Original Research Article

A study of clinico-hematological profile of pancytopenic patients in Central India

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Abstract

Background: To segregate the causes of pancytopenia which are easily treatable, from those requiring sophisticated investigations & vigorous treatment, based on clinico-hematological profile of the patients and affordable diagnostic mathods. **Materials and Methods:** 180 Pancytopenic patients were evaluated clinically along with hematological parameters, peripheral smears and bone marrow aspiration in the Department of Pathology, GMC, Bhopal for 3 years. **Result:** Age range was 5 months-70 years (mean- 26.6 years), with Male preponderance (M: F: 1.76:1). Commonest symptoms were weakness (97.8%), breathlessness (75%) and signs were pallor (98.3%) and splenomegaly (25.5%). Patients who presented with per rectal bleeding, in 46.2% diagnosis was dimorphic anemia thus per rectal bleeding was cause of associated iron deficiency. Commonest peripheral smear finding was microcytic hypochromic picture (27.22 %) & bone marrow was hypercellular (70.00%). Bone marrow aspiration revealed commonest cause was megaloblastic anemia (25%) followed by dimorphic anemia (17.2%) and infections (17.2%). In our study MCV was <100 fl in 12/45 (26.66%) cases of megaloblastic anemia so even if a patient presents with MCV < 100 fl megaloblastic anemia should not be ruled out only on this basis. Sensitivity of peripheral smear for dimorphic anemia was very low 35.48% and specificity was 82.55%, so bone marrow examination should be investigation of choice for inconclusive peripheral smears. **Conclusion:** Common causes of pancytopenia were easily treatable and reversible & can be detected by early and affordable diagnostic methods therefore should be considered at higher order in differential diagnosis of pancytopenia.

Key words: Bone marrow aspiration, Megaloblastic anemia, Pancytopenia

Introduction

Pancytopenia is the simultaneous presence of anemia, leucopenia and thrombocytopenia. Pancytopenia therefore exists in the adult when the hemoglobin level is less than 13.5gm/dl in males or 11.5gm/dl in females, the leucocyte count is less than $4x10^9$ /L and the platelet count is less than $150x10^9$/L [1].

Although all the cell-lines of the blood are depleted, the clinical manifestations are mutually different from one another due to various causes of pancytopenia. The etiology of pancytopenia varies in different populations depending upon age, prevalence of infections, nutritional status and environment. So this study is conducted to evaluate the clinical profile, etiological spectrum and, peripheral blood & bone marrow findings of pancytopenic patients, with the aim to segregate easily treatable and reversible causes of pancytopenia from the diseases which need vigorous treatment in the central part (Bhopal region of Madhya Pradesh) of India, as there is paucity of authentic data about this.

Bone marrow aspiration, relatively safe and easy test, except for a mild discomfort to the patients, plays important role in determining the cause for pancytopenia, unexplained cytopenias, storage disorders and hematological malignancies [2]. As our hospital is a tertiary care centre, most of the patients belong to

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nearby rural areas, have poor socioeconomic status and they are unable to afford costly investigations. Thus there is a need to identify a pattern in the symptoms so as to pick up the clues to find out the exact etiology. A correlation between the clinical features of individual patients and the blood picture, bone marrow examination is being reviewed in this study in order to achieve the same. Such a correlation will help the clinicians to reach at the diagnosis easily and early. So this study is conducted to find out the causes of pancytopenia at earliest with minimal economical resources. Importance of the study lies in the timely intervention for the etiology of pancytopenia which can either bring about a complete cure or at least a remission from the disease.

Materials and Methods

Patients with pancytopenia underwent a detailed history, clinical examination, complete Blood Count, peripheral smear and bone marrow aspiration as per proforma specially designated for this study. Assessment of hematological parameters was done by automated cell counter (Mindray BC 5300). Peripheral smears were prepared and stained by Leishman stain for all the cases and examined in detail. Bone marrow aspiration was subsequently performed under aseptic precaution using Salah's bone marrow aspiration needle after obtaining written consent from the patient or guardian. Bone marrow aspiration smears were also stained by Leishman stain and Perl's staining was done for assessment of iron status.

