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PATHOLOGICAL STUDY OF BONE MARROW MORPHOLOGY IN CASES OF PANCYTOPENIA AT A TERTIARY HOSPITAL

Vaddadi Vishali[1], V Jyothi[2], Sudharani S[3], Satishkumar D[4], V Manoja[5]

[1]Assistant Professor, Department of Pathology, Khaja Banda Nawaz University-Faculty of Medical Sciences, Kalaburagi, Karnataka, India.
[2]Associate Professor, Department of Pathology, Government Medical College, Nandyal, Andhra Pradesh, India.
[3]Assistant Professor, Department of Pathology, Khaja Banda Nawaz University-Faculty of Medical Sciences, Kalaburagi, Karnataka, India.
[4]Professor, Department of Biochemistry, ESIC MC PGIMSR, Bemgaluru, Karnataka, India.
[5]Associate Professor, Department of Pathology, Sri Venkata Sai Medical College, Mahbubnagar, Telangana, India.

Corresponding Author:
Dr. V Manoja. Associate Professor, Dept of Pathology, Sri Venkata Sai Medical College, Mahbubnagar, Telangana, India.
Email: vaddadi71@gmail.com

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Abstract

Background: Pancytopenia is defined as reduction of all the three formed elements of blood below the normal reference range. The severity of pancytopenia and the underlying pathology determines the management and prognosis. Present study was aimed to study bone marrow morphology in cases of pancytopenia at a tertiary hospital. **Material and Methods:** Present study was single-center, prospective, observational study conducted in patients with hematological diagnosis of pancytopenia (Haemoglobin < 10 gms, Total leucocyte count < 4000/cumm & Platelet count < 100,000/cumm) after complete hemogram study, **Results:** Most of the patients were in the age group of 11-20 years (32.7%) and male to female ratio was 1.16:1. The predominant blood picture was dimorphic anemia (48.1%), followed by Microcytic hypochromic anemia (30.1%). In present study, bone marrow cellularity Hypercellular (64.1 %) in majority of cases followed by Normocellular (17.9%), Unsatisfactory (9.6 %) & Hypocellular (8.3 %). A total of 156 cases were diagnosed on bone marrow aspiration with dimorphic anemia as the commonest diagnosis (41%). Megaloblastic anemia was diagnosed as the second common diagnosis (25%). Among 156 cases which were diagnosed on aspiration we have done bone marrow biopsy for103 cases. Dimorphic anemia was the commonest diagnosis (43.6%). **Conclusion:** Dimorphic anemia was the commonest cause of pancytopenia in the present study followed by megaloblastic anemia. This seems to reflect higher prevalence of nutritional anaemia in the Indian subjects.

Keywords: Pancytopenia, hematological entity, dimorphic anemia, megaloblastic anemia

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Introduction

Pancytopenia is a relatively common haematological entity. It presents with a triad of findings that may result from a number of disease processes.[1,2] The spectrum of disorders primarily or secondarily affecting the bone marrow may manifest with peripheral pancytopenia.[3]

Pancytopenia is defined as reduction of all the three formed elements of blood below the normal reference range. The presenting symptoms are often attributable either to the anaemia or thrombocytopenia. Leukopenia is an uncommon cause of initial presentation, but can become a serious threat to life during the course of the disorder.[4,5]

Pancytopenia is a serious hematological problem, the underlying cause of which is diagnosed by bone marrow aspiration and biopsy. Bone marrow examination is extremely helpful in the evaluation of pancytopenia.[6,7] The severity of pancytopenia and the underlying pathology determines the management and prognosis. Thus, identification of the correct cause will help in implementing the appropriate therapy. Present study was aimed to study bone marrow morphology in cases of pancytopenia at a tertiary hospital

Material And Methods

Present study was single-center, prospective, observational study conducted in department of pathology, Mahadevappa Rampure medical college, from Basaveshwar and Sangameshwar Teaching and General Hospital and Government General Hospital, Kalaburagi, India. Study duration was of 2 years (August 2015 to July 2018). Study approval was obtained from institutional ethical committee.

