and platelet count between 1,00,001-1,50,000cumm. Peripheral smear study of all the cases had marked anisopoikilocytosis with microcytic hypochromic red cells with mild leucopenia and thrombocytopenia [Table/Fig-7]. Bone marrow study of most cases were hypercellular

Sl. No.	Causes	No of Cases (%)
1	Megaloblastic anemia	77 (72.6%)
2	Iron deficiency anemia	13 (12.2%)
3	Malaria	4 (3.7%)
4	Leukemia	3 (2.8%)
5	SLE	3 (2.8%)
6	Aplastic anemia	2 (1.8%)
7	Multiple myeloma	1 (0.9%)
8	Myelofibrosis	1 (0.9%)
9	MDS	1 (0.9%)
10	Hyperspleenism	1 (0.9%)
	Total	106 (100%)

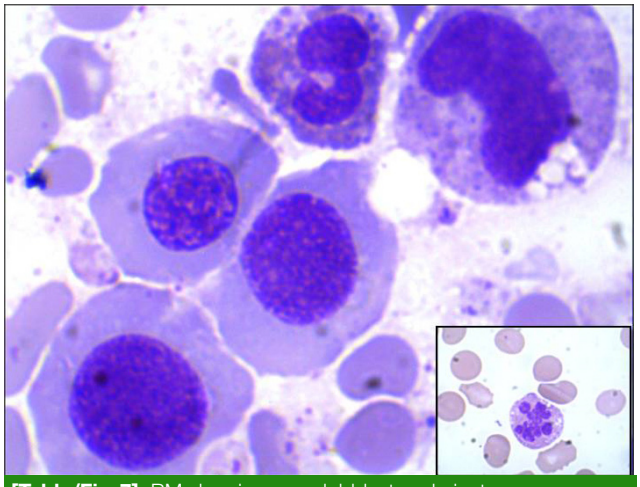
[Table/Fig-4]: Distribution of various causes of pancytopenia

Study	Tilak et al., 1999	Kumar et al., 2001	Khunger et al., 2002	Gupta et al., 2008	Present study 2011
Age group	5-70	All ages	2-70	1.5-18	Adults
No of cases	77	166	200	105	106
commonest cause	Megaloblastic anemia	Aplastic anemia	Megaloblastic anemia	Aplastic anemia	Megaloblastic anemia
Second common cause	Aplastic anemia	Megaloblastic anemia	Aplastic anemia	Acute leukemia	Iron deficiency anemia

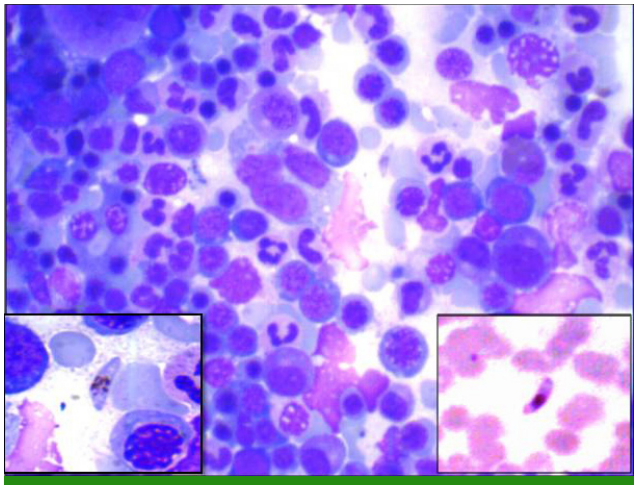
[Table/Fig-5]: Comparison of various studies



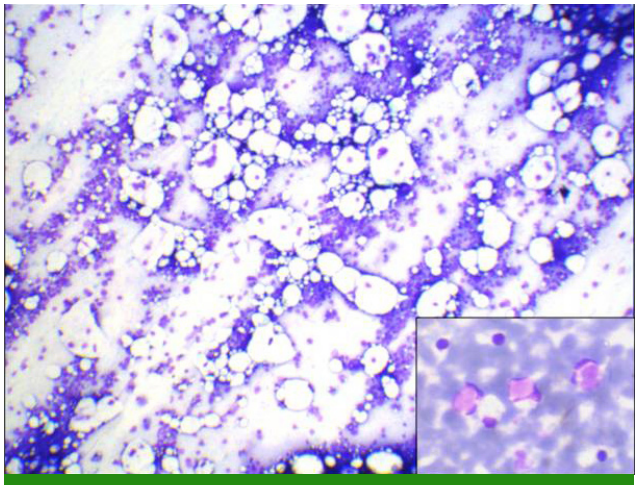
[Table/Fig-6]: BM showing erythroid hyperplasia in iron deficiency anemia. Geisma stain 400X. Inset: PS severe anisopoikilocytosis microcytic hypochromic anemia due to IDA



