ISSN: 0975-3583,0976-2833 VOL13,ISSUE05,2022

TITLE PAGE
// ORIGINAL RESEARCH ARTICLE//

Title of article: "BONE MARROW EVALUATION IN PANCYTOPENIA"

Type of Article: Original Study

Author's Sequence and Affiliations:
Rani S. S. Sabitha[1], Vamshidhar I. S. [2]

1. **Dr.S.S.SABITHARANI**,MD.Pathology; Assistant Professor, Department of Pathology, Government Medical College, BhadradriKothagudem, Telangana-507101, India.
2. **Dr.I.S.VAMSHIDHAR*,**MD.Physiology,Assistant Professor,Department of Physiology, Government Medical college, Mahabubabad, Telangana-506101, India.

Corresponding author:Dr. I.S.VAMSHIDHAR,H.No: 30-2-701/A, West City,Road No:3,Kazipet, Madikonda-506142, Warangal Urban, Telangana, India. Mob. No: +919000685980**E-mail address:** vamshiscorpio2004@gmail.com

Source of Support: *Nil*
Conflict of Interest: *None*

Ethical Permission: *Obtained*

Acknowledgment: Authors thank the Dept of Pathology, Kakatiya Medical College and MGM Hospital, Warangal, Telangana State, India for their support in the conduction of this study.

ABSTRACT

Background: After the blood, the bone marrow is the biggest and most extensively distributed organ in the body, and it is the primary location for blood cell creation. Peripheral pancytopenia can be caused by a variety of illnesses that affect the bone marrow either directly or indirectly. As a result, a bone marrow examination is particularly useful in the diagnosis of pancytopenia. This study focuses on the etiologic of pancytopenia as well as bone marrow morphology in pancytopenia patients.

Methods: A total of n=70 samples with pancytopenia were identified and included in the study. A complete clinical examination including lab investigations was done. The Laboratory Investigations include a complete hemogram, serum B12, folate estimation, and other biochemical investigations wherever necessary. The peripheral smear was studied after staining with Leishman's stain. Special stains Periodic acid Schiff reagent stain, Myeloperoxidase, Sudan black and Perl's stains were used wherever indicated.

ISSN: 0975-3583,0976-2833 VOL13,ISSUE05,2022

Results: Predominant blood picture in our study was dimorphic anaemia followed by macrocytic anaemia. Macrocytic anaemia cases showed macro-ovalocytes and hypersegmented Neutrophils, nucleated RBC showing nuclear budding, irregular nuclei, and Howell-jolly bodies. Leucopenia and thrombocytopenia were seen in all the cases. The bone marrow cellularity was hypercellular in 59% of cases.

Conclusion: A comprehensive clinical and haematological study of patients with pancytopenia usually helps in arriving at the etiological diagnosis. Since megaloblastic anaemia and vitamin B12/folate deficiency are common in pancytopenia in the Indian population and this is easily reversible with appropriate treatment. Therefore, megaloblastic anaemia should be first ruled out in cases of pancytopenia in the Indian population.

Keywords: Bone Marrow Aspiration, Pancytopenia, Anaemia, Thrombocytopenia, Leucopenia

MAIN TEXT

Introduction

The bone marrow is the body's biggest and most extensively dispersed organ. It is the primary place for the production of blood cells. The average adult produces and exports around 2.5 billion red cells, 2.5 billion platelets and 1.0 billion granulocytes per kilogram of body weight daily. [1] Peripheral pancytopenia can be caused by a variety of illnesses that affect the bone marrow either directly or indirectly. [2] Pancytopenia is characterized as a decrease in all three blood components below the normal reference range. The presenting symptoms are frequently due to anaemia or thrombocytopenia. Leucopenia is a common complication of the disorder's progression. Pancytopenia manifests in a variety of haematological and non-hematopoietic disorders. Reduced hematopoietic cell production, marrow replacement by abnormal cells, suppression of marrow growth and differentiation, ineffective haematopoiesis with cell death, defective cell formation, antibody-mediated sequestration or destruction of cells in a hypertrophied and overactive reticuloendothelial system are all mechanisms that contribute to pancytopenia. [3] Pancytopenia is a significant haematological condition that is identified by bone marrow aspiration and biopsy. A bone marrow examination is particularly useful in determining pancytopenia. [4]The occurrence of pancytopenia diseases may vary according to a variety of reasons including regional dispersion and genetic abnormalities. [5-7] There are a few similar studies in the literature.Although it is a common clinical pattern with a broad differential diagnosis, it receives minimal attention in major textbooks of internal medicine and haematology.Because the underlying pathophysiology of pancytopenia impacts patient therapy and prognosis, there is a clear need for research into pancytopenia.[8]

Material and methods

This cross-sectional study was conducted in the Department of pathology, Kakatiya Medical College and MGM Hospital, Warangal, Telangana. Institutional Ethical committee permission was obtained for the study. Written consent was obtained from all the participants (or parents/guardians in case of minors) of the study.

