Original research article

A Clinico-hematological Study of Pancytopenia In all Age groups.

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Abstract

Background: Pancytopenia is a relatively common hematological entity characterized by the reduction of three formed elements of blood. It may be a manifestation of a wide variety of disorders, which primarily or secondarily affect the bone marrow. Hematological investigation plays an important part in the management of patients with pancytopenia and therefore needs a detailed study of bone marrow. We in the current study tried to evaluate the clinical presentations and hematological profile of cases with pancytopenia due to various causes.

Methods: A complete hematological workup was done which included hemoglobin estimation, RBC count, RBC indices (MCH, MCV, MCHC), WBC count, differential count, reticulocyte count, ESR, PCV, Platelet count, Bleeding time, clotting time and clot retraction test. Peripheral smear was stained by Leishman stain for all the cases. To determine the cause, this was done for every patient. The material was aspirated from the tibial tuberosity in infants under the age of two or the posterior iliac crest in adults using a Jemshidi needle.

Results: A total of n=40 cases were studied in this study. Megaloblastic anemia was commonest cause constituting 70.0% followed by aplastic anemia 17.5%, Malaria 5.0% subleukemic leukemia 2.5%, multiple myeloma 2.5%, and storage disorder 2.5%. In the study majority of megaloblastic erythropoiesis occurs in the hypercellular bone marrow. Gigantic megakaryocytes and giant band metamyelocytes were also seen. The majority of patients had reduced fat cells and hypercellular bone marrow 62.5%. 10% of cases had normocellular bone marrow. In all the cases there was erythroid hyperplasia with megaloblastic maturation.

Conclusion: when a patient presents with unexplained anemia, prolonged fever, and a tendency to bleed. The physical findings and peripheral blood picture provide valuable information in the work of cytopenic patients. The typical presentation in the peripheral blood picture will be anemia, presence of nucleated RBCs, and immature myeloid cells which may be suggestive of marrow infiltration of primary hematologic disorder.

Keywords: Pancytopenia, megaloblastic anemia, aplastic anemia, myeloid erythroid ratio

Introduction

Cytopenia is a condition when the production of one or more blood cell types stops or is significantly diminished. [1] All three of the primary blood components red blood cells, white blood cells, and platelets are lowered more than usual when someone has pancytopenia. [2] It is not a distinct illness but rather a trio of results that may be caused by several disease processes, some of which may directly or indirectly affect the bone marrow. The symptoms that are now present are typically caused by leucopenia, thrombocytopenia, or anemia. [3] From modest drug-induced bone marrow hypoplasia and megaloblastic marrow to lethal bone marrow aplasias and leukemias, pancytopenia is a distinguishing hallmark of many serious and life-threatening disorders. [4] Because various demographic groups have varied age distributions, dietary conditions, and rates of infectious illness, it is predicted that the pattern of diseases causing pancytopenia will vary among them. [4] Reduced production of hematopoietic cells, marrow replacement by abnormal cells, inhibition 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 are some of the underlying mechanisms of pancytopenia. [5] A bone marrow aspiration and biopsy are used to identify the underlying cause of pancytopenia, a dangerous hematological condition. Pancytopenia can be evaluated greatly using a bone marrow test. [6] There is very little mention of this aberration in major textbooks of internal medicine and hematology. [7, 8] Despite the fact that it is a frequent clinical pattern with a wide differential diagnosis. The care and prognosis of patients are based on the underlying pathology. The treatment plan for these individuals and their prognosis depends on the degree of pancytopenia and the underlying condition. [9] Pancytopenia's causes are not clearly understood in India. Megaloblastic anemia is a key contributor to pancytopenia, according to earlier Indian investigations. [10] Therefore, the current investigation was conducted to assess the possible causes of pancytopenia and to compare the results of a bone marrow aspiration with peripheral blood.

Material and methods

This cross-sectional study was conducted in the Department of Pathology (hematology unit) of RVM Medical Institute of Medical Sciences and Research Center, Laxmakkapally Village, Mulugu, Siddipet, Telangana State. Institutional Ethical Committee approval was obtained for the study. Written consent was obtained from all the patients or parents/guardians of the cases in the study.

Inclusion criteria

1. Presence of three of the following
2. Haemoglobin < 9 g/dl
3. TLC <4000/cumm and
4. Platelet count <1,00,000 / cumm
5. All age groups
6. Males and Females

Exclusion criteria

1. Patients on myelotoxic chemotherapy.
2. Those on Radiation Therapy
3. Those on drugs known to cause cytotoxicity

Three ml of blood was collected by venipuncture under aseptic precaution in a vacutainer. A complete hematological workup was done which included hemoglobin estimation, RBC count,

RBC indices (MCH, MCV, MCHC), WBC count, differential count, reticulocyte count, ESR, PCV, Platelet count, Bleeding time, clotting time and clot retraction test. Peripheral smear was stained by Leishman stain for all the cases and examined in detail.