[Table/Fig-7]: BM showing megalobblast and giant metamyelocyte. Geisma stain (1000X). Inset shows hypersegmented neutrophill on PS



[Table/Fig-8]: BM showing P. Falciparum gametocytes. Geisma stain (400X). Inset: Left showing P. Falciparum (1000x), Right: PS with gametocyte



[Table/Fig-9]: BM: Hypocelluiar marrow in SLE Geisma stain (400X). Inset: LE cell demonstrated on buffy coat smear (Leishman stain 1000X)

with altered M:E ratio, increased erythropoiesis showing micronormaoblasts. Myelopoiesis was normal and there was slight increase in megakaryocytes [Table/Fig-6]. Bone marrow iron stores were within 0-2 grade that is complete of absence reduced iron stores.

Pancytopenia Due to Other Causes

In present study, malarial infestation was seen in 4 cases. They presented with fever, chills, rigor, vomiting and headache. Clinical examination revealed pallor and hepato-splenomegaly.

PS showed macrocytic hypochromic anaemia with neutropenia, thrombocytopenia and gametocytes of Plasmodium falciparum were seen in all cases. BM was hypercellular, P.falciparum gametocytes were seen on bone marrow smears [Table/Fig-8]. In three patients, hypercellular marrow due to leukemia, 2 cases were AML and 1 case was CLL were noted. SLE was seen in 3 cases with pancytopenia, all females, aged between 15-35 years. Clinical features included fever, weakness and skin rashes, one case with classical butterfly rash. Pallor and lymphadenopathy noted on physical examination. Peripheral smear was microcytic hypochromic picture. Bone marrow aspirate had scanty material and reported as hypoplastic bone marrow. LE cell phenomenon: Test was carried out as per standard procedure and 2 cases were positive for LE cell and advised further for ANA antibody detection [Table/Fig-9].

In present study 2 cases, 40 years old male and 18 years old were diagnosed as aplastic anemia.

Single case of Multiple Myeloma, Myelofibrosis, Myelodysplastic syndrome (MDS) and Hyperspleenism were obtained in the study. In myelofibrosis Bone marrow aspirate had scanty cellularity, hence bone marrow biopsy done which had increased fibrosis.

Reticulin stain: Increased fibrosis. Diagnosis of myelofibrosis was considered.

DISCUSSION

Although pancytopenia is common clinical problem with an extensive differential diagnosis, there is a relatively little discussion of this abnormality in major textbooks of internal medicine and hematology and there are limited number of the studies on the frequency of various causes of pancytopenia. There are limited numbers of studies on the frequency of various causes of pancytopenia [8].

Common age group affected was 1st-3rd decade in the studies done by Kishore et al., [9], Khunger et al., [3], Niazi et al., [2]. In present study, 15-25 years age group was most commonly affected.

In the present study, most common clinical manifestation was generalized weakness (86.79%) followed by pallor (83.96%) and fever (58.49%). Similar features were noted in studies done by Tilak et al., [8], Khunger et al., [3] and Nanda et al., [10].

The presenting symptoms were usually attributed to anaemia, or thrombocytopenia. Leucopenia was an uncommon cause of the initial presentation of the patient, but can become the most serious threat to life during course of the disorder [3,5]. Physical findings included hepatomegaly (24.52%), Splenomegaly (17.92%), lymphadenopathy (5.66%) and sternal tenderness (4.71%) in our study, which was comparable with Santra et al., [15] who noted hepatomegaly (24.32%), splenomegaly (44.14%) and lymphadenopathy (6.31%) as common clinical manifestations in his study.

The variation in the frequency of various diagnostic entities causing pancytopenia has been attributed to differences in methodology and stringency of diagnostic criteria, geographic

area, period of observation, genetic differences and varying exposure to myelotoxic agents, etc. [2,8,9].

