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Pancytopenia: Clinical approach

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INTRODUCTION

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.

AETIOLOGY OF PANCYTOPENIA

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 $170x10^9$ RBC, $100x10^9$ neutrophils, and $200x10^9$ 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 (Tables 1 & 2). 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.

According to a study of 200 cases of pancytopenia conducted by Khunger et al at a large general hospital in North India, the commonest cause found was megaloblastic anaemia seen in 72% cases, followed by aplastic anaemia (14%). The other causes included subleukaemic leukaemia (10 cases), myelodysplastic syndrome (4 cases), hypersplenism due to kala azar (4 cases), hypersplenism due to malaria (2 cases), Non-Hodgkin's lymphoma, myelofibrosis, multiple myeloma (2 cases each), Waldenstrom macroglobulinaemia and disseminated tuberculosis (1 case each). In another prospective study of 104 pancytopenic patients conducted by Gayatri and Rao at a teaching institute in South India for a period of two years, commonest cause of pancytopenia was megaloblastosis (74%) followed by aplastic anaemia (18%).

In another prospective study of 250 cases of pancytopenia conducted by Jain and Naniwadekar at a tertiary care hospital in Maharashtra hypersplenism (29.2%), infections (25.6%), myelosuppression (16.8%), megaloblastosis (13.2%), and hypoplastic/aplastic anaemia (4.8%) were found to be the common causes of pancytopenia. In this study a male preponderance was observed, male to female ratio being 2.6:1 and majority of cases were encountered in third and fourth decade of life. In an analysis of 166 cases of pancytopenia conducted by Kumar et al at two tertiary care hematology centers where patients receiving myelotoxic chemotherapy or those with leukaemic cells in peripheral blood smears were excluded from the study, it was observed that aplastic anaemia (49 cases) was most common cause followed by megaloblastic anaemia (37cases), aleukaemic leukaemia or lymphoma (30 cases), and hypersplenism (19 cases). Megaloblastosis was not commonest cause of pancytopenia in these series probably because many cases of megaloblastic anaemia need not be referred to a tertiary care centre and could easily be treated at general hospitals.

OVERVIEW OF COMMON CAUSES OF PANCYTOPENIA

Megaloblastic anaemia (Tables 3 & 4)

Megaloblastic haematopoiesis is a hypercellular bone marrow failure due to deficiency of vitamin B_{12} (cobalamin) and folate. These nutrients have important role in synthesis of DNA. Megaloblastic anaemia is a predominant cause of pancytopenia in India because of high prevalence of nutritional anaemia in Indian subcontinent. Vitamin B_{12} deficiency may also cause subacute combined degeneration of cord and psychiatric illness (megaloblastic madness). The degree of bone marrow suppression is inversely related to presence and severity of neurological dysfunction. The coexistence of significant anaemia and neurological deficit is thought to be rare.

Table 1: Causes of pancytopenia with cellular bone marrow

Primary bone marrow disease	Secondary to systemic disease
Myelodysplasia	Vitamin B_{12} deficiency, folate deficiency
Paroxysmal nocturnal haemoglobinuria	Hypersplenism
Myelophthisis	Alcoholism
Myelofibrosis	Sepsis, enteric fever
Subleukaemic leukaemia	HIV infection, hepatitis B, hepatitis C, Ebstein-Barr virus, cytomegalovirus
Bone marrow lymphoma	Malaria, leishmaniasis, filariasis
Hairy cell leukaemia	Systemic lupus erythematosus, sarcoidosis

Hypersplenism

Hypersplenism is characterized by splenomegaly, cytopenia(s), normal or hyperplastic bone marrow, and a response to splenectomy. In hypersplenism there is peripheral pooling and destruction of cells in enlarged spleen resulting in pancytopenia. Causes of hypersplenism include congestive splenomegaly (cirrhosis, congestive heart failure), malaria, hyperreactive malarial splenomegaly, leishmaniasis, thalassaemia, and Hodgkin's disease. Hypersplenism can rarely be idiopathic.

