Original Article

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# Diagnostic Approach of New-Onset Pancytopenia: Study from A Tertiary Care Center

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## ABSTRACT

**Background:** Pancytopenia is a disorder associated with decrease in number of all three major formed elements of blood (red blood cells, white blood cells and platelets). It is a triad of findings resulting from many disease processes. This may be associated with various hematopoietic and non-hematopoietic condition involving the bone marrow. The management and prognosis of patients with pancytopenia is based on the severity of pancytopenia and the underlying disease process. The present study helps in highlighting the role of various laboratory parameters in conjunction with clinical findings in evaluation of these patients.

**Methods:** The present retrospective study was done for 2 years from April 2015 to April 2017 in Department of Pathology. A total of 210 patients with pancytopenia were included in the study. A detailed history and hematological findings including the bone marrow findings and flow cytometric results were noted in these patients, wherever available.

**Result:** Out of 210 patients, 136 were adults and 74 were children. The mean age of the patients was 42 years. The male to female ratio was 2.7:1. The most common clinical feature in our study was pallor (in all cases) followed by fatigue (70.9%). Most of the cases had severe anemia (57.8%). Megaloblastic anemia was found to be the most common cause, constituting 61.9% of all cases, followed by hypersplenism (25.7%), aplastic anemia (9%), leukemia (1.9%). Occasional cases of kala-azar, typhoid and malaria was also noted.

**Conclusion:** A proper clinico-hematological examination aids in making a proper diagnosis in patient presenting with pancytopenia. Sometimes, specific clinical features point towards a certain disease process. Various laboratory parameters can help in diagnosing the underlying disorder leading to pancytopenia. This diagnostic approach will facilitate to highlight the significance of various diagnostic modalities.

***Keywords:*** Pancytopenia, Bone Marrow, Leukemia, Infection

## Introduction

Pancytopenia is the disorder with simultaneous reduction in red blood cells (RBCs), white blood cell (WBC) and platelet count. It should be suspected on clinical grounds when the patient is presenting with pallor, recurrent and prolonged fever and tendency to bleed. Various hematopoietic and non-hematopoietic conditions are associated with pancytopenia. The severity of the pancytopenia and underlying disease decides the management and prognosis of the patients. [1,2] This study is conducted to identify the clinical profile, etiological agents, peripheral and bone marrow findings of the patients with pancytopenia.

## Materials and Methods

The present retrospective study was done for 2 years from April 2015 to April 2017 in Department of Pathology. A total of 210 patients were included in the study. A detailed history including drug history was noted in these patients. Complete clinical findings were noted along with complete blood count which was performed using automated cell counter. The patients whose hemoglobin was less than 9 gm/dl, total leukocyte count was lower than 4000/ $mm^3$ (Absolute neutrophil count <1500/µL) and platelet count less than 1,00,000/$mm^3$ were included in the study. Bone marrow aspirate and biopsy findings were also analysed. Other informations in selected cases according to their provisional diagnosis, like malaria parasite, enteric fever, blood culture, anti-nuclear factor (immunofluorescent method), rheumatoid factor, liver function test, vitamin B12 and folic acid, human immunodeficiency virus (HIV) I and II, and the hepatitis B surface antigen (HBsAg) were also collected. The data of history, physical examination, complete blood count and bone marrow findings were recorded, tabulated and analysed.

## Result

Out of 210 patients, 136 were adults and 74 were children. The mean age of the patients was 42 years. The male to

female ratio was 2.7:1. The most common clinical feature in our study was pallor (in all cases) followed by fatigue (70.9%). Other features included fever (48.5%), weakness (67.6%) and breathlessness (43.8%). Splenomegaly and hepatomegaly were noted in 40.4% and 33.8%cases respectively. Presenting complaints and physical findings are shown in Table 1.

The haematological parameters revealed a mean haemoglobin concentration of 5.6gm/dl. Most of the cases had severe anemia (57.8%). The leucocyte count varied between 750-3800/$mm^{3.}$ The platelet count was 10,000-1,00,000/mm3 of blood. Most of the patients had moderate thrombocytopenia (48.8%).

There was the spectrum of causes which led to pancytopenia. Megaloblastic anemia was found to be the most common cause, constituting 61.9% of all cases. The distribution of various causes of pancytopenia is shown in Table 2.