Inclusion criteria

- patients with hematological diagnosis of pancytopenia (Haemoglobin < 10 gms, Total leucocyte count < 4000/cumm & Platelet count < 100,000/cumm) after complete hemogram study, willing to participate in present study

Exclusion criteria

- Chemotherapy induced pancytopenia
- Patients with severe thrombocytopenia
- (< 10,000 cells/cumm) or any bleeding disorder
- Non co-operative patients.
- Critically ill patients.

Study was explained to patients in local language & written consent was taken for participation & study. Clinical history and examination of all the identified cases of pancytopenia were done as per the proforma. Two ml of blood was collected in a vacutainer for complete hemogram. Investigations done were haemoglobin percentage, total leucocyte count, platelet count and red cell indices using automated hemogram Sysmex Kx 21. Bleeding and clotting time were done. Peripheral smears were studied using fields and Leishman's stain. Special stains like perls, PAS. Reticulin stains were used where ever necessary.

Bone marrow aspiration was done where ever indicated. Written consent was taken from the patient or guardian before the procedure. The site for aspiration was posterior superior iliac spine. Skin over the chosen site was cleaned with spirit and infiltrated with 2%

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xylocaine. Aspiration was performed using Salah needle (16G) with drilling action through skin and periosteum needle was entered in to the marrow, which was appreciated by lack of resistance. Once in marrow cavity stylet was removed and 0.3-0.5 ml of material aspirated with a 10ml syringe. Smears were immediately made over the slides and then stained with Giemsa stain.

Bone marrow biopsy was performed following aspiration if indicated using Jamshidi needle(11 G). By drilling action needle was pushed in to the marrow, following loss of resistance stylet was removed and needle was further advanced in to with clock wise-anticlockwise movements up to 2-3 cm. Once marrow core was sampled the needle withdrawn by rotating movements. Using a probe he core was extracted and imprints were taken on slides.

Tissue obtained was fixed in 10% aqueous formalin solution, followed by decalcification and then subjected for tissue processing. The processed tissue was then embedded in paraffin to obtain 3 to 5 micron thin sections. These sections were stained with routine hematoxylin and eosin stain and then examined under microscope. Bone marrow cellularity and morphology was then assessed.

Statistical analysis was done using descriptive statistics.

Data was collected and compiled using Microsoft Excel, analysed using SPSS 23.0 version. Frequency, percentage, means and standard deviations (SD) was calculated for the continuous variables, while ratios and proportions were calculated for the categorical variables. Difference of proportions between qualitative variables were tested using chi-square test or Fisher exact test as applicable. P value less than 0.5 was considered as statistically significant.

Results

The present study includes 156 patients with a hematological diagnosis of pancytopenia during the period. Most of the patients were in the age group of 11-20 years (32.7%) and least occurrence was seen in the age group of 71-80 years (0.6%). The sex distribution of pancytopenia showed a male preponderance. The male to female ratio was 1.16:1.

Table 1: Age and sex distribution

Age (Year)	Male	Female	Total cases	%
0-1	2	0	2	1.2%
1-10	16	8	24	15.4%
11-20	16	35	51	32.7%
21-30	17	10	27	17.3%
31-40	15	09	9	15.4%
41-50	6	3	9	5.8%
51-60	7	2	9	5.8%
61-70	4	5	9	5.8%
71-80	1	0	1	0.6%
Total	84	72	156	100%

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Generalized weakness was the commonest symptom (52.6%), followed by fever (44.2%). In all the cases pallor was noted (100%) followed by splenomegaly (32%) and hepatomegaly (18%).

Table 2: Clinical features

Clinical presentation	No of cases	Percentage
Generalized weakness	82	52.6%
Fever	69	44.2%
Easy fatigability	40	25.6%
Gastrointestinal symptoms	36	23.1%
Breathlessness	34	21.8%
Cough	25	16%
Giddiness	12	7.7%
Yellow discoloration of sclera	8	5.1%
Headache	8	5.1%
Bleeding	8	5.1%

The hemoglobin percentage varied from 1 gm% to 10 gm%. Majority (40.3%) of the patients had hemoglobin ranging from 3.1-5 gm%. 7.6% of the patients had hemoglobin values between 1% and 3%. The total leukocytic count was in the range of 500-4000 cells/cumm. Most (39.1%) of the patients had values in the range of 2100-3000 cells/cumm. 2.6% of the patients had values in between 500 and 1000 cells/cumm.