In India with diverse ethnic populations, different dietary and social customs, the incidence of megaloblastic anemia and its associated problem have not been adequately documented [11]. The incidence of megaloblastic anaemia varies from 0.8% to 32.26% of all pancytopenia patients [3,8] and the incidence of aplastic anaemia varies from 10% to 52.7% of all pancytopenic patients [3,8].

In present study, megaloblastic anaemia was commonest cause of pancytopenia with 74% cases which coincides with studies done by Khunger et al., [3] (72%) and Tilak et al., [8] (68%). Second most common cause of pancytopenia in our study was iron deficiency anemia which was in sharp contrast with the studies done by other authors. This seems to reflect the higher prevalence of nutritional anaemia in Indian subjects [8,9].

Osama Ishtiaq et al., [7] studied 100 patients having panytopenia and encountered five cases of iron deficiency anemia (5%) as 4th common cause in his study which was comparable with our study where we also encountered 13 cases of iron deficiency anemia manifesting with pancytopenia. Iron deficiency anaemia is the second most common cause of nutritional deficiency in USA [12]. Iron deficiency anaemia can be associated with Pancytopenia. Though iron deficiency is associated with a reactive thrombocytosis, increasing severity of iron deficiency leads to normalisation and occasionally even decrease platelet counts. The exact mechanism of this is unclear but may be related to the alteration in the activity of iron dependant enzymes in thrombopoiesis and leucopoiesis [13,14].

Four cases of malaria were noted in present study comparable with the studies done by Osama et al., [7] and Tilak et al., [8]. Aplastic anemia was second most common cause in the studies done by Tilak et al., [8] and khunger et al., [3 but in present study we had only two cases of aplastic anemia and thus prevalence of aplastic anemia varies.

Santra et al., studied 8 cases of SLE out of which 3 cases had hypocellular marrow and 5 cases had cellular marrow [15]. We had 3 cases of SLE, all three had hypocellular marrow, 2 cases had LE cell positive on smear. We had one case of multiple myeloma, incidence being 1% compared to Khunger JM et al., [3] who also reported 1% cases of multiple myeloma in his study. Khunger et al., [3] encountered 1% case of myelofibrosis in his study comparable with the present study which also had one case of myelofibrosis with pacytopenia.

Hemophagocytic syndrome, tuberculosis, AML, hairy cell leukemia, multiple myeloma, myelofibrosis and drug induced pancytopenia were rare causes of pancytopenia in Khunger JM et al., [3] and Tilak et al., [8] study. Thus in present study myelofibrosis, MDS, hypersplenism and multiple myeloma were rare causes leading to pancytopenia.

In present study 73 cases (68.8%) had hypercellular marrow, followed by normocellular (15%) and hypocellular marrow (16.2%)which was comparable with the studies done by Santra et al., [15], who had 60/111 cases of cellular marrow and 50 cases of hypocellular marrow.

Bone marrow aspiration was diagnostic value to the patients of pancytopenia [15]. Most common cause for hypercellular bone marrow was megaloblastic anemia in present study. Hypercellular marrow was noted in cases of MDS, multiple myeloma, NHL and subleukemic leukemia in the study done by khunger et al., [3].

The diagnosis of AML was based on bone marrow aspiration study, and we reported 2 cases of AML-M2 and 1 case of CLL. Khodke K et al., reported a single case of AML-M2 of 50 cases of pancytopenia. Kumar R et al., reported 5 cases of ALL, 13 cases of AML, 2 cases of hairy cell leukemia out of 166 cases of pancytopenia, over a 6 year study period [5].

In present study we had one case of MDS with pancytopenia. Pancytopenia with few abnormal cells as seen in MDS was also noted in 2% cases by khunger et al., [3].

Hence pancytopenia has either cellular or hypocellular bone marrow morphology and there are few studies in the literature that explore the various aetiological factors of pancytopenia with hypocellular or cellular marrow [15].

CONCLUSION

Bone marrow aspiration is an important diagnostic tool in haematology which helps to evaluate various cases of Pancytopenia. Bone marrow aspiration is sufficient to make a diagnosis in cases of nutritional anaemia and initial diagnosis of leukemia.

Tuberculosis being highly prevalent and endemic in India, it is essential to be aware of its manifestation as pancytopenia.

Present study concludes that detailed primary haematological investigations along with bone marrow aspiration in cytopenic patients is helpful for understanding of the disease process, to diagnose or to rule out the causes of Pancytopenia and helpful in planning further investigations and management of Pancytopenic patients.

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