Bone marrow aspiration: To determine the cause, this was done for every patient. The material was aspirated from the tibial tuberosity in infants under the age of two or the posterior iliac crest in adults using a Jemshidi needle. After giving a test dosage, local infiltration anesthesia was employed. There were sterile precautions taken. After positioning the stylet and needle, the cap was fastened. The periosteum and cortex were punctured with a drilling action after the skin and subcutaneous tissue was punctured. Once within the marrow cavity, the stylet was taken out and a sterile, single-use 10 ml syringe was used to aspirate 0.2–0.3 ml of marrow fluid. A series of slides were used to transfer and smear the aspirate. The needle was withdrawn and a tincture benzoin seal was applied. Slides were stained with Leishman's stain. In case of failure, bone marrow aspirations were done at different sites.

Bone marrow biopsy: Aspiration and biopsies were carried out in the same location as needed. The stylet was removed from the needle after aspiration, and the cap was then fastened. It was now rotated for a further 0.5–1 cm, pushing it deeper into the cavity. The marrow core sample was obtained within the needle by this method. The needle was then removed while rotating in the other direction. On a sterile gauge, a wire probe was placed at the needle's hub. The material was decalcified with 6% EDTA for 72 hours after being fixed in 10% formalin overnight. It was then treated in a manner similar to that of a histopathological sample, and H&E sections were examined. When necessary, special stains like PAS and reticulin were performed.

Results

Based on the inclusion and exclusion criteria n=40 patients who presented with pancytopenia were studied. Primary hematological procedures were carried out in all n=40 patients, which included ten hematological parameters. Peripheral smears were examined in detail. Bone marrow aspiration yielded adequate material in all cases. Bone marrow aspirations were also obtained from n=40 cases. The highest incidence of pancytopenia was found in the age group 21 – 30 years with 22.5% of all the cases followed by 0 – 10 years with 17.5% of cases (table 1). The mean age of the group was 26.5 ± 10.5 years. Out of the n=40 cases, n=24(60%) were males and n=16(40%) were females. The male to female ratio was 3:2.

Table 1: Incidence of pancytopenia in different age groups of the cases

Age group (years)	*Frequency*	*Percentage*
0 – 10	7	17.5
11-20	6	15.0
21-30	9	22.5
31-40	5	12.5
41-50	4	10.0
51-60	6	15.0
61-70	2	05.0
71-80	1	02.5
Total	40	100.00

The commonest presentation was generalized weakness, Fever, and pallor which was present in all patients constituting 100%. Dyspnoea was found in 55.0% of patients. Splenomegaly was noted in 35.0% cases, Hepatomegaly was noted in 30.0% of cases of megaloblastic anemia and

malaria. Bony tenderness was noted in one case of multiple myeloma the other details have been depicted in table 2.

Table 2: Presenting Complaints and Physical Findings in the cases of Pancytopenia

Physical Findings	*Frequency*	*Percentage*
Generalized weakness	40	100.0
Dyspnoea	22	55.0
Fever	40	100.0
Bleeding manifestation	1	2.5
Weight loss	3	7.5
Chills and rigor	2	5.0
Pallor	40	100.0
Splenomegaly	14	35.0
Hepatomegaly	12	30.0
Jaundice	2	5.0
Bony tenderness	1	2.5
Lymphadenopathy	0	0.0

Hemoglobin percentage varied from 1.8 – 9.2 gm%. Most patients had hemoglobin percentages between 5.1- 8 gm%. The lowest count of 1.8 gm% was seen in a case of megaloblastic anemia. Total leucocyte count ranged from 500 – 3,900 cells/mm^3. Most patients had white cell count in the range of 2501 – 3,900 cells/mm^3. The lowest count of 500 cells/mm3 was seen in a case of megaloblastic anemia. Reticulocyte count ranged from 0.5 – 2%. Most patients had reticulocyte counts between 0.6 – 1%. Platelet count ranged from 10,000 – 95,000 cells/mm^3. Most of patients had platelet count between 51,000 – 80,000 cells/mm^3. The lowest platelet counts of 10,000 cells/mm^3 were seen in a case of aplastic anemia. All the details have been depicted in table 3.