Infections

Haematologic abnormalities have been frequently observed in patients with HIV infection. Pancytopenia is usually seen in advanced stage of HIV infection. Aetiology of pancytopenia in advanced HIV stage is multi-factorial

Table 2: Causes of pancytopenia with hypocellular marrow

Acquired aplastic anemia
Congenital aplastic anaemia (Fanconi's anaemia)
Some myelodysplasias
Acute myeloid leukaemia
Acute lymphoid leukaemia
Lymphoma of bone marrow

Table 3: Causes of Vitamin B_{12} deficiency

Food	Decreased consumption-vegan diet, cobalamin malabsorption (common in elderly)
Stomach	Pernicious anaemia, atrophic gastritis, gastrectomy, gastric bypass, H. pylori infection, Zollinger-Ellison syndrome
Intestine	Chronic pancreatitis, tropical sprue, celiac disease, ileal resection, fish tapeworm infestation, bacterial overgrowth syndrome, HIV infection
Drugs	Metformin, Proton pump inhibitors, H_2 blockers

Table 4: Causes of folate deficiency

Nutritional deficiency	Dietary deficiency
Increased requirements	Infancy and childhood, pregnancy, malignancy, chronic haemolytic anaemia, chronic exfoliative dermatitis, chronic inflammatory disorders (tuberculosis, rheumatoid arthritis, Crohn's disease), CHF, chronic liver disease, haemodialysis, homocystinuria
Malabsorption	Tropical and non-tropical sprue, gluten sensitive enteropathy
Drugs	Antiepileptic drugs, methotrexate, pyrimethamine, alcohol
Congenital	Abnormalities of folate metabolism

in nature and includes high viral load, use of antiretroviral therapy, and presence of acute or chronic opportunistic infection. Viral hepatitis has been known to cause transient pancytopenia during the course of illness and has also been associated with aplastic anaemia. Hepatitis associated with pancytopenia and aplastic anaemia is usually fatal. Whereas hepatitis B and hepatitis C are common causes; Epstein-Barr virus, cytomegalovirus, and rarely hepatitis A and dengue virus can also cause pancytopenia. Mild blood count depression is common in course of many viral and bacterial infections but resolves with the resolution of infection. Sepsis and enteric fever continue to be important public health problems in India and have been associated with pancytopenia which has been attributed to bone marrow suppression, disseminated intravascular coagulation, and infection associated haemophagocytic syndrome. Tuberculosis is a common disease in India. Disseminated miliary tuberculosis is known to cause pancytopenia. Although pancytopenia is a rare presentation of tuberculosis, it should always be considered in patients presenting with pancytopenia, unexplained pyrexia and weight loss. Both tuberculous bacilli and anti-tuberculous therapy have been implicated in pathogenesis of pancytopenia. *Wuchereria bancrofti* is an endemic filarial nematode spread by a mosquito vector. The clinical manifestations vary from asymptomatic microfilaraemeia to lymphoedema. Cases of microfilaria in bone marrow aspirate presenting as pancytopenia in peripheral blood have been reported. Though, aetiopathological correlation of pancytopenia with microfilaria infection is not clear.

Aplastic anaemia

Aplastic anaemia is defined as pancytopenia with hypocellular marrow in absence of abnormal infiltrate or increased fibrosis. It is a normocytic normochromic anaemia that results from a loss of blood cell precursors, causing hypoplasia of bone marrow leading to pancytopenia. Severe aplastic anaemia is defined as a bone marrow cellularity < 25% with at least two of the three criteria i.e., neutrophils < 500/mcL, platelets < 20,000/mcL, and reticulocyte count < 20,000/mcL. It is a potentially life threatening failure of bone marrow. Most cases of aplastic anaemia are acquired and T-cell mediated autoimmune disease. Triggering factors may include drugs, viruses, and toxins but most cases are idiopathic. In some cases radiation, drugs, toxic chemicals and viruses induce depletion of haematopoietic stem cells by direct toxicity.

Paroxysmal nocturnal haemoglobinuria (PNH)

PNH is an acquired chronic haemolytic anaemia characterized by persistent intravascular haemolysis with recurrent exacerbations due to activation of complement C. In addition to haemolysis, there is often pancytopenia and a risk of venous thrombosis. Haemolysis in PNH is due to an intrinsic abnormality of the red cell, which makes it sensitive to activated complement C. Diagnostic gold standard of PNH is flow cytometry which can be carried out on granulocytes as well as on RBC. A bimodal distribution of cells with a discrete population that is CD59 and CD55 negative is diagnostic of PNH.