Inclusion Criteria

1. Patients showing peripheral blood picture of pancytopenia

ISSN: 0975-3583,0976-2833 VOL13,ISSUE05,2022

2. Males and Females
3. All age groups
4. Patients who have given written consent for bone marrow aspiration and/ or biopsy

Exclusion criteria

1. Patients with haemorrhagic disorders.
2. Patients who did not give consent for the procedure
3. Pancytopenia patients with known etiological cause

Based on the inclusion criteria and exclusion criteria during the study period, a total of n=70 samples with pancytopenia were identified and included in the study. The detailed demographic profile of the cases including family history and relevant clinicalhistory was obtained in a pre-tested and pre-structured questionnaire. A complete clinical examination including lab investigations was done. The Laboratory Investigations include a complete hemogram, serumB12, folate estimation, and other biochemical investigationswherever necessary. The peripheral smear was studied after staining with Leishman's stain. Special stainsPeriodic acid Schiff reagent stain, Myeloperoxidase, Sudan black and Perl's' stains were used wherever indicated.

Bone Marrow Aspiration: AJamshidi needle was used to aspirate material from the Posterior iliac crest. Following the administration of a test dosage, local anaesthetic infiltration was performed under sterile conditions. The needle and stylet were positioned and the cap was closed. The periosteum and cortex were penetrated with a drilling action after the skin and subcutaneous tissue was pierced. After inserting the stylet into the marrow cavity, 0.2-0.3 ml of marrow fluid was aspirated with a sterile disposable 10 ml syringe. The aspirate was spread over a series of slides. The needle was removed and a tincture benzoin seal was placed. The slides were dyed using Leishman's stain. In case of failure, bone marrow aspirations were done at different sites.

*Bone marrow biopsy:*When necessary, biopsies and aspirations were performed in the same session. After aspiration, the stylet was removed from the needle and the cap was closed. It was now pushed deeper into the cavity by rotating motions of around 0.5-1 cm. The marrow core sample is captured within the needle throughout this process. The needle was then extracted by spinning in the opposite direction. A wire probe was placed into the needle hub of a sterile gauge. The material was fixed overnight in 10% formalin and decalcified for 72 hours in 6% EDTA. The material was then treated similarly to a histopathology sample and H&E sections were examined. When necessary, special stains such as PAS and reticulin were used.

Results

Our study included n=70 cases of pancytopenia with most of the patients being females n=38(54.28%) and 32(45.71%) cases were males. The age range in our study was 5- 80 yrs. Most of the patients were in the second decade of life, accounting for 22.85% of cases followed by 31 – 40 years with 21.43% of cases. The youngest case was a female of age 6 years and the oldest was a male of age 69 years. The mean age of the cases in the study was 28.56 ± 8.5 years. The detailed distribution of cases age-wise is given in table 1.

ISSN: 0975-3583,0976-2833 VOL13,ISSUE05,2022

Table 1: Age-wise prevalencein our study

Age in years	*Frequency*	*Percentage*
0-10	05	07.14
11-20	16	22.85
21-30	09	12.86
31-40	15	21.43
41-50	13	18.57
51-60	10	14.28
>60	02	02.86
Total	70	100.0

Generalized weakness and pallor were the commonest presentations in 68% of the cases. The second common finding reported in the study was fever in 48% of cases followed by Dyspnoeafound in 34% of cases and icterus and organomegaly in 15% of cases each, details depicted in table 3.

Table 2: Clinical manifestation of the cases with pancytopenia

Clinical manifestation	*Frequency*	*Percentage*
Pallor	47	68
Icterus	11	15
Organomegaly	11	15
Dyspnoea	24	34
Fever	33	48
Generalized weakness	47	68

Generalized weakness and pallor were the commonest presentations in 68% of the cases. The second common finding reported in the study was fever in 48% of cases followed by Dyspnoea found in 34% of cases and icterus and organomegaly in 15% of cases each, details depicted in table 3. Most patients in our study showed haemoglobin levels of 6-7.9 gm/dl. accounting for n=26 cases and n=2 cases manifested severe anaemia with Hb below 2gm/dl.

