

CLINICAL ASSESSMENT OF PANCYTOPENIA IN PERIPHERAL BLOOD SMEARS IDENTIFIED IN PATIENTS FROM BIHAR REGION

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Abstract

The severity of pancytopenia and the underlying pathology determine the management and prognosis. [3] Thus, identification of exact cause will help in implementing appropriate therapy. The major diagnostic problems occur when there are no specific features in the peripheral smear to point the cause. In India the causes of pancytopenia are not well defined, so the present study has been undertaken to evaluate the various causes and to correlate the peripheral blood smear findings.

The present study was planned in Department of Pathology, Anugrah Narayan Magadh Medical College, Gaya, Bihar from July 2017 to Dec 2017. Total 50 cases of the clinical suspicion of a hematological disorder and demonstrating pancytopenia in the peripheral blood smears were enrolled in the present study.

All participants underwent a detailed history, clinical examination and investigations which included complete blood picture with red cell indices and peripheral smear, liver function test, renal function test, ultrasound abdomen and bone marrow examination in all cases. Cause of pancytopenia was ascertained and data was analysed on SPSS on the basis of etiology, clinical and haematological findings.

The data generated from the present study concludes that systematic and thorough workup is required in patients presenting with pancytopenia, so that elimination of the cause is needed to treat the condition. Among them, megaloblastic anaemia and infections are early treatable and reversible.

Keywords: Pancytopenia, Bone Marrow Aspiration, Megaloblastic Anemia, Hypoplastic Marrow, etc

INTRODUCTION

Pancytopenia is a medical condition in which there is a reduction in the number of red and white blood cells, as well as platelets. If only two parameters from the complete blood count are low, the term bicytopenia can be used. The diagnostic approach is the same as for pancytopenia.

Pancytopenia is a descriptive term referring to the combination of low levels of all of the types of blood cells including red blood cells (anemia), white blood cells (leukopenia), and platelets (thrombocytopenia). Symptoms often occur due to the reduced levels of these cells such as fatigue, infections, and bruising. There are a number of different conditions that can cause pancytopenia, including bone marrow diseases, some cancers, and some infections. Medical conditions such as chemotherapy can also cause pancytopenia. The treatment options for

pancytopenia depend on the specific causes and usually focus on increasing the levels of cells to improve symptoms while treating the underlying cause.

Pancytopenia may be caused by anything which interferes with the formation of blood cells in the bone marrow or their availability in the bloodstream (such as if they are held in the spleen). This may involve bone marrow destruction by toxins, bone marrow suppression, such as during chemotherapy, or the replacement of bone marrow by other cells resulting in the disruption of blood cell production as can occur with some cancers. Destruction or suppression may occur from inflammation, infections, or autoimmune conditions. Most of these conditions are acquired later in life, but a few are inherited and present from birth. At least 50 percent of the time there is not an obvious cause—something doctors refer to as "idiopathic."

Pancytopenia may be noted on a complete blood count (CBC). A first step in evaluating low levels of all cells is to repeat the CBC. A peripheral blood smear is then done to look further at each of the different types of cells. If it appears that you truly have pancytopenia, the first step is often a bone marrow biopsy. A bone marrow aspiration and biopsy look at the components of bone marrow, that include hematopoietic stem cells (the cells that differentiate into all of the different types of blood cells), blood cells in various phases of maturation, and supplies used to make blood cells, such as iron and vitamin B12. In addition to looking at B12 levels in the blood, special stains and tests are done on the cells looking for changes such as chromosomal and gene changes often found with leukemias. [1]

Pancytopenia is a common haematological condition often encountered in day to day clinical practice. It is defined as a decrease in all the three cell lines of blood viz., red blood cells, leucocytes, and platelets. Many diseases affect production of these cells by bone marrow resulting into pancytopenia i.e., simultaneous presence of anaemia, leucopenia, and thrombocytopenia. Pancytopenia is defined as haemoglobin of < 9 gm/dl, WBC < 4,000/cmm, and platelets < 100,000/cmm. Severe pancytopenia is defined as absolute neutrophil count < 500/cmm, platelet count < 20,000/cmm, and corrected reticulocyte count < 1%. Presenting symptoms of pancytopenia may be attributable to anaemia, leucopenia, and/or thrombocytopenia. Anaemia may present with fatigue, breathlessness, and cardiac symptoms. Neutropenia may present with febrile illness due to increased susceptibility to infections. Patients with thrombocytopenia may present with mucocutaneous bleed or bruising. Pancytopenia should be suspected on clinical grounds in any patient presenting with unexplained anaemia, prolonged fever and bleeding tendency. The severity of pancytopenia and underlying aetiology determine the management and prognosis. Pancytopenia usually presents with the clinical sign and symptoms of bone marrow failure such as pallor, easy fatigability, dyspnoea, bleeding or bruising, and increased tendency to infection. As platelets have shortest half life, platelet count is first to be affected leading to thrombocytopenia. Mucocutaneous bleed is typical manifestation of decreased platelet count with petechial haemorrhages in the skin and mucous membrane. Epistaxis, haematuria, gastrointestinal bleeding, menorrhagia, and rarely intracranial