The range of platelet count varied from 10,000-1,00,000 cells/cumm. Most (35.2%) patients had platelet counts in the range of 25,000-50,000 cells/cumm. The predominant blood picture was dimorphic anemia (48.1%), followed by Microcytic hypochromic anemia (30.1%).

Table 3: Hematological parameters

	No. of patients	Percentage
Hemoglobin (gm%)		
< 3	12	7.6%
3.1 to 5	63	40.3%
5.1 to 7	52	33.3%
7.1 to 10	29	18.5%
Leukocyte count (cells/cumm)		
500-1000	4	2.6
1001-2000	43	27.6
2001-3000	61	39.1
3001- 4000	48	30.8
Platelet count (cells/cumm)		
Up to 25000	17	10.9%
25000-50000	55	35.3%
50000-75000	35	22.4%
75000-1,00,000	49	31.4%

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Peripheral smear finding		
Normocytic normochromic	18	11.5%
Microcytic hypochromic	47	30.1%
Macrocytic	16	10.3%
Dimorphic	75	48.1%

In present study, bone marrow cellularity Hypercellular (64.1 %) in majority of cases followed by Normocellular (17.9%), Unsatisfactory (9.6 %) & Hypocellular (8.3 %).

Table 4: Bone marrow cellularity in patients with pancytopenia

Type of cellularity	**No. of cases**	**Percentage**
Hypocellular	13	8.3%
• Aplastic anemia	2	15.3%
• Hypoplastic marrow	11	84.6%
Normocellular	28	17.9%
• Megaloblastic anemia	9	32.1%
• Microcytic anemia	7	25%
• Dimorphic anemia	11	39.2%
• Multiple myeloma	01	3.5%
Hypercellular	100	64.1%
• Dimorphic anemia	53	53%
• Megaloblastic anemia	30	30%
• Microcytic anemia	14	14%
• Multiple myeloma	01	1%
• ALL	01	1%
• Malaria	01	1%
Unsatisfactory	15	9.6%

A total of 156 cases were diagnosed on bone marrow aspiration with dimorphic anemia as the commonest diagnosis (41%). Megaloblastic anemia was diagnosed as the second common diagnosis (25%). As the p is 0.00 (less than 0.05) all the etiological factors have statistical significant association with pancytopenia.

Table 5: Causes of pancytopenia

Causes	**Number of cases**	**Percentage**
Dimorphic anemia	64	41%
Megaloblastic anemia	39	25%
Microcytic hypochromic anemia	21	13.4%
ALL	02	1.2%
AML	01	0.6%
Multiple myeloma	04	2.5%
Metastasis	01	0.6%
Aplastic anemia	05	3.2%
Hypoplastic marrow	11	7%

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Malaria	01	0.6%
Undiagnosed	07	4.4%

Chi-Square = 251.692,'p' value is 0.00

Among 156 cases which were diagnosed on aspiration we have done bone marrow biopsy for103 cases. Dimorphic anemia was the commonest diagnosis (43.6%).Cases which were unsatisfactory/undiagnosed on bone marrow aspiration were diagnosed on trephine biopsy are 1 case of AML, 1 case of ALL, 2 cases of multiple myeloma,1 case of metastasis.