Table 3: Haemoglobin levels

Hb values (gm/dl)	*Frequency*	*Percentage*
8-9.9	5	07.14
6-7.9	26	37.14
4-5.9	17	24.28
2-3.9	20	28.57
1-1.9	2	02.85
total	70	100.0

Total Leucocyte Count: the total leucocyte count in the stud was between 3000 – 3999 cells/mm^3 in n=21(30.0%) of cases followed by a count between 2000 – 2999 cells/mm^3in n=26(37%) of cases. The counts between 1000 – 1999 cells/mm^3 was found in n=18(25.7%) cases and between 300 = 999 cells/mm^3 was in n=5(7.14%) cases respectively.*The mean plateletcounts:* The mean platelet counts were between 5000 – 25000 in n=6(8.57%) cases,

ISSN: 0975-3583,0976-2833 VOL13,ISSUE05,2022

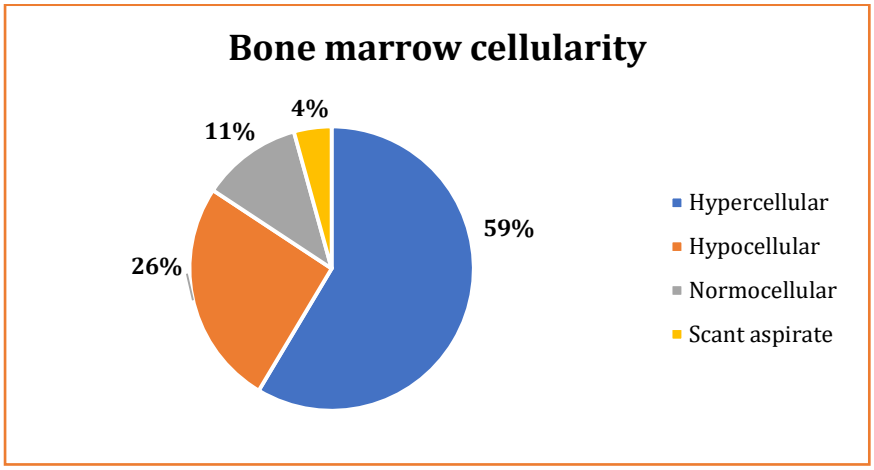
25001 – 50000 in n=12 (17.14%) cases 50001 – 75000 in n=25 (70.0%) cases and between 75001 – 100000 in n=20 (28.57%) cases. *Peripheral smear:*Predominant blood picture in our study was dimorphic anaemia followed by macrocytic anaemia. Macrocytic anaemia cases showed macro-ovalocytes andhyper segmented Neutrophils,nucleated RBC showing nuclear budding, irregular nuclei, and Howell-jolly bodies. Leucopenia and thrombocytopenia were seen in all the cases. The bone marrow cellularity was hypercellular in 59% of cases and the distribution of BM cellularity is depicted in figure 1.

Table 4: Predominantperipheral blood picture in the cases

RBC picture	*Frequency*	*Percentage*
Dimorphic anaemia	28	40.0
Macrocytic anaemia	26	37.14
Normocytic normochromic anaemia	07	10.0
Normocytic hypochromic anaemia	09	12.85
Total cases	70	100.0

All the cases showing hypocellular marrow were diagnosed as aplasticanaemia as there was no evidence of fibrosis in the marrow. Out of n=52 cases showing normal and hyper-cellular marrow n=27 cases were showing megaloblastic erythroid hyperplasia, n=5 cases were showingmixed nutritional deficiency, n=17 cases showed normoblastic erythroidhyperplasia, n=3 cases showed scant aspirate details depicted in figure 1.

Figure 1: Showing the bone marrow cellularity obtained in the cases



Out of n=70 cases of pancytopenia, n=27(38.57%) cases were megaloblastic anaemia which included n=18(25.71%) cases were females and n=9(12.85%) cases were males.Based on the diet out of the total of n=27 cases n=23(85.18%) were vegetarians and n=2(7.4%) cases were chronic alcoholics. The mean Hb levels in the cases of megaloblastic anaemia was 5.76 ± 2.29 gm/dl. The mean Total leucocyte count in megaloblastic anaemia cases was 2897.47 ± 503.6cells/mm^3. The mean platelet counts in these cases were 65698.25 ± 3501.36 cells / mm^3.