Study Design- Retrospective and prospective observational study.

Setting- Department of Pathology, Gandhi Medical College, Bhopal during a period of 1st August 2013 to 31st August 2016.

Inclusion Criteria- The study included all cases of pancytopenia i.e. hemoglobin less than 13.5gm/dl in males or 11.5gm/dl in females, total leucocyte count less than 4,000 cells/ cumm and platelet count less than 1, 50, 000 / cu mm.

Exclusion Criteria- Patients who had received chemotherapy, radiotherapy or immunosuppressive drugs were excluded from the study.

Participants- Patients diagnosed with pancytopenia and referred to our department for further work up.

Data Source- Data was collected from history, clinical examination, case files as well as investigations done in these patients.

Bias- Referral Bias.

Study size- This Study included 180 cases of pancytopenia.

Statistical Methods- For statistical analysis Chi square test was applied. P value was considered significant if $p<0.05$, and highly significant if $p<0.01$. Percentage, mean, standard deviation, sensitivity and specificity were also calculated.

Results

Table-1: Age group wise distribution of cases according to final diagnosis.

Final Diagnosis	0-14 years (n= 48)	15-34years (n=72)	>34years (n= 60)	Total
Megaloblastic Anemia	9(5%)	18(10%)	18(10%)	45(25%)
Dimorphic Anemia	8(4.4%)	13(7.2%)	10(5.6%)	31(17.2%)
Marrow Reactive to infection	6(3.3%)	14(7.7%)	11(6.2%)	31(17.2%)
Aplastic/ Hypoplasic Anemia	8(4.4%)	11(6.1%)	4(2.3%)	23(12.8%)
Acute Myeloid Leukemia	5(2.8%)	6(3.3%)	6(3.3%)	17(9.4%)
Acute Lymphoblastic Leukemia	4(2.3%)	3(1.6%)	1(0.6%)	8(4.4%)
Trilineage Hyperplasia with hypersplenism	1(0.6%)	3(1.6%)	3(1.6%)	7(3.8%)
Lymphoma	0(%)	1(0.6%)	3(1.6%)	4(2.6%)
Plasma Cell Dyscrasia	1(0.6%)	1(0.6%)	1(0.6%)	3(1.6%)
Gaucher's Disease	2(1.1%)	0(0%)	1(0.6%)	3(1.6%)
Others	4(2.2%)	2(1.1%)	2(1.1%)	8(4.4%)
Total	48(26.7%)	72(39.8%)	60(33.5%)	180(100%)

Most common cause of pancytopenia found was megaloblastic anemia (25%), followed by dimorphic anemia (17.2%) and bone marrow reactive to infection (17.2%). Age range was 5 months to 70 years (Mean-26.6 years). Males were 63.8% and females were 36.2%. A definite male preponderance (Male: Females: 1.76:1) was observed. Male: female ratio in pediatric age group was slightly higher (2.69:1). Most common age group (39.8%) was 15-34 years. In all the age groups most common cause of pancytopenia was megaloblastic anemia. (Table-1).

Table 2-Gender wise distribution of symptoms.

Symptoms	Males No. (%)	Females No. (%)	Total No. (%)	P value
Weakness	113(62.8%)	63(35.0%)	176(97.8%)	0.558
Breathlessness	93(51.7%)	42(23.3%)	135(75%)	0.015**
Fever	83(46.2%)	43(23.8%)	126 (70%)	0.397
Epistaxis	20(11.2%)	8(4.4%)	28(15.6%)	0.366
Hematemesis	7(3.9%)	9(5.0%)	16(8.9%)	0.078
Per rectal bleeding	12(6.7%)	1(0.5%)	13(7.2%)	0.026**
Gum bleeding	7(3.9%)	3(1.7%)	10(5.6%)	0.678
Ecchymosis	6(3.4%)	3(1.6%)	9(5.0%)	0.858
Rashes	7(3.9%)	2(1.1%)	9(5.0%)	0.373
Petechiae	5(2.7%)	3(1.6%)	8(4.4%)	0.933
Hematuria	0(0%)	2(1.1%)	2(1.1%)	NA
Pedal edema	9(5.0%)	6(3.3%)	15(8.3%)	0.743

**Statistically significant as p <0.05

NA- No. of cases were <5, so chi square test was not applied.