bleeding are the presenting features of thrombocytopenia. Anaemia develops slowly because RBC has longest half life. Early manifestation of neutropenia is often a sore throat, or chest or soft tissue infection with poor response to antibiotics. Patients with pancytopenia may develop overwhelming sepsis without any focal sign of infection, with malaise and fever being the only clinical features.

Normal marrow has tremendous capacity to increase the output of peripheral blood cells whenever necessary with the help of growth factors and cytokines. All the peripheral cells arise from common progenitor pluripotent cells having enormous capacity of self renewal. The normal adult marrow produces about 170×10^9 RBC, 100×10^9 neutrophils, and 200×10^9 platelets daily. Defects in the stem cells or in the stroma or microenvironment of bone marrow can lead to bone marrow failure and pancytopenia. Pancytopenia is not a disease by itself but a triad of haematological finding that can result from a number of disease processes. It can be a feature of many serious and life threatening illnesses like drug induced bone marrow hypoplasia, fatal bone marrow aplasia, and leukaemias. It can result from failure of production of stem cells in bone marrow, infiltration of bone marrow by malignant cells or fibrosis, immune mediated bone marrow suppression, ineffective erythropoiesis and dysplasia, peripheral sequestration of blood cells by overactive reticuloendothelial system, and immune or non-immune mediated increased destruction of blood cells. Marrow damage may be caused by infiltration of marrow with tumour or fibrosis that crowds normal marrow cells. Tumour or fibrosis that infiltrates the marrow may originate in the marrow as in leukaemia or myelofibrosis or be secondary to process originating outside marrow as in metastatic cancer or myelophthisis. Incidence of various disorders causing pancytopenia varies according to geographical distribution and genetic mutations. Main causes of pancytopenia in our country are megaloblastic anaemia due to nutritional deficiencies, hypersplenism (congestive splenomegaly, malaria, and leishmaniasis), aplastic anaemia, myelodysplastic syndrome, subleukaemic leukaemias, military tuberculosis, multiple myeloma, paroxysmal nocturnal haemoglobinuria.

Evaluation of pancytopenia requires a careful history and physical examination. The causes of pancytopenia are diverse. Attention must be paid to

history of the patient and the family. Nutritional history, drug history and history of alcohol intake should always be assessed. History suggestive of previous pancytopenia, aplastic anaemia, inherited bone marrow failure syndrome, repeated early foetal loss, cancer, liver disease, metabolic disorders, or connective tissue disorder is important. Cytotoxic chemotherapy and radiotherapy are important cause of transient pancytopenia. History of weight loss and anorexia may suggest underlying infection or malignancy. Recurrent oral ulcers and chronic diarrhoea may point towards HIV infection. Recurrent oral ulcers, malar rash and joint pain may suggest SLE. Bone pain and loss of height indicate multiple myeloma. A thorough physical examination is of paramount importance in evaluation of pancytopenia. It should include assessment of jaundice, clubbing of fingers, lymphadenopathy and splenomegaly (underlying infection, infectious mononucleosis, lymphoproliferative disorder, and malignancy), loss of height (suggestive of multiple myeloma), malar rash, retinal haemorrhage, oral petechiae, gingival hyperplasia, stomatitis or cheilitis, oropharyngeal candidiasis, RUQ abdominal tenderness, signs of chronic liver disease. Laboratory evaluation: A routine complete blood count (CBC) is required as a part of initial evaluation of pancytopenia. CBC should include red cell indices, peripheral blood film, reticulocytes count and absolute reticulocyte count. A very high MCV (>110fl) indicates megaloblastic anaemia. In addition liver function test, viral markers for hepatitis, coagulation profile, fibrinogen, D-dimer, serum B12, folate levels, HIV serology, antinuclear antibodies (ANA) should be done. Serum ferritin levels should also be assessed. Low levels of serum ferritin along with low serum B12 and/or folate levels may indicate mixed anaemia/pancytopenia. Peripheral blood smear provides important information in pancytopenia and it should always be done prior to transfusion of blood. Blood smear may reveal polychromasia—red cells that are slightly larger than normal and greyish blue in colour. These cells are reticulocytes that have been prematurely released from the bone marrow. These cells may appear in circulation due to architectural damage of the bone marrow caused by fibrosis or malignant cell infiltration. Bone marrow examination is almost always indicated in cases of pancytopenia unless the cause is otherwise apparent (e.g., chronic liver disease with portal hypertension, deficiency of vitamin B12 or folate). In megaloblastic