Acute leukaemias

Acute myeloid leukaemia occurs in all age group but predominantly in older adults. Acute lymphoblastic leukaemia is the most common acute leukaemia in childhood. Clinical history and symptoms usually indicate bone marrow failure. These include fatigue, dyspnoea, dizziness, bleeding, easy bruising, and recurrent infections. Cytogenetic abnormalities are prognostically important and affect patient management. In all cases of severe pancytopenia (symptomatic anaemia, WBC < 500/mcL, and platelets < 20,000/mcL) investigations are mandatory within first 24-48 hrs. Supportive therapy with RBC, platelets, and broad spectrum antibiotics may be initiated before underlying cause has been ascertained.

Myelodysplastic syndrome

Myelodysplastic syndromes (MDS) are the common haematological diseases characterized by cytopenias associated with abnormal appearing cellular marrow producing ineffective red blood cells. The incidence of MDS increases with advancing age. Median age at disease onset is 70 years with only about 10% of the patients below 50 years. MDS are diseases of haematopoietic stem cells. They are characterized by disturbance of differentiation and maturation, and by changes in the bone marrow stroma. MDS are accompanied not only by reducing blood cell counts but also with an increased risk (20-25%) of developing acute myeloid leukaemia. The disease course varies greatly from patient to patient, with median survival time from few months to years.

Systemic lupus erythematosus (SLE)

Haematologic abnormalities such as anaemia, leucopenia, and thrombocytopenia secondary to peripheral destruction are commonly seen in SLE. Most frequent haematologic manifestation of SLE is normocytic and normochromic anaemia. Leukopenia is also common and almost always consists of lymphopenia and not granulocytopenia. Thrombocytopenia may be a recurring problem in SLE. Cytopenias from autoimmune myelofibrosis are also uncommonly seen in SLE.

Idiopathic cytopenia of undetermined significance

Idiopathic cytopenia of undetermined significance (ICUS) is a recently proposed provisional diagnosis that recognizes patients who present with cytopenias of undetermined aetiology. Diagnostic criteria for ICUS include: i) persistent cytopenia for 6 months (Hb < 11mg/dl, neutrophil < $1.5x10^9$ /L, and platelets < $100x10^9$/L); ii) No morphologic feature of myelodysplasia; iii) normal chromosome analysis; and iv) A detailed clinical history and investigation that excludes other secondary causes of cytopenias. The diagnosis of ICUS should only be established when all possible differential diagnosis have been excluded. The term Idiopathic fatal pancytopenia has been suggested for ICUS because it is a fatal disease with no definite cause.

Drug Induced pancytopenia (Table 5)

Drugs are the common cause of pancytopenia. Drug induced pancytopenia can be dose dependent or immune

Table 5: Common drugs causing pancytopenia

a.	By bone marrow suppression: Cytotoxic drugs
b.	By dose dependent effect: Chloramphenicol
c.	By immune mediated idiosyncratic reaction
	NSAIDS, chloremphenicol, sulphonamide, phenothiazines, thiazides, anti-thyroid drugs, anti-epileptics, anti-diabetic drugs, colchicine, azathioprine

mediated (idiosyncratic). Chloramphenicol can cause pancytopenia by both the mechanisms. Azathioprine, an immunosuppressive drug used for treatment of various diseases, usually causes leucopenia and rarely pancytopenia.

CONGENITAL CAUSES OF PANCYTOPENIA

Fanconi's anaemia is an autosomal recessive disorder and manifests as congenital developmental anomaly, progressive pancytopenia, and an increased risk of malignancy. Patients typically have short stature, *café au lait* spots, and anomalies involving thumb, radius, and genitourinary tract. Dyskeratosis congenita is characterized by mucous membrane leukoplakia, dystrophic nails, reticular hyperpigmentation, and development of aplastic anaemia during childhood.

APPROACH TO A CASE OF PANCYTOPENIA

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 mononucelosis, 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 B_{12}, 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 B_{12} 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 B_{12} 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.