Megaloblastic anemia was observed in 84 males and 46 females, their age ranging from 12 years to 84 years. Out of all these cases 46% had decreased levels of vitamin B12 and folic acid. Peripheral smear showed moderate anisopoikilocytosis with predominantly macrocytic normochromic cells, few macro-ovalocytes and polychromatic cells. Bone marrow aspiration showed megaloblastic erythroid hyperplasia. Megaloblasts had the characteristic feature of dyserythropoiesis, sieved nuclear chromatin and asynchronous nuclear-cytoplasmic maturation Figure 1]. Giant metamyelocytes and band forms were noted in all the cases.

In the present study, 25.7 % cases were due to hypersplenism when compared to the study by Jain et al where hypersplenism was the cause of maximum number of cases. In this study hypersplenism was the second most common cause of pancytopenia.

Aplastic anemia was seen in 19 cases (9.0% cases), majority of them were in pediatric age group. Mild female preponderance was noted (11 were males and 15 were females). Bone marrow aspirate and biopsy were hypocellular for the age of the patient. There was suppression of erythropoiesis, myelopoiesis and megakaryopoiesis with relative lymphoplasmacytosis. [Figure2].

Four cases of hypocellular marrow (post chemotherapy related) were also seen. All 4 cases were on the treatment of ALL. Two cases of subleukemic leukemia were also analysed; one was acute myeloid leukemia and other was acute lymphoblastic leukemia. Bone marrow aspirate and biopsy in both the cases showed complete replacement of the bone marrow elements by the blast cells. Flowcytometric immunophenotyping delineated further characterisation of blast cells of acute lymphoblastic leukemia. [Figure- 3]

Under the category of infective etiology; one case each of kala azar, malaria falciparum and typhoid were seen. Bone marrow aspiration smears of the kala azar patient showed many intra- and extracellular LD bodies. [Figure 4] RK 39 was positive in this case. Gametocytes of malaria parasite were seen in the aspiration smears. Bone marrow aspirate smears of the typhoid patient showed histiocytic prominence.

**Table 1: Clinical presentation of the patients with pancytopenia**

| Clinical features | Number of patients | % of patients |
|---|---|---|
| Pallor | 210 | 100% |
| Fatigue | 149 | 70.9% |
| Fever | 102 | 48.5% |
| Weakness | 142 | 67.6% |
| Breathlessness | 92 | 43.8% |
| Splenomegaly | 85 | 40.4% |
| Hepatomegaly | 70 | 33.8% |

**Table 2: Table showing the spectrum of causes of pancytopenia.**

| Spectrum of causes of pancytopenia | Number of cases | Percentage of cases |
|---|---|---|
| Megaloblastic anemia | 130 | 61.9% |
| Hypersplenism | 54 | 25.7% |
| Aplastic anemia | 19 | 9.0% |
| Leukemia | 04 | 1.9% |
| Kala azar | 01 | 0.47% |
| Enteric fever | 01 | 0.47% |
| Malaria | 01 | 0.47% |

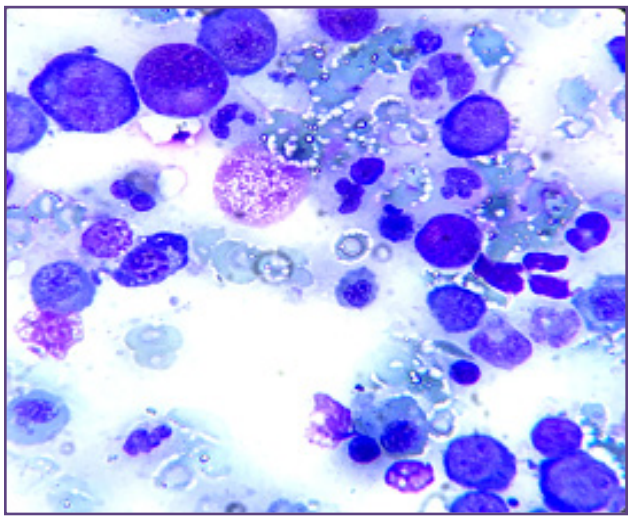

**Fig. 1: Photomicrograph shows bone marrow aspirate smear with many megaloblasts with sieve-like chromatin (Giemsa, 400X).**