anaemia bone marrow shows megaloblastic erythroid hyperplasia, sieved nuclear chromatin, asynchronous nuclear maturation, bluish cytoplasm with cytoplasmic blebs. Giant metamyelocytes and band forms are predominant in granulocyte series. Bone marrow in aplastic anaemia is hypocellular with suppression of erythropoiesis, myelopoiesis, and megakaryopoiesis with relative lymphoplasmacytosis. In acute leukaemias, bone marrow is hypercellular with reduced erythroid and megakaryocytic series and majority of cells are myeloblast or lymphoblast. Bone marrow aspiration in AML shows myeloblast with Auer rods. [2]

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Methodology:

The present study was planned in Department of Pathology, Anugrah Narayan Magadh Medical College, Gaya, Bihar from July 2017 to Dec 2017 Total 50 cases of the clinical suspicion of a hematological disorder and demonstrating pancytopenia in the peripheral blood smears were enrolled in the present study.

All participants underwent a detailed history, clinical examination and investigations which included complete blood picture with red cell indices and peripheral smear, liver function test, renal function test, ultrasound abdomen and bone marrow examination in all cases. Cause of pancytopenia was ascertained and data was analysed on SPSS on the basis of etiology, clinical and haematological findings.

Bone marrow aspiration was done under aseptic conditions after infiltrating the sight of aspiration with xylocaine. Common sites of aspiration were posterior iliac crest and sternum. Bone marrow aspiration was done from tibial tuberosity in infants and young children. Sterilised Salah's needle and 10cc syringe were used for aspiration. Aspirated material was expressed on to clean glass slides and smears prepared. Simultaneously complete blood count was done and peripheral blood smear was prepared. Bone

marrow aspiration smears and peripheral blood smears were stained with leishman stain. Marrow smears were examined for cellularity, megakaryocytes, erythroid myeloid ratio, erythropoiesis, myelopoiesis, other cells such as plasma cells, lymphocytes, blasts and parasites. Perl's stain was done in all cases for iron stores. When required special stains, periodic acid-Schiff stain and myelo peroxidase stain were used. Reticulin stain was done in suspected myelofibrosis cases. Trepine biopsy was done in 18 cases for further evaluation of bone marrow. Trepine biopsy specimens were fixed in formalin processed and the sections were stained with haematoxylin and eosin. Flow cytometry was advised in some cases of leukaemia for typing.

All the patients were informed consents. The aim and the objective of the present study were conveyed to them. Approval of the institutional ethical committee was taken prior to conduct of this study.

Following was the inclusion and exclusion criteria for the present study.

Inclusion Criteria

Patients of all age groups and both sexes who fulfilled the criteria for pancytopenia diagnosis; haemoglobin level less than 13.5 g/dL in males or 11.5 g/dL in females; leukocyte count less than $4 \times 10^9/L$ (Absolute neutrophil count $1.5 \times 10^9/L$) and Platelet count less $100 \times 10^9/L$ were included.

Exclusion Criteria

Cases of chemotherapy (myelotoxic) induced pancytopenia were excluded.

Results & Discussion:

Pancytopenia is a broad vague haematological finding under which many aetiologies are hidden to be further evaluated with bone marrow examination, which give accurate diagnosis in many cases. This will help in further management of patients. Pancytopenia is a common manifestation of varied disease entities. Etiological evaluation of pancytopenia using easily available and less invasive diagnostic tools is very important. Age, gender and clinical presentation do aid in planning investigations in a case of pancytopenia.

Most studies especially from western world have reported aplastic anemia as a common cause. On the contrary many studies from Indian subcontinent have shown megaloblastic anemia to be a common cause

of pancytopenia in this region [4, 5, 6]. This seems to reflect the higher prevalence of nutritional deficiency in India. A study from Aden revealed that most common causes of pancytopenia in their patients were malaria and hypersplenism (> 45%), followed by megaloblastic anemia (14.7%), aplastic anemia and acute leukemia [7].