Most common symptom was generalized weakness (97.8%) followed by breathlessness (75%), fever (70%) and bleeding tendencies. Most common bleeding tendency was epistaxis (15.6%) followed by hematemesis (8.9%), per-rectal bleeding (7.2%) and others. Breathlessness (p= 0.015) and per rectal bleeding (p= 0.026) were more significant in males as compared to females. (Table-2).
13/180 patients presented with per rectal bleeding and 6/13 (46.2%) were diagnosed as case of Dimorphic anemia thus, per rectal bleeding is a cause of associated iron deficiency.

Table-3: Gender wise distribution of clinical signs.

Signs	Males No. (%)	Females No. (%)	Total No. (%)	P value
Pallor	112(62.2%)	65(36.1%)	177(98.3%)	0.189
Splenomegaly	29(16.1%)	17(9.4%)	46(25.5%)	0.889
Hepatomegaly	24(13.3%)	9(5.0%)	33(18.3%)	0.242
Icterus	20(11.2%)	12(6.6%)	32(17.8%)	0.856
Lymphadenopathy	20(11.1%)	3(1.6%)	23(12.7%)	0.013**
Ascites	6(3.3%)	9(5.0%)	15(8.3%)	0.044**

**Statistically significant as p <0.05

NA- No. of cases were <5, so chi square test was not applied.
Most common sign was pallor (98.3%) followed by splenomegaly (25.5%), hepatomegaly (18.3%) and others.
Lmphadenopathy (p=0.013) was more significant in males and ascites (p=0.044) was more significant in females.
In present study among 8 cases of ALL, one patient (12.5%) presented without hepatosplenomegaly and lymphadenopathy (Aplastic Presentation).

Table-4: Comparison of hematological parameters among leading causes of pancytopenia.

Diagnosis	Hemoglobin Mean ±SD	Total RBC Count Mean ±SD	Total WBC Count Mean ±SD	Platelet Count Mean ±SD
Megaloblastic Anemia	5.57 ± 1.93 gm/dl	2.15 ± 0.84 x10^6 /cumm)	2700 ± 853 cells/cumm)	47000 ± 28,000 /cumm)
Dimorphic Anemia	5.69 ± 1.95 gm/dl	2.62 ± 0.84 x10^6 /cumm	3000 ± 800 cells/cumm)	66,000 ± 28,000 /cumm)
Marrow Reactive to infection	6.19 ± 1.93 gm/dl	2.55± 0.84 x10^6 /cumm	3000 ± 800 Cells/cumm)	49000 ± 28,000 /cumm)
Aplastic/ Hypoplasic Anemia	4.73 ± 1.94 gm/dl	1.21 ± 0.84 x10^6 /cumm	2300 ± 800 Cells/cumm)	40000 ± 28,000 /cumm)
Acute Myeloid Leukemia	5.58 ± 1.95 gm/dl	1.98 ± 0.84 x10^6 /cumm	2700 ± 800 cells/cumm)	36000 ± 28,000 cumm)
Acute Lymphoblastic leukemia	5.28 ± 1.94 gm/dl	2.18 ± 0.84 x10^6 /cumm	2600 ± 800 Cells/cumm)	48000 ± 28,000/cumm
Trilineage Hyperplasia with hypersplenism	6.42 ± 1.92 gm/dl	2.60 ± 0.77 x10^6 /cumm	2500 ± 800 Cells/cumm	59000 ± 28,000 /cumm

In Aplastic/Hypoplastic anemia Mean hemoglobin percentage (4.73±1.94 gm/dl), Mean RBC count (1.21± 0.84x10^6/cumm) and mean total leucocyte count (2300±800 cells/cumm) were lowest in comparison to other causes of pancytopenia.