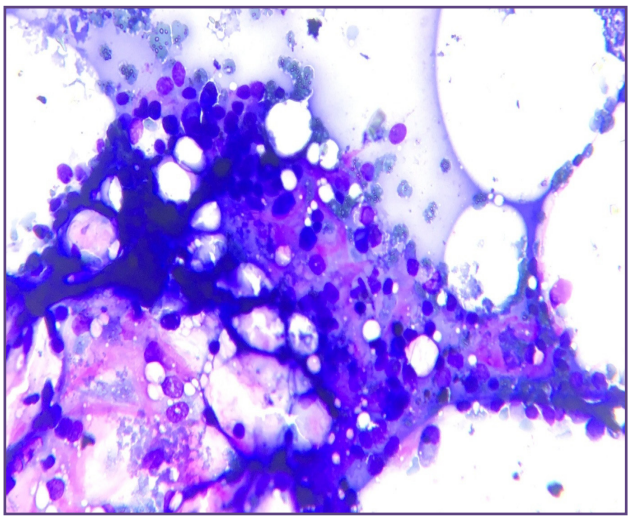

**Fig. 2: Photomicrograph showing bone marrow aspirate smear with hypocellular bone marrow fragments in a six years old child.**

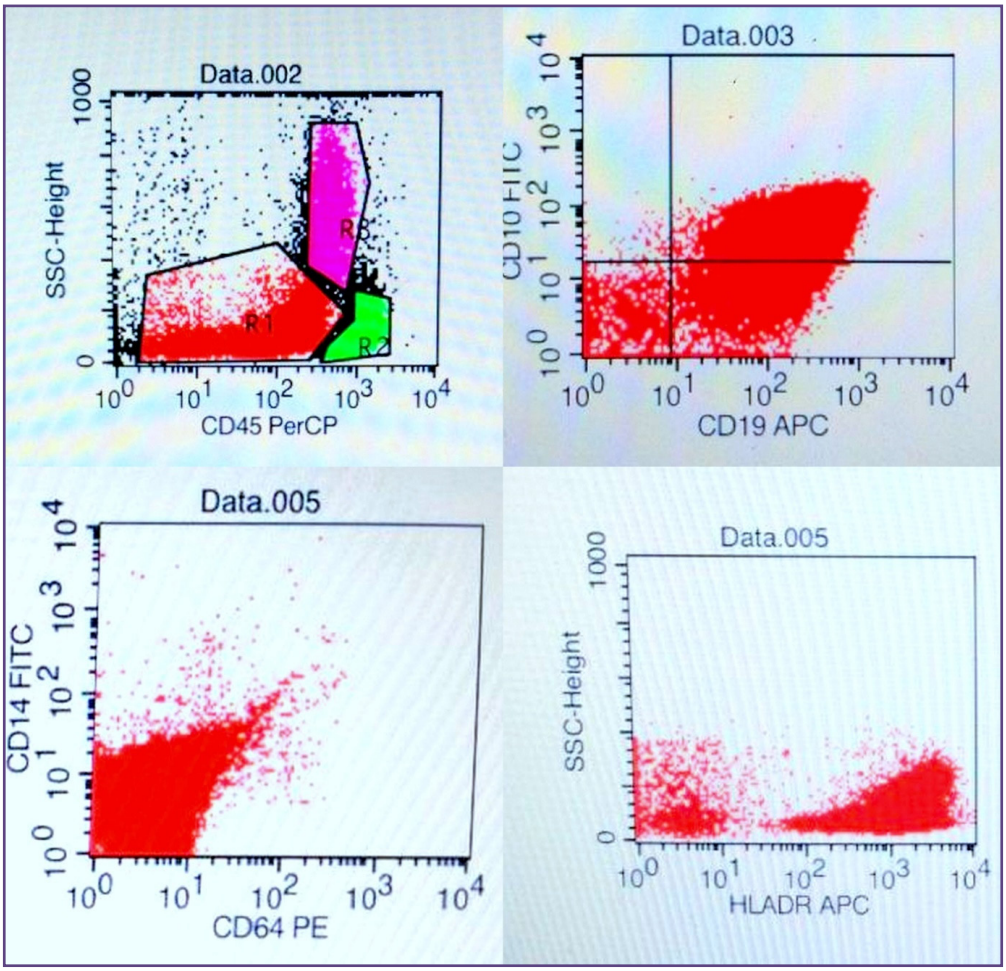


**Fig. 3: Photomicrograph showing a case of B-ALL with blast cells (R1) with dim CD45 positivity. Blast cells were positive for CD19 and CD 10, along with strong positivity for HLA-DR, explaining blast nature of the cells.**