Table 1: Indications for Bone Marrow Examination

Indication	No. of Cases
Anemia Under Evaluation	23
Pancytopenia Under evaluation	10
Suspected Leukemia	5
Thrombocytopenia	5
Hepatosplenomegaly Under evaluation	2
Pyrexia Under Evaluation	1
Others	4
Total	50

Table 2: Haematological Disorders behind Pancytopenia Blood Picture in Adults

Haematological disorder	No. of Cases
Megaloblastic anemia	31
Hypoplastic marrow	6
Acute myeloid leukemia	5
Dimorphic anemia	4
Lymphoproliferative disorder	3
Idiopathic thrombocytopenic purpura	1
Total	50

Table 3: Spectrum of Haematological Disorders Causing Pancytopenia in Children

Haematological disorder	No. of Cases
Hypoplastic marrow	15
Megaloblastic anemia	10
Dimorphic anemia	10
Idiopathic thrombocytopenic purpura	5
Acute myeloid leukemia	5
Hypersplenism	5
Total	50

We have found primary bone marrow/ hematological malignancies (leukemia, lymphoma & MM) to be the most common cause of pancytopenia (39.1%) in this state (Tripura) of North East India. This experience is even different from that of Raphael, Khonglah and Dey et al. who found megaloblastic anemia to be the commonest etiology (41.2%) of pancytopenia in the state of Meghalaya, another nearby north-eastern state mostly tribal inhabited [8]. In their study at Davangere in Karnataka, Gayathri and Rao also found

megaloblastic anemia had been the commonest cause (74% cases) of pancytopenia [9]. But in a study in another north-eastern state, Manipur the investigators found that hypoplastic anemia had been the most common cause (22%) followed by megaloblastic anemia and myelodysplastic syndrome(18% each). [10]

In India poor eating habits, poverty, poor quality of foods, and lack of education and self-avoidance of necessary foods may be the cause of nutritional deficiency leading to megaloblastic anaemia. Megaloblastic anaemia can be prevented by improving the nutritional status of our population. The incidence of hypoplastic anaemia in our study was 14%, which correlated with the studies of Gayathri and Kadam (19%) [11]; Khodke, et al. (14%) [2]. A higher incidence (29.5%) was reported by Kumar, et al. [13]. Aplastic anaemia may be due to environmental factors or exposure to pesticides/ drugs/ toxic chemicals, infections.

Hematopoietic neoplasms may sometimes present with pancytopenia in both children and adults and acute leukemias are among the most common of these. Fever and bleeding may be present. Organomegaly or lymphadenopathy may or may not be present. Meticulous examination of peripheral smear for immature cells may give a clue. But if there is no obvious cause for pancytopenia bone marrow aspiration must be done early.

Variations in the frequency of various diagnostic entities causing pancytopenia have been attributed to difference in methodology and stringency of diagnostic criteria, geographic area, period of observation, genetic differences and varying exposure to myelotoxic agents, etc. [14] The haematological parameters were usually nonspecific in many cases and showed considerable overlap. In all these cases a peripheral blood film was important in indicating towards a diagnosis of megaloblastic anaemia or leukaemia. Bone marrow aspiration and biopsy are important diagnostic tools in the diagnosis of various haematological disorders, including pancytopenia. Both of these procedures are complimentary to each other.

Many infections cause bone marrow necrosis and bone marrow suppression alongwith increased peripheral destruction due to persistent congestive splenomegaly. Pancytopenia in enteric fever is mostly due to histiocytic hyperplasia in bone marrow, with marked phagocytosis of platelets, leukocytes and

RBCs but sometimes due to myelosuppression also as seen in our study. Most of the cases are reversible with treatment of infection. Autoimmune diseases can also present with new-onset pancytopenia, which can have multiple etiologic factors like autoimmune destruction, vasculitis, myelosuppression, inflammation and nutritional deficiency. Indeed, hematologic abnormalities are often part of the diagnostic criteria for such diseases, including systemic lupus erythematosus.

Pancytopenia is a common haematological entity that we come across in our routine practice. There are numerous causes of pancytopenia which include both non-neoplastic and neoplastic conditions. With the help of detailed clinical history, physical examination, and haematological investigations, pancytopenia can be diagnosed and the causes can be ascertained. Megaloblastic anaemia due to vitamin B12/folate deficiency seems to reflect the higher prevalence of pancytopenia in Indian subjects, putting nutritional anaemia and hypoplastic/aplastic anaemia in the second and third position respectively. Pancytopenia due to megaloblastic anaemia is albeit transient and easily reversible with appropriate treatment. Thus, megaloblastic anaemia should always be considered in the evaluation of pancytopenia in Indian settings. However, other important causes of pancytopenia should be kept in mind while planning investigations for the complete workup of pancytopenic patients.

Hence, while approaching patients of pancytopenia attention should be paid to demographic details like age and gender. Also clinical characteristics like constitutional symptoms, weight loss, presence of bleeding, organomegaly, lymphadenopathy and sternal tenderness should be taken into account. Serious disorders like aplastic anemia and leukemia may be missed if index of suspicion is not kept high. Bone marrow aspiration should be done early if indicated after baseline evaluation.

Conclusion:

The data generated from the present study concludes that systematic and thorough workup is required in patients presenting with pancytopenia, so that elimination of the cause is needed to treat the condition. Among them, megaloblastic anaemia and infections are early treatable and reversible.

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