Our study found n=18(25.71%) cases of aplastic anaemia and n=5(7.14%) cases of mixed nutritional anaemia with pancytopenia. N=3(4.28%) cases showed hypercellular marrow with reversal of M: E ratio and n=2(2.86%) cases showed normocellular marrow. There were n=17 cases of normoblastic and micro-normoblastic erythroid hyperplasia. One case out of them

ISSN: 0975-3583,0976-2833 VOL13,ISSUE05,2022

showed dyserythropoietic features. Normoblastic erythroid hyperplasia by itself is not a cause of pancytopenia. Proper clinical history and laboratory investigations should be done and hypersplenism and haemolyticanaemia's should be ruled out in cases of erythroid hyperplasia. Splenomegaly was found in a 22-year female patient but no evidence of hypersplenism was seen.

Discussion

Although pancytopenia is not a disease in and of itself, however, may be a prominent symptom of many serious and fatal illnesses and is brought on by a variety of different ailments. [9-11] Various etiological causes for pancytopenia, such as megaloblastic anaemia, aplastic anaemia, leukaemia, myelodysplastic syndrome, etc., have been the subject of much research. Since there is a paucity of data on this subject in our area, we conducted this study. Out of n=70 cases of pancytopenia, n=27(38.57%) cases were megaloblastic anaemia. Our study found n=18(25.71%) cases of aplastic anaemia and n=5(7.14%) cases of mixed nutritional anaemia with pancytopenia. N=3(4.28%) cases showed hypercellular marrow with reversal of M: E ratio and n=2(2.86%) cases showed normocellular marrow.Tilak et al.,[12] in their study found the most common cause of pancytopenia was megaloblastic anaemia (68%) followed by aplastic anaemia (7.70%) cases agreeing with the observations of the current study. According to Savage et al.,[13] and Khunger et al.,[14] megaloblastic anaemia, hypoplastic anaemia and acute leukaemia were the most frequent causes of pancytopenia, with megaloblastic anaemia coming in second.N=5(7.14%) cases of mixed nutritional anaemia with pancytopenia were found in this study. This seems to reflect the higher prevalence of nutritional anaemia in Indian subjects as well as in developing countries. However, similar results have been reported in studies from other Indian studies. [12, 14, 15]In the current study we found a slightly higher female prevalence of 54.28% as compared to males 45.71%, and the highest prevalence in ages 1-30 years. However, several studies of pancytopenia across India have found male predominance although the differences have been only marginal. Tilak et al., [12]found the maximum number of cases under the age of 20 years (32.47%) with male to female ratio being 1.13:1. Niazi et al.,[16] in a similar study have also reported the commonest age group between 21 and 30 years of age with male to female ratio being 1.7:1 in their study of n=89 cases of pancytopenia. In the present study based on the clinical presentation, generalized weakness and pallor were the commonest presentations in 68% of the cases. The second common finding reported in the study was fever in 48% of cases followed by Dyspnoea found in 34% of cases and icterus and organomegaly in 15% of cases. A similar frequency of reported clinical symptoms has been reported by Tilak et al., [12]Khungar et al.,[14] and Santra et al., [13]Most of the presenting symptoms are attributed to anaemia and thrombocytopenia. Leucopenia may be uncommon in the initial presentation of the patients however, it can become more serious during the course of the disease [2, 14] organomegaly was found in 15% of the cases in this study. Santra et al., [17] found hepatomegaly in 24.32% of cases, splenomegaly was found in 44.14% and lymphadenopathy was found in 6.31% of the cases of their study. Erythroid hyperplasia was frequently encountered in the cases of the study however, its exact relationship to pancytopenia is unclear. Marrow is usually hypercellular with predominantly megaloblastic erythropoiesis. Giant band forms, metamyelocytes and giant megakaryocytes were also seen. Some of these

ISSN: 0975-3583,0976-2833 VOL13,ISSUE05,2022

cases might be a stage in the development of hypoplasia, while others might be refractory anaemia cases. These patients should be monitored because the criteria for differentiating these groups are still poor. [2]Pancytopenia patients with inadequate haematopoiesis and marrow cell loss can also present with hypercellular or normocellular marrow.[18]Similar to hypercellular marrow, the paraneoplastic syndrome can show as peripheral pancytopenia and paraneoplastic syndrome.[19]The peripheral picture of the majority of cases showed varying degrees of anaemia, Leucopenia, thrombocytopenia, anisopoikilocytosis, macroovalocytosis and hyper segmented neutrophils. K Khodke et al., [2]showed 90.9% of cases with anisocytosis, 45.54% cases of the dimorphic picture and 90.9% with hyper segmented neutrophils. In our study, we found macro-ovalocytes with anisopoikilocytosis were seenin most of the cases and hypersegmented neutrophils were seen inalmost all the cases.