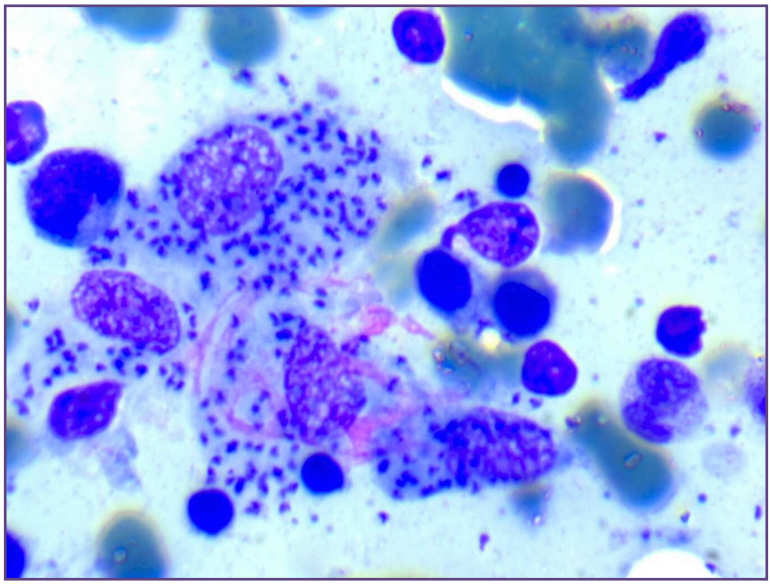

**Fig. 4: High power view showing extracellular and intracellular LD bodies within the myeloid and erythroid cells (Leishmania X 400).**