Table-5: Diagnosis wise MCV values among leading causes of pancytopenia.

	MCV			
Diagnosis	**<80 fl**	**80-100 fl**	**>100 fl**	**Total cases(n)**
Megaloblastic Anemia	4(8.9%)	8(17.77%)	33(73.33%)	45(100%)
Dimorphic Anemia	15(48.38%)	12(38.7%)	4(12.92%)	31(100%)
Marrow Reactive to infection	14(45.17%)	15(48.38%)	2(6.45%)	31(100%)
Aplastic/ Hypoplasic Anemia	6(26.08%)	16(69.56%)	1(4.36%)	23(100%)
Acute Myeloid Leukemia	3(17.66%)	10(58.82%)	4(23.52%)	17(100%)
Acute Lymphoblastic Leukemia	3(25%)	7(58.3%)	2(16.7%))	12(100%)
Trilineage Hyperplasia with hypersplenism	6(85.71%)	1(14.29%)	0(0%)	7(100%)

12/45(26.66%) cases of megaloblastic anemia presented with MCV values <100 fl. So even if a patient presents with MCV value of <100 fl, megaloblastic anemia should not be ruled out only on this basis.

Table-6: Comparison of organomegaly in cases of cellular and hypocellular marrow.

	Hypercellular + Normocellular	Hypocellular	Total	P value
Hepatomegaly	28 (84.8%)	5(15.2%)	33(100%)	0.279642.
Splenomegaly	42(91.3%)	4(8.7%)	46(100%)	0.01054**.

**Statistically significant as p <0.05

Bone marrow was hypercellular in 70.00% cases followed by hypocellular marrow (22.22%) and normocellular marrow (7.78%). We also found association of organomegaly with bone marrow cellularity. There was significant association of splenomegaly with cellular marrow (p= 0.010).

Peripheral smear findings- In peripheral smear anisopoikilocytosis was predominant finding. Microcytic hypochromic RBCs were seen in 27.22% cases followed by normocytic hypochromic RBCs (26.67%), Macrocytic picture were seen in 25% cases and dimorphic picture was seen in 20.56% cases.

Table-7: Sensitivity and specificity of Peripheral smear examination as compared to bone marrow examination

A. Megaloblastic Anemia.

Peripheral smear	Final Diagnosis by bone marrow -Megaloblastic Anemia (+)	Final Diagnosis by bone marrow- Megaloblastic Anemia (-)	Total
Macrocytic picture(+)	31	14	45
Macrocytic picture(-)	14	121	135
Total	45	135	180

- Sensitivity- 68.89%
- Specificity- 89.63%

B. Dimorphic Anemia-

Peripheral smear	Final Diagnosis by bone marrow-Dimorphic Anemia (+)	Final Diagnosis by bone marrow- Dimorphic Anemia (-)	Total
Dimorphic picture(+)	11	26	37
Dimorphic picture(-)	20	123	143
Total	31	149	180

- Sensitivity- 35.48%
- Specificity- 82.55%

Sensitivity of peripheral smear for diagnosis of megaloblastic anemia was 68.89% and specificity was 89.63 % whereas for dimorphic anemia sensitivity was very low 35.48% and specificity was 82.55% (bone marrow aspiration was taken as gold standard test) so bone marrow examination should be investigation of choice in case of inconclusive peripheral smears.

Discussion

In our study megaloblastic anemia (25%) was most common cause of pancytopenia. Tilak & Jain et al. reported higher incidence of megaloblastic anemia 68%. Jha et al. found 23.64% and Bhatnagar et al. found 28.4% cases of megaloblastic anemia in their studies. [3, 10, 11]

In pediatric age group also most common cause was megaloblastic anemia (18.75%). Similar result was seen by Bhatnagar et al., they also reported megaloblastic anemia (28.4%) as most common cause. In contrast Gupta et al. found aplastic anemia (43%) as most common cause in children. [11, 13]

Very few studies have reported Dimorphic Anemia to be a major cause of pancytopenia. Dimorphic anemia (17.2%) was second most common cause of pancytopenia in present study. Raphael et al. found 8.7% cases of dimorphic anemia as second most common cause and Prabhala et al. Found 14.53% cases of dimorphic anemia as third most common cause of pancytopenia in their study. [8, 14].