Table 6: Diagnosis on Bone marrow aspiration & Histopathology

Diagnosis	**Bone marrow aspiration diagnosis**		**Histopathological diagnosis**	
	No of cases	**Percentage**	**No of cases**	**Percentage**
Dimorphic anemia	64	41%	45	43.6%
Micronormoblastic anemia	21	13.4%	12	11.6%
Megaloblastic anemia	39	25%	24	23.3%
ALL	01	0.6%	02	1.9%
Aplastic anemia	02	1.2%	05	4.8%
Hypoplastic marrow	11	7%	11	10.6%
Multiple myeloma	02	1.2%	02	1.9%
Malaria	01	0.6%	-	-
Undiagnosed	15	9.6%	-	-
AML	-	-	01	0.9%
Metastasis	-	-	01	0.9
Total	156 cases	100%	103 cases	100%

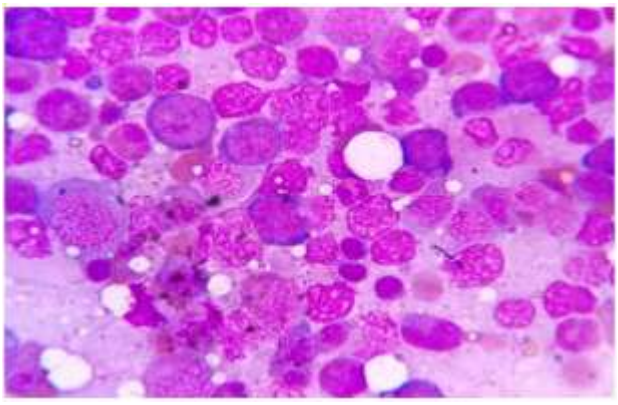

Figure 1. Megaloblastic anemia. Bone marrow aspiration showing erythroid hyperplasia with megaloblasts giemsa. 40x

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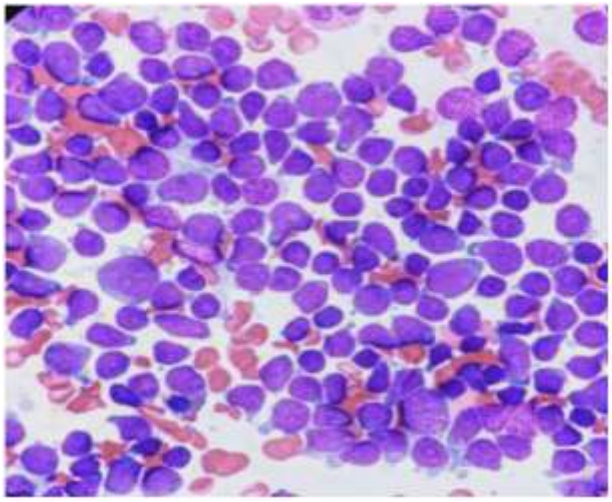

Figure 2: ALL. Bone marrow aspirate showing lymphoblasts, Giemsa 100x

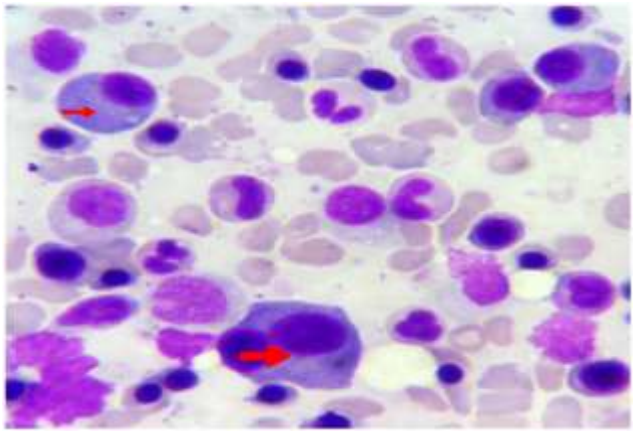

Figure 3: Multiple myeloma. Bone marrow aspirate showing increased number of plasma cells; thin arrow shows plasma cells, thick arrow shows binucleate forms geimsa 100x