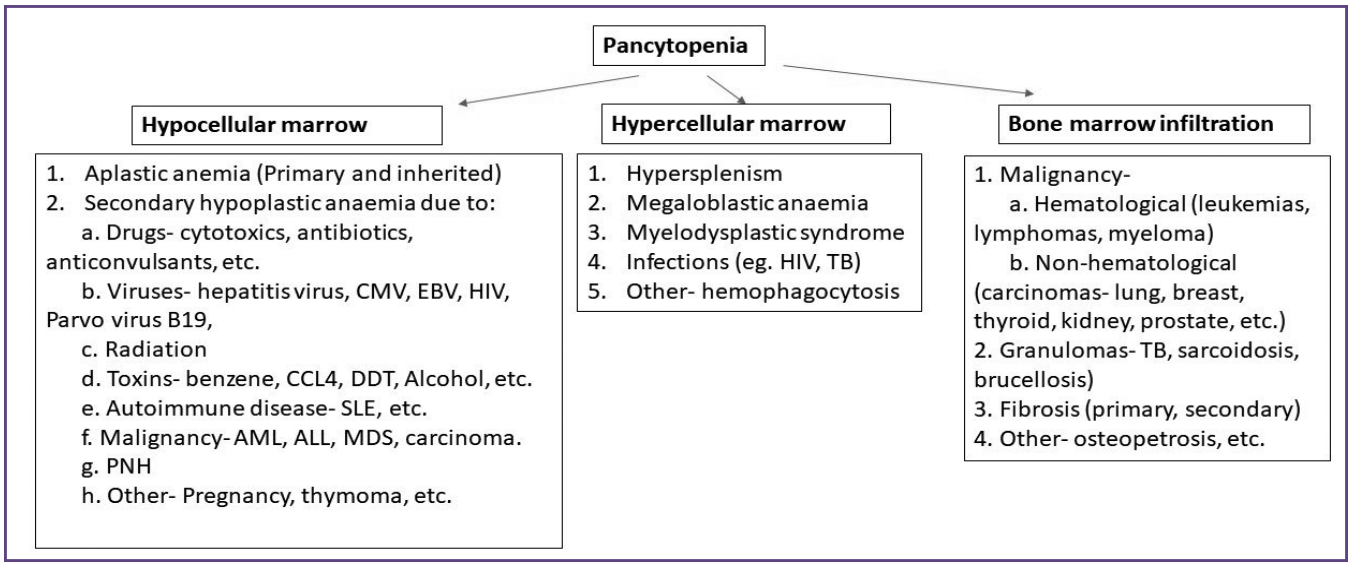


**Fig. 5: Flowchart enumerating various etiologies leading to pancytopenia.**

## Discussion

There are various causes of pancytopenia, which can present with either cellular or hypocellular marrow. Pancytopenia is usually a common indication for bone marrow examination. The frequency and pattern of diseases causing pancytopenia varies in population and can be due to differences in methodology and stringency of diagnostic criteria, geographic area, period of observation, genetic differences, nutritional status, prevalence of infections and varying exposure of different drugs. [3] A definite male predominance observed in our study has been reported by many other similar studies. [4,5]

New-onset pancytopenia, in both children and adults can pose diagnostic challenge. A detailed clinical, medical, drug and environmental exposure history along with bone marrow examination is required for an extensive work-up of new-onset pancytopenia. Family history is pertinent in cases of inherited aplastic anemia like Fanconi's anemia, Dyskeratosis congenita and storage disorder, SLE. Various etiologies can be considered for pancytopenia. [Figure 5]. Pancytopenia can result from disease/ phenomenon involving the bone marrow or peripheral consumption.

The most common cause of pancytopenia varied in different studies. In our study megaloblastic anemia was the most common cause of pancytopenia. Similar results were seen in the Indian studies done by Savage et al, Khunger et al and Khodke et al, suggesting that nutritional anemia is the most common cause of pancytopenia in India. [6,7,8]

Megaloblastic anemia commonly presents with anemia, thrombocytopenia, and occasionally pancytopenia. Folate deficiency is rare in West; however, it is much more common in India. A study done in 1989 in India, showed that of 139 patients, 43.8% had pancytopenia. In another study, 72% of cases of pancytopenia were attributed to megaloblastic anemia caused by folate and/or B12 deficiency. Bone marrow aspiration and biopsy are quite characteristic with folate/Vitamin B2 deficiency and show a hypercellular marrow with erythroid hyperplasia and megaloblastic maturation. Additionally, other laboratory findings can be diagnostically helpful like peripheral macrocytosis, hypersegmented neutrophils, decreased serum cobalamin and red cell folate levels.

Santra et al reported aplastic anemia as the most common cause of pancytopenia accounting 22.72% of all cases. [9] Aplastic anemia was seen in 9.0% cases and showed hypocellular marrow, thus constituting second most common cause of pancytopenia. These results are in concordance with the studies done by Khunger et al and Khodke et al. Both found the incidence of 14%. [7,8] The incidence of aplastic anemia varied from 10 to 52% in various studies. [9] Majority of cases of aplastic anemia are idiopathic; however multiple etiologies include drugs, chemicals, radiation, viruses, anorexia, and even pregnancy. The peripheral blood typically shows pancytopenia, with a relative lymphocytosis and without definite morphologic abnormalities. Bone marrow typically demonstrates a markedly hypocellular marrow with a reduction of all cell lines.

We noted two cases of acute leukemia in the present series [one case of each AML & ALL]. Among the hematopoietic neoplasms causing pancytopenia, acute leukemias are among the most common. [9-10] The clinical presentation is variable but often a manifestation of patient's underlying pancytopenia resulting from the replacement of bone marrow by lymphoblasts. Usually patients present with fatigue, easy bruising, infection, lymphadenopathy, hepatosplenomegaly and bone pain. Peripheral blood often shows B-lymphoblasts and leukocyte count may be decreased, normal, or markedly elevated. Bone marrow examination is usually diagnostic and generally shows replacement of marrow space sheets of B lymphoblasts. Flowcytometric and cytogenetic studies help in further characterization along with bone marrow aspirate & biopsy findings. Subleukemic leukaemia was seen in 0.9% of our cases which is in contrast to the study done by Kumar et al who reported 12% of subleukemic leukaemia, out of which 5 cases were ALL, 13 were AML and 2 cases were hairy cell leukemia. Khunger et al reported subleukemic leukemia in 5% of all cases. [7] This difference was primarily explained by difference in their patient profile.