Table 3: Vital Haematological Parameters in Cases of Pancytopenia

Parameter	*Range*	*Frequency*	*Percentage*
Hemoglobin (gm%)	< 5.0	14	35.0
	5.1 – 8.0	16	40.0
	8.1 – 9.2	10	25.0
Total leucocyte count (cells/mm^3)	500 – 1000	1	2.5
	1001 – 2500	3	7.5
	2501 – 3900	36	90.0
Reticulocyte count (%)	< 0.5	12	30.0
	0.6 – 1.0	19	47.5
	1.1 – 2.0	09	22.5
Platelet counts (cells/mm^3)	10000 – 50000	16	40.0
	51000 – 80000	13	32.5
	81000 - 95000	11	27.5

In present study megaloblastic anaemia was commonest cause constituting 70.0% followed by aplastic anaemia 17.5%, Malaria 5.0% subleukemic leukemia 2.5%, multiple myeloma 2.5% and storage disorder 2.5% (table 4).

Table 4: Distribution of various causes of pancytopenia

Causes	*Frequency*	*Percentage*
Megaloblastic anemia	28	70.0
Aplastic anemia	7	17.5
Subleukemic leukemia	1	2.5
Malaria	2	5.0
Multiple myeloma	1	2.5
Storage disorder	1	2.5
Total	40	100.0

In the study majority of megaloblastic erythropoiesis occurs in the hypercellular bone marrow. Gigantic megakaryocytes and giant band metamyelocytes were also seen. The majority of patients had reduced fat cells and hypercellular bone marrow 62.5%. 10% of cases had normocellular bone marrow. In all the cases there was erythroid hyperplasia with megaloblastic maturation. The predominant peripheral blood picture was Dimorphic anemia constituting 37.5% followed by macrocytic anemia 30.0% Neutropenia and thrombocytopenia were seen in all cases details have been depicted in figure 1.

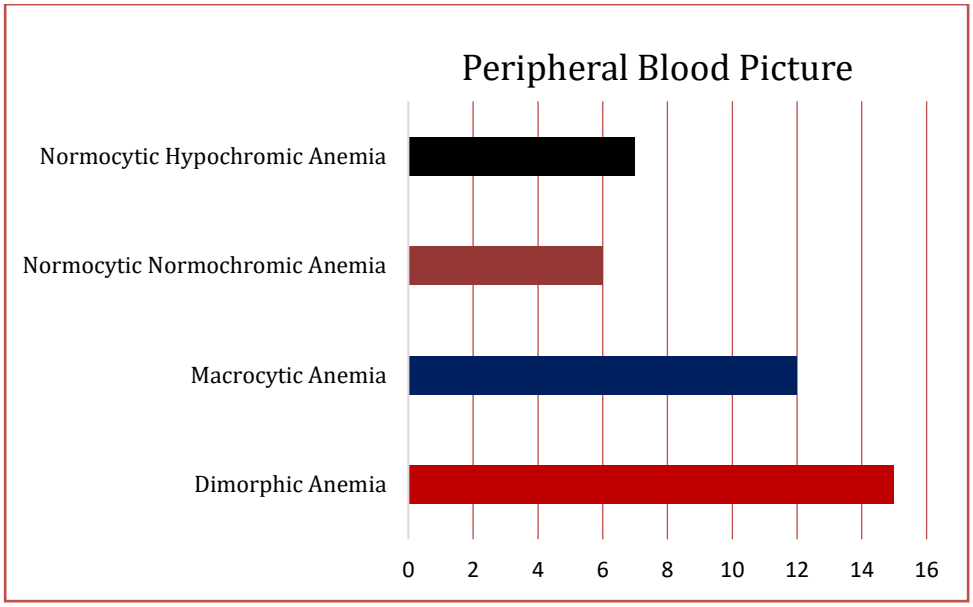

Figure 1: Peripheral Blood Picture in pancytopenia patients

In the present study subleukemic leukemia of myeloblastic type was seen in an n=1 case in an adult male. The common presentation was fever and generalized weakness and abdominal discomfort. Clinical examination revealed pallor with hepatosplenomegaly. There were bleeding tendencies with bleeding time prolonged in the case. Peripheral blood picture showed myeloblasts with Auer rods up to 20% of bone marrow was hypercellular. E/M ratio was reduced majority of cells were myeloblasts up to 40% diagnosis of AML-M2 was made. The malarial infestation was seen in n=2(5.0%) cases in the present study that presented pancytopenia. They presented with fever, chills, rigor, vomiting, and headache. Clinical examination revealed pallor and hepatosplenomegaly. Peripheral smear showed normocytic hypochromic anemia with marked anisopoikilocytosis, neutropenia, thrombocytopenia, and gametocytes of Plasmodium falciparum seen in both cases. BM was hypercellular with megaloblastic change. No malarial parasites were seen on bone marrow smears. In the current investigation, a 14-year-old boy had hepatosplenomegaly, pallor, and fever. PS revealed

neutropenia, thrombocytopenia, and normocytic hypochromic anemia. The BM was acceptable and normocellular. Megakaryopoiesis, leucopoiesis, and erythropoiesis all occurred normally. Numerous big cells with nuclei positioned peripherally and multivacuolated foamy cytoplasm was seen. Niemann-Pick disease diagnosis was made.