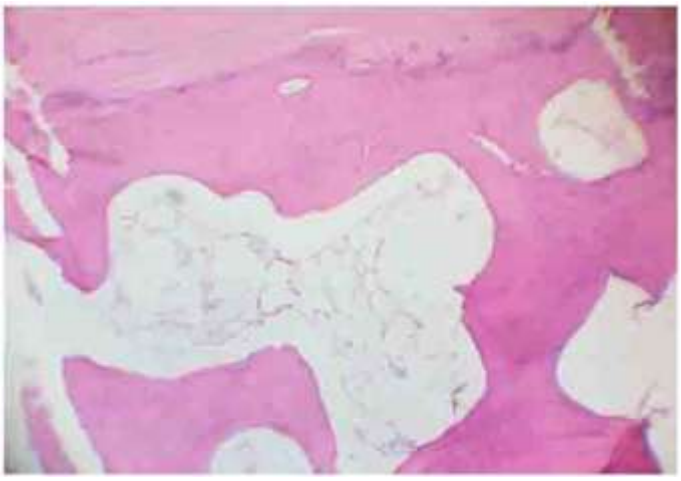

Figure 4: Aplastic anemia. Bone marrow biopsy showing hypocellularity H & E stain 40x

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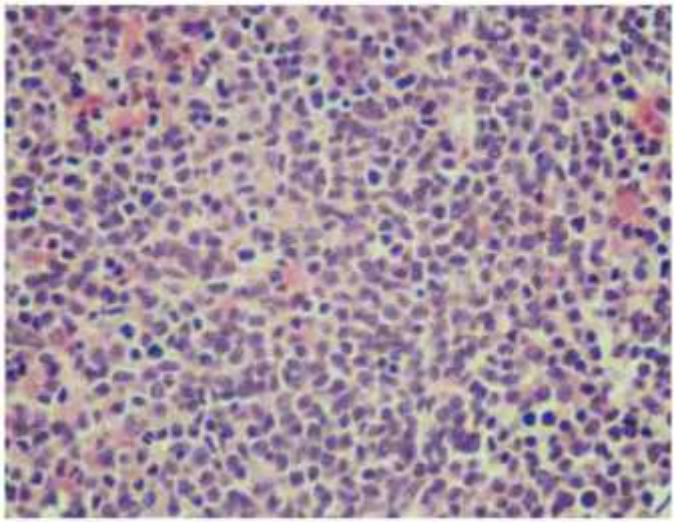

Figure 5: AML. Bone marrow biopsy showing blast cells H&E100X

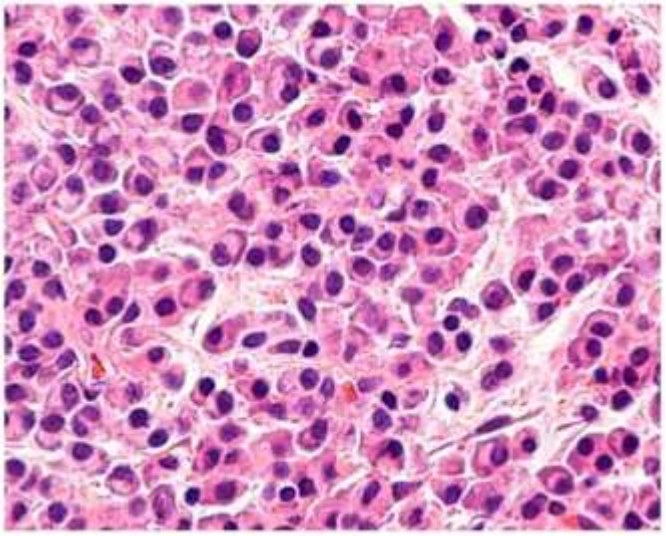

Figure 6: Multiple myeloma. Bone marrow biopsy showing mature plasma cells H&E 100X

Discussion

The mechanisms contributing to pancytopenia include, decrease in haematopoietic cell production, marrow replacement by abnormal cells, suppression of marrow growth and differentiation, ineffective hematopoiesis with cell death, defective cell formation, antibody mediated sequestration or destruction of cells in a hypertrophied and overactive reticuloendothelial system.[5]

Pancytopenia is a common hematological entity encountered in day –day clinical practice. pancytopenia has a wide array of underlying causes. Symptoms are attributable to anemia and thrombocytopenia. Leucopenia is an un common in initial presentation, but can become most serious threat to life during subsequent course of disorder. Pancytopenia should be suspected on clinical grounds when a patient presents with un-explained anemia, prolonged fever and tendency to bleed.