A single case of enteric fever showing pancytopenia with hypocellular marrow was also noted. Pancytopenia was primarily constituted by hemophagocytosis. The other mechanism of pancytopenia varied from histiocytic hyperplasia with hemophagocytosis to immune mediated destruction of the cells to necrosis. [10] The patients usually present with fever, headache, vomiting, abdominal pain, generalized body pain, loss of appetite, weight loss and diarrhea followed by yellowish discoloration of eyes & urine and hepatosplenomegaly. Though liver is commonly involved but severe hepatic derangement simulating acute viral hepatitis is rare. Mild derangements of liver enzymes are noted. The frequency of liver enzymes elevation in typhoid fever has been reported as 22%, 26% and 52% in various case series. Peripheral smear usually shows anemia, leucopenia, thrombocytopenia and eosinophilia along with sub clinical disseminated intravascular coagulation. Bone marrow suppression and hemophagocytosis are important mechanism in producing hematological changes.

Similarly, one case of falciparum malaria causing pancytopenia was seen showing hyper cellular marrow. In malarial infection various mechanisms are associated with pancytopenia which includes direct bone marrow invasion by parasite, immune mediated haemolysis, disseminated intravascular coagulation, hypersplenism, bone marrow necrosis or haemophagocytosis. [11,12,13,14] Usually patients present with fever associated with chill. A triad of thrombocytopenia, elevated lactate dehydrogenase (LDH) levels and reactive/ atypical lymphoid cells prompt to obtain malarial smears. Hemoglobin level is usually decreased. Thick and thin films are routinely used for diagnosis of malaria. Ring and trophozoites forms of parasite are noted in smear.

In this series, a case of visceral leishmaniasis was also noted showing pancytopenia. The patient presented with fever, hepatomegaly with moderate to marked splenomegaly along with cutaneous manifestations. Progressive leucopenia and severe anemia are striking features of L. donovani infection. There is a progressive decline in total leucocyte count along with decreased erythrocyte and platelet counts. Differential leucocyte count gives a higher monocyte and lymphocyte count. Detection of L. donovani is the most specific test for diagnosis of kala-azar. Spleen and bone marrow are the preferred sites for detecting these organisms; showing scattered histiocytes with LD

bodies lying intracellularly and extracellularly. LD body is small, oval to round shaped with nucleus and kinetoplast. rK39 antibody kit is used for detecting leishmaniasis. Leishmania-HIV coinfection is a major concern as there can be wide dissemination of organisms to various other organs and patients can present with atypical features.

In our study, pediatric patients comprised 32.8% of all the patients. Similar to the adult age group, megaloblastic anemia (60%) was the most common cause of the pancytopenia followed by aplastic anemia. This compared favourably with Bhatnagar et al (46 pediatric patients). [15] However some of the studies which are in variance includes Gupta et al who found aplastic anemia being the most common cause of pancytopenia (105 cases) followed by acute leukemia [16] and Tilak et al, who observed megaloblastic anemia (64 cases) as the most common cause of pancytopenia. [17]

## Conclusion

The present study highlights the significance of detailed analysis of clinico-hematological profile and laboratory parameters in the evaluation of pancytopenia. It paves the way for the systematic planning for diagnostic algorithm to ascertain the cause and thereby avoiding unnecessary tests which are not only additive to the cost of treatment but may also result in the delayed diagnosis and prolonged treatment.

Majority of the etiological factors leading to pancytopenia are reversible and treatable and hence precise diagnosis and timely intervention will be life-saving and improve the prognosis. Infective causes like enteric fever, malaria etc. are easily treatable and hence early and aggressive management will lead to an excellent outcome. As the causes and hence the prognosis of the pancytopenia is varied, comprehensive evaluation of the different laboratory parameters, peripheral blood and bone marrow findings and its correlation with clinical profile is of utmost importance to arrive at a correct diagnosis and impart an adequate management.

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