ABSOLUTE RETICULOCYTE COUNT

An accurate reticulocyte count is the key to initial evaluation of pancytopenia. Normally, reticulocytes are the red cells that have been recently released from the bone marrow. Normal reticulocyte count ranges from 1-2% and reflects the daily replacement of 0.8-1% of the circulating RBC population. Reticulocyte count provides a reliable measure of RBC production. Reticulocyte count and absolute reticulocyte count (ARC) should be done on day one along with CBC in order to avoid therapy related changes in reticulocyte count particularly with nutritional anaemia. Absolute reticulocyte count (ARC) is a calculated index derived from the product of reticulocyte count percentage and RBC count (Normal; Male: 4.32-5.72 million/cmm, Female: 3.90-5.03 million/cmm). ARC is a marker of red cell production by bone marrow. It plays important role in establishing the cause of pancytopenia and helps in distinguishing between hypoproliferative and hyperproliferative anaemias. Normal range of absolute reticulocyte count is 50,000-100,000/cmm. All cases of pancytopenia with very low ARC (< 25,000/cmm) should be examined by bone marrow aspiration for aplastic anaemia. All cases of pancytopenia with high ARC (> 100,000/cmm) should also be evaluated by bone marrow aspiration unless there is a history suggestive of sepsis or malaria. Pancytopenia with ARC 25,000-50,000/

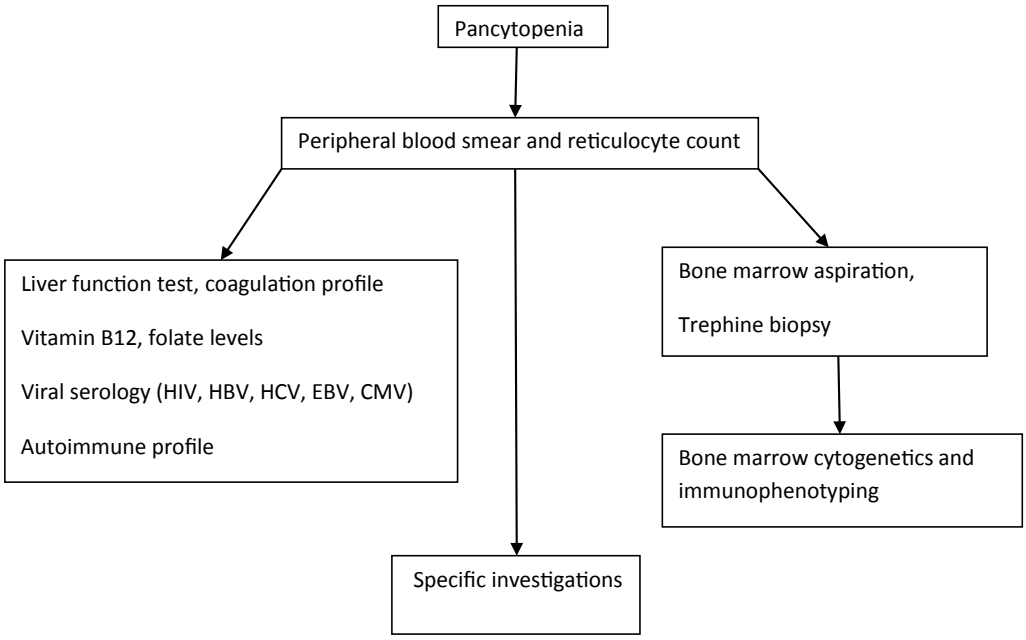

Fig. 1: Flow diagram for Evaluation of Pancytopenia

cmm should initially be evaluated with serum B_{12}, folate and ferritin assays and if any one of these is found to be low, bone marrow aspiration is not needed.

SPECIFIC EVALUATION

Lymphoproliferative disorders: immunophenotyping, cytogenetics, lymph node biopsy

Multiple myeloma: serum electrophoresis, bone marrow aspiration

Paroxysmal nocturnal haemoblobinuria: flow cytometry (CD55, CD59)

Cytomegalovirus infection: serology for CMV (IgG, IgM)

Epstein-Barr virus: Serum monospot, viral capsid antigen, EB nuclear antibody

Leishmaniasis: Blood and bone marrow culture, LD bodies

Carcinoma prostate: Serum PSA

Fanconi's anaemia: diepoxybutane test for chromosomal breakage in peripheral blood lymphocyte

CONCLUSION (FIGURE 1)

Pancytopenia is not a disease itself. It is a haematological feature of varying aetiology with slight male preponderance. Megaloblastic anaemia along with mixed nutritional anaemia is leading cause of pancytopenia in India followed by hypersplenism and aplastic anaemia being second and third common causes respectively. Absolute reticulocyte count is an important indicator for determining the cause of pancytopenia and should be done along with peripheral smear in the very beginning. Serum B_{12}, folate and ferritin assays should be done for evaluation of mixed nutritional anaemia. Bone marrow aspiration is an important and safe invasive procedure for evaluation of cause of pancytopenia.

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