The age distribution of the patients in the present study ranged from 1- 80 years.

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There was a male preponderance and the male to female ratio was 1.16:1. Similar findings were noted by Tilak *et al.*,[8] (1.14:1), Khodke K *et al.*,[9] (1.3:1), Khunger J M *et al.*,[10] (1.2:1), Gayatri B. N *et al.*,[11] (1.2:1), Jha *et al.*,[12] (1.5:1) & Melina. D *et al.* [3] (1.8:1).

The most common presenting symptom in the present study was generalized weakness (52.5%) and fever (44.2%) followed by easy fatigability, gastro intestinal symptoms, breathlessness, cough, yellowish discoloration of sclera, bleeding, giddiness, headache. Pallor was seen in all cases (100%) followed by splenomegaly (32%) and hepatomegaly (18%)

Similar findings were noted in the studies by B.N Gayatri *et al.*,[11] generalized weakness (100%) followed by breathlessness and pallor clinically. Melina D *et al.*,[3] study also showed similar findings. The presenting symptoms are usually attributed to anemia or thrombocytopenia. Leucopenia is an un common cause of the initial presentation of the patient but can become the most serious threat to life during the course of the disorder.

In the present study, dimorphic anemia (41%) was the commonest cause of pancytopenia, followed by megaloblastic anemia (24.3%), aplastic anemia (3.2%), leukemias - AML (0.6%) and ALL(1.2%), multiple myeloma (2.5%),malaria(0.6%) and metastasis (0.6%). In the present study thirty nine cases of megaloblastic anemia were noted (24.3%)and it was the 2nd commonest cause of pancytopenia.

Megaloblastic anemia is common in India. This seems to reflect the higher prevalence of nutritional anemia in Indian subjects.[13,14] Though, bone marrow aspiration studies are un common in suspected cases of megaloblastic anemia, it is indicated only when hematological assays are not available and if patient requires urgent treatment.[13]

Khodke *et al.*,[9] observed hypoplastic anemia (29.5%) as the common causes of pancytopenia followed by megaloblastic anemia (22.3%). Osama *et al.*,[1] found megaloblastic anemia (39%) as the commonest cause of pancytopenia followed by hypersplenism (19%).

Peripheral smear study showed microcytic hypochromic anemia in 2 cases, and normocytic normochromic in 3 cases. Bone marrow was mostly hypocellular and the aspirate was composed of fat cells in all the patients. Bone marrow biopsy revealed bony trabeculae with 5-10% cellularity and predominant fat spaces were seen. Naeem Khan *et al.*,[15] found aplastic anemia (20%) as the commonest cause of pancytopenia followed by megaloblastic anemia (16.7%). Aplastic anemia is the commonest cause of pancytopenia reported in various studies throughout the world.[13]

Bone marrow examination showed blasts with increased N:C ratio, multi nuclearity and nuclear lobulation. Bone marrow aspiration in one case was hypercellular and lymphoid hyperplasia with immature cells were seen. Another case which was un satisfactory on aspiration was diagnosed on biopsy

In the study of Varma and Dash[16] Acute leukemia was the third common cause of pancytopenia which is similar to the study by Savage *et al.*,[17] & Kishore Khodke *et al.*,[9] found one case of acute myeloid leukemia with immature cells in the peripheral blood. In the study by Tilak Jain *et al.*,[8] one case of acute myeloid leukemia with anisocytosis, circulating erythroblasts and immature cells was reported.

A detailed work up of clinical, haematological and bone marrow study of patients with pancytopenia usually helps in identification of the underlying cause which are useful for planning further investigations and management. However, in view of a wide array of

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etiologies, pancytopenia continues to be a challenge for hematologists.

Conclusion

Pancytopenia is a common hematological entity encountered in day to day clinical practice. Dimorphic anemia was the commonest cause of pancytopenia in the present study followed by megaloblastic anemia. This seems to reflect higher prevalence of nutritional anaemia in the Indian subjects. Uncommon etiological factors like malaria was also identified in this study.

Conflict of Interest: None to declare
Source of funding: Nil

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