Discussion

Out of the n=40 cases in the study, the age range was from 3 – 75 years the mean age was 26.5 ± 10.5 years the male to female ratio was 3:2. In similar studies on pancytopenia Khuger JM et al., [10] found the age range from 2 – 70 years and the male to female ratio of 1.2:1. Kumar R et al., [4] found the age of cases ranged from 12 – 73 years and the male to female ratio was 2.1:1. Khodke K et al., [5] found the range of age from 3 – 69 years and the male to female ratio of 1.14:1. In our investigation, generalized weakness, fever, and Pallor in 100% of cases and dyspnea in 55% of cases were the most often reported presenting complaints. Splenomegaly (35.0%) and hepatomegaly (30.0%) were the most frequent physical findings. Thrombocytopenia or anemia was frequently blamed for the symptoms. Although leucopenia was a rare cause of the patient's initial presentation, it can become the most significant threat to life as the condition progresses. In the current study, we found megaloblastic anemia in 70% of cases, Aplastic anemia in 17.5% of cases, malaria in 5% of cases, and others as depicted in table 4. Tilak V et al., [9] The common cause of pancytopenia was megaloblastic anemia 68.0% followed by aplastic anemia 7.70%. Their study also revealed a few uncommon and rare, but interesting causes of pancytopenia like drug-induced agranulocytosis, hemophagocytic syndrome, and Waldenstroms macroglobulinemia. In the current study, the majority of megaloblastic erythropoiesis occurs in the hypercellular bone marrow. Gigantic megakaryocytes and giant band metamyelocytes were also seen. The majority of patients had reduced fat cells and hypercellular bone marrow 62.5% and 10% of cases had normocellular bone marrow. This study found the peripheral blood picture was Dimorphic anemia constituting 37.5% followed by macrocytic anemia 30.0% Neutropenia and thrombocytopenia were seen in all cases. K Khodke et al., [5] 90.9% cases showed anisocytosis, 45.45% cases showed dimorphic blood picture, and 90.9% cases showed hypersegmented neutrophils. In this study, we did not find any case of hypersegmented neutrophils in megaloblastic anemia. Khunger JM et al., [10] also did not report the presence of hypersegmented neutrophils in megaloblastic anemia. Relative anemia and lymphocytosis were noted in 57.14% of cases of aplastic anemia. Tilak V et al., [9] and Khunger JM et al., [10] reported similar findings in 50% cases and 85.71% cases respectively. Most of the cases of aplastic anemia were idiopathic. History of drug intake was seen in n=2(5%) cases. Hepatitis was noted in one case. Marrow aspirates in all were hypocellular with fragments composed largely of fat. Normoblastic erythropoiesis was seen with a normal M: E ratio and there was a mild increase in lymphocytes and plasma cells. dyserythropoiesis was the feature in a few cases. Also, relative lymphocytosis in aplastic anemia was noted in 52.63% in our study compared to 50% in Tilak V et al., [9] study and 85.71% in Khunger JM et al., [10] study. Aplastic anemia and bone marrow failure may be inherited or acquired and can involve just one or all three cell lines. Hypoplastic myelodysplastic syndrome is one of the differential diagnoses of aplastic anemia. [11] But the presence of blasts is required for the diagnosis of hypoplastic MDS. [12, 13] The incidence of aplastic anemia is higher in western literature as compared to this study probably due to higher exposure to toxic chemicals in the western population. [5] We encountered n=1(2.5%) of subleukemic leukemia which was diagnosed as AML-M2 based on the bone marrow aspiration study. Khodke K et al reported a single case of AML-M2 of 50 cases of pancytopenia. Khunger JM et al., [10] who has reported 5% of subleukemic leukemia. Kumar R et al., [4] reported 12% of aleukemic leukemia. Pancytopenia was the common feature in these cases which can be

correlated with Kumar R et al and Khunger JM et al., [10] studies.4, 7 We encountered n=1(2.5%) case of multiple myeloma compared to Khodke K et al reported an incidence of 4%, Tilak V et al., [9] reported 1.3% and Khunger JM et al., [10] reported 1% in his study.

Conclusion

Pancytopenia is a common hematological issue encountered in clinical practice and should be suspected on clinical grounds when a patient presents with unexplained anemia, prolonged fever, and a tendency to bleed. The physical findings and peripheral blood picture provide valuable information in the work of cytopenic patients. The typical presentation in the peripheral blood picture will be anemia, presence of nucleated RBCs, and immature myeloid cells which may be suggestive of marrow infiltration of primary hematologic disorder. Bone marrow aspiration is an important diagnostic tool in hematology that helps to evaluate various causes of cytopenia.

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