A possible explanation of such large number of patients presenting with nutritional anemia in our study can be extremes of age, nutritional deficiencies, malabsorption, parasitic infestations, chronic gastrointestinal, genitourinary bleed etc. Since nutritional disorders are easily treatable therefore should be considered at higher order in differential diagnosis list of pancytopenia.

Table-8: Comparison with other studies.

Study	Country	Year	No. of cases	Age group	M:F ratio	Commonest cause	2nd common cause	3rd common cause
Tilak and Jain et al. [3]	India-Chandigarh	1999	77	5 - 70	1.1 : 1	Megaloblastic Anemia (68%)	Aplastic Anemia (7.7%)	Other causes (24.3%)
Kumar et al. [4]	India-Delhi	2001	166	12- 73	2.1: 1	Aplastic Anemia (29.5%)	Megaloblastic Anemia (22.3%)	Aleukemic leukemia (12%
Hamid et al. [5]	Yemen	2008	75	3 - 85	1.03: 1	Hypersplenism (45.3%)	Megaloblastic Anemia (14.7%)	Aplastic Anemia (13.3%)
Santra & Das et al. [6]	India-Kolkata	2010	111	13-65	1.5 : 1	Aplastic Anemia (22.72%)	Hypersplenism (15%)	Drug induced (13%)
Gayathri & Rao et al. [7]	India-Karnataka	2011	104	2-80	1.2:1	Megaloblastic Anemia (74.04%)	Aplastic Anemia (18.3%)	Subleukemic Leukemia (3.8%)
Raphael et al. [8]	India-Meghalaya	2012	80	1-79	1 : 1.2	Megaloblastic Anemia (41.2%)	Dimorphic Anemia(8.7%), Aplastic Anemia / Hypoplastic Anemia (8.7%)	Acute Leukemia (7.5%)
Jain and Naniwadekar [9]	India-Maharashtra	2013	250	All	2.6 : 1	Hypersplenism (29.2%)	Infections (25.6%)	Myelosuppressants (16.8%)
Present Study	India - central India	20l6	180	5 months - 70years	1.76:1	Megaloblastic anemia (25%)	Dimorphic anemia and Bone Marrow reactive to infection (17.2% each)	Aplastic /Hypoplastic Anemia (12.8%)

Bone marrow reactive to infections (17.2%) was also second most common cause of pancytopenia in present study. Our finding is similar to Jain & Naniwadekar et al. also found infective etiology (25.6%) to be 2nd most common cause of pancytopenia in their study. Gupta et al. concluded infection (19.1%) to be third most common cause of pancytopenia. Pine & Walter et al. found infectious etiology (64%) to be most common cause of pancytopenia [9,13,15].

Infective etiology being a major cause of pancytopenia points towards poverty and poor hygienic conditions as our hospital is a tertiary care centre and majority of our patients belong to poor socioeconomic status. Aplastic anemia/hypoplastic anemia (12.8%) was third most common cause of pancytopenia in our study. Similar result were seen in studies of Khodke et al. and Khunger et al., both observed an incidence of 14% and it was second most common cause of pancytopenia in their study [12, 16]. In contrast a higher incidence was reported by Kumar R et al. 29.5%, Jha et al. found 29% *and* Verma N & Dash S et al. reported 40.6% cases of aplastic anemia. Higher incidence of Aplastic / Hpoplastic anemia in other studies may be related to environmental factors such as increased exposure to toxic chemicals in industrialized areas, pesticide exposure and overuse and easy availability of over the counter drugs. [4, 10, 17].

In present study there was 13.8% incidence of Aleumic Leukemia (9.4% incidence of Acute Myeloblastic Leukemia and 4.4% incidence of Acute Lymphoblastic Leukemia) compared to 12% reported by Kumar *R et al.* and Khunger *JM et al.* reported 5% incidence of aleukemic leukemia [4, 16].