Conclusion

Pancytopenia is a common haematological disorder however; it has not received adequate attention. Therefore, studies on pancytopenia using readilyavailable diagnostic equipment techniques are necessary. A comprehensive clinical and haematological survey of patients withpancytopenia usually helps in arriving at the etiological diagnosis. However,given a wide range of clinical conditions leading to pancytopenia, itremains a challenge to haematologist's to determine the etiological causes in some cases. Since megaloblastic anaemia and vitamin B12/folate deficiency are common in pancytopenia of the Indian population and this is easily reversible with appropriate treatment. Therefore, megaloblastic anaemia should be first ruled out in cases of pancytopenia in the Indian population.

REFERENCES

1. Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, Siberstein LE.Haematology. Basic Principles and practice. 3rd ed. USA: Churchill Livingstone; 2005.
2. Khodke K, Marwah S, Buxi G, Yadav RB, Chaturvedi NK. Bone MarrowExamination in Cases of Pancytopenia. JIACM 2001; 2:55-59.
3. Jha A, Sayami G, Adhikari RC, Panta AD, Jha R. Bone marrow examination in cases of pancytopenia. J Nepal Med Assoc 2008; 47(169):12 – 17.
4. Dodhy MA, Bokhari N, Hayat A. Aetiology of Pancytopenia, A five-yearexperience. Ann Pak Inst Med Sci 2005;1 (2):92-95.
5. International agranulocytosis and aplastic anaemia study. Incidence of aplastic anaemia, the relevance of diagnostic criteria. Blood 1987; 70:1718-21.
6. Wintrobe MM Clinical Haematology. 8th ed. Philadelphia: Lea and Febiger 1981; p 699-15.
7. Keisu M, Ost A. Diagnosis in patients with severe pancytopenia suspected of having aplastic anaemia. Eur J Haematol 1990; 45:11-14.
8. Verma N, Dash. Reappraisal of underlying pathology in adult patients presentingwith pancytopenia. Trop Geog Med 1992;44:322-17.
9. Firkin F, Chesterman C, Penington D, Rush B, editors. De Gruchy's Clinical Haematology in Medical Practice. 5th ed. United States: Blackwell Science; 1989.
10. Kumar R, Kalra SP, Kumar H. Anand AC, Madan H. Pancytopenia - a six-year study. J Assoc Physicians India 2001; 49:1078-81.

ISSN: 0975-3583,0976-2833 VOL13,ISSUE05,2022

11. Manzoor F, Karandikar MN, Nimbargi RC. Pancytopenia: A clinico-hematological study. Med J DY Patil Univ 2014; 7:25-28.
12. Tilak V, Jain R. Pancytopenia - a clinicohematologic analysis of 77 cases. Indian J PatholMicrobiol 1999; 42:399-04.
13. Savage DG, Allen RH, Gangaidzo IT, et al. Pancytopenia in Zimbabwe. Am J Med Sci 1999; 317:22-32.
14. Khunger JM, Arulselvi S, Sharma U, Ranga S, Talib VH. Pancytopenia - a clinico-hematological study of 200 cases. Indian J PatholMicrobiol 2002; 45:375-79.
15. Bhatnagar SK, Chandra J, Narayan S, Sharma S, Singh V, Dutta AK. Pancytopenia in children: Etiological profile. J Trop Pediatr 2005; 51:236-39
16. Niazi M, Fazl-i-Razia. The incidence of underlying pathology in pancytopenia - an experience of 89 cases. J Postgrad Med Inst 2004; 18:76-79.
17. Nanda A, Basu S, Marwaha N. Bone marrow trephine biopsy as an adjunct to bone marrow aspiration. J Assoc Physicians India 2002; 50:893-95.
18. Williams DM. Pancytopenia, Aplastic anaemia, and Pure red cell aplasia. In: Wintrobe's Clinical Haematology, 10th ed. Baltimore: William and Wilkins. 1993; p.1449-84.
19. Raz I, Shinar E, Polliack A. Pancytopenia with Hypercellular bone marrow- a possible paraneoplastic syndrome in carcinoma of the lung: a report of three cases. Am J Hematol 1984; 16:403-08.