Most common symptom was generalised weakness (97.8%) followed by breathlessness (75%), fever (70%) and bleeding tendencies -epistaxis (15.6%) followed by hematemesis (8.9%), per-rectal bleeding (7.2%), gum bleeding(5.6%), ecchymosis(5.0%), rashes(5.0%) and petechiae(4.4%). Santra & Das et al. found weakness in 45%, fever in 50.4 % and bleeding tendencies in 41.4% cases. Khodke et al. found was fever in 40% followed by weakness in 30% and bleeding manifestations in 20% of the cases [6, 12].

Most common sign was pallor, which was present in 98.3% of cases followed by splenomegaly (25.5%), hepatomegaly (18.3%), icterus (17.8%), lymphadenopathy (10.6%) and ascites (8.3%). Lmphadenopathy was more significant in males and ascites was more significant in females ($p<0.05$). Similarly Santra et al. found pallor in 84.6%, hepatomegaly in 24% and splenomegaly in 44% cases. Khodke et al. reported pallor was universally present in all the patients, splenomegaly was seen in 40% and hepatomegaly in 38% of cases [6,12].

Among hematological parameters in present study in Aplastic/Hypoplastic anemia Mean hemoglobin percentage (4.73 gm/dl), Mean RBC count (1.21x106/cumm) and mean Total leucocyte count (2300cells/cumm) were lowest in comparison to other causes of pancytopenia. Azad et al. found in their study that mean Total leucocyte count (1600cells/cumm and platelets (25000/cumm) were lowest among patients of aplastic anemia [18]. 12/45(26.66%) cases of megaloblastic anemia presented with MCV values <100 fl. So even if a patient presents with MCV value of <100 fl, megaloblastic anemia should not be ruled out only on this basis.

In present study in peripheral smear anisopoikilocytosis was predominant finding. Microcytic hypochromic RBCs were seen in 27.22 % cases followed by normocytic hypochromic RBCs (26.67%), Macrocytic picture were seen in 25% cases and Dimorphic picture was seen in 20.56% cases. Gayathri & Rao et al. found in their study,the predominant blood picture was dimorphic anemia (37.5%), followed by macrocytic anemia (31.7%). Khodke et al. also found most common finding in peripheral blood film was dimorphic anaemia (20%). [7, 12].

In present study bone marrow was hypercellular in 70.00% cases followed by hypocellular marrow (22.22%) and normocellular marrow (7.78%). Imbert M et al. found normo or hypercellular bone marrow in 66% cases. [19].

Present study shows significant association of splenomegaly with cellular marrow ($p=0.010$). Santra G et al. found hepatomegaly and splenomegaly both were significantly more common in patients with cellular marrow ($p < 0.05$) [6].

Thus present study stresses the importance of physical and peripheral blood findings in the management of pancytopenic patients. Sensitivity of peripheral smear examination was 68.89% and specificity was 89.63% for megaloblastic anemia. Sensitivity of peripheral smear for diagnosis of dimorphic anemia was very low (35.48%) and specificity was 82.55%, so bone marrow examination should be investigation of choice in case of inconclusive peripheral smears.

Conclusion

Our study reflects higher prevalence of nutritional anemia and infections in central part of India.These causes are easily treatable and reversible & detected by early and affordable diagnostic methods therefore should be considered at higher order in differential diagnosis of pancytopenia. Present study stresses the importance of association of physical and peripheral blood findings in the management of pancytopenic patients.

Thus sophisticated & costly investigations and vigorous treatment, which may not be accessible everywhere and also not mandatory for all pancytopenic patients, especially in underdeveloped and developing countries, so there is need to conduct more studies on pancytopenia with larger cohort in future, which will definitely be helpful to decrease cost particularly for individuals, society and overall for countries with constraint resources like our India.

Abbreviations

Hb Haemoglobin, TLC Total Leucocyte Count, MCV Mean Corpuscular Volume, MCH Mean Corpuscular Hemoglobin, MCHC Mean Corpuscular Hemoglobin Concentration.

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