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"A STUDY OF CLINICAL PROFILE OF 50 PATIENTS HAVING PANCYTOPENIA IN SIR T. GENERAL HOSPITAL, BHAVNAGAR"

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ABSTRACT

Objective: To study clinical and investigative profile of patients having pancytopenia. To study the predictive power of investigative parameters (clinical, haematological, biochemical, radiological) including Bone marrow aspiration which are responsible for pancytopenia. **Materials and methods:** 50 patients having pancytopenia, from April 2015 to March 2016 were studied. Those who had age <12 years and not giving consent were excluded. Detailed clinical history was taken and physical examination was done. Patients underwent the required haematological, biochemical, radiological investigations and Bone marrow aspiration (if required).

KEYWORDS: Pancytopenia, Bone marrow aspiration, Megaloblastic anemia, Aplastic anemia.

INTRODUCTION

Normal haematopoiesis occurs within a specialised microenvironment, where humoral factors also play an important role. Haematopoiesis will increase markedly in response to increased demands. Mature blood cells derived from pleuripotent stem cells are then released into circulation.

Cytopenia is a disorder in which production of one or more blood cell types ceases or is greatly reduced than normal levels.[1]

Pancytopenia is a disorder in which all three major formed elements of blood (red blood cells, white blood cells and platelets) are decreased than normal.[2]

Pancytopenia is not a disease entity, but a triad of findings that may result from a number of diseases processes–primarily or secondarily involving the bone marrow.

The presenting symptoms like weakness, fatigue, dyspnoea, fever, bleeding manifestations are usually attributable to presence of anaemia, leucopenia or thrombocytopenia.[3] Leucopenia is an uncommon cause of initial presentation but can become the most serious threat to life during the course of disorder.

Pancytopenia is a striking feature of many serious and life threatening illnesses ranging from simple drug induced bone marrow hypoplasia, megaloblastic marrow to fatal bone marrow aplasias and leukemias.[4]

The underlying mechanisms of pancytopenia are decrease in haematopoietic cell production, marrow replacement by abnormal cells, suppression of marrow growth and differentiation, ineffective haematopoiesis with cell death, defective cells formation, antibody mediated sequestration or destruction of cells in a hypertrophied and overactive reticuloendothelial system.[5]

The pattern of diseases leading to pancytopenia is expected to vary in different population groups with their difference in age pattern, nutritional status and prevalence of infective disorder.[4]

Careful assessment of the blood elements is often the first step in assessment of hematologic function and diagnosis of disease.[4]

Physical findings and peripheral blood picture provide valuable information in the work up of pancytopenic patients and help in planning investigations on bone marrow samples.[6]

Bone marrow evaluation is an invaluable diagnostic procedure in practice of medicine which may confirm the diagnosis of suspected cytopenia, from the clinical features and peripheral blood examination or occasionally give a previously unsuspected diagnosis.[7]

The severity of pancytopenia and the underlying pathology determine the management and prognosis of these patients.[6]

Studies done elsewhere in the world show aplastic anemia and hypoplastic anemia as the most common cause of pancytopenia which is contrast with the studies done in India.

In India, the causes of pancytopenia are not well defined.[4]

Previous studies done in India stress the importance of megaloblastic anaemia as being the major cause of pancytopenia.[6,8] Megaloblstic anemia and other Nutritional anemias are easily preventable and treatable so it is very important to identify and treat those conditions.

Although it is a common clinical pattern with an extensive differential diagnosis, there is a relatively little discussion of this abnormality in major textbooks of internal medicine and haematology.

So the present study has been undertaken to evaluate the various causes of pancytopenia and to correlate the peripheral blood findings with bone marrow findings.

Thereby, this data would help in planning the diagnostic and therapeutic approach in patients with pancytopenia.

AIMS

Study of clinical profile of patients having pancytopenia.

OBJECTIVES

- To study the etiology of pancytopenia.
- To study the clinical presentation of patients having pancytopenia due to various causes.
- To correlate peripheral smear findings with bone marrow aspiration studies of the patients presenting with pancytopenia.
- To correlate hematological parameters with clinical findings in differentiating causes of pancytopenia.

REVIEW OF LITERATURE

The work of Neumann and Bizzozero established the relationship between blood and the bone marrow. In 1868, Neumann noted that bone marrow was an important organ for formation of red blood cells.[9]

Haematopoiesis involves the process in the production of all blood cells from hematopoietic cells. These processes include the self-renewal of stem cells, the commitment of most progeny of stem cells to differentiate ultimately into a particular cell type and the proliferation of progenitor cells and their differentiation to a particular kind of mature blood cell.

Hematopoiesis, the production of blood cells, a fundamental concept in hematology and aplastic anaemia, a disease due to the absence of hematopoiesis have had parallel histories since the discovery of the function of bone marrow in the mid-nineteenth century.

Various studies are available in the literature, to know the commonest causes of cytopenias and cytopenia as the manifestations of various systemic disorders.

Anatomy of Bone Marrow

The bone marrow provides a unique microenvironment for the orderly proliferation, differentiation, and release of blood cells.[10]

Under the electron microscope, the marrow cavity is a vast network of thin walled sinusoids lined by a single layer of endothelial cells under laid by a discontinuous layer of basement membrane and adventitial cells. Within the interstitium lie clusters of haemopoietic cells and fat cells. Differentiated blood cells enter sinusoids by transcellular migration through the endothelial cells. The normal marrow is organized anatomically in subtle but important ways.[10]

Normal megakaryocytes lie next to sinusoids and extend cytoplasmic processes the bud off in to the blood stream to produce platelets.[10]

Similarly, normal immature granulocytic myeloid forms are concentrated next to bone trabeculae, while mature granulocytes are located more centrally.[10]

It is estimated that the weight of the marrow in an adult is 1300 to 1500 gm. The marrow can undergo complete transformation in few days and occasionally even in few hours. The rapid transformation involving the whole organ as evidenced by the fact that a small sample represented by a biopsy or aspiration is usually fairly representative of the whole marrow.[11]

The formed elements of blood – red cells, granulocytes, monocytes, platelets and lymphocytes – have a common origin from pleuripotent haemopoietic stem cells. The pleuripotent stem cell gives rise to two types of multipotent progenitors, the common lymphoid and the common myeloid stem cells. The common lymphoid stem cell in turn gives rise to precursors of T – cells (pro-T-cells), B-cells (pro-B-cells), and natural killer cells.[10]

From the common myeloid stem cell arise at least three types of committed stem cells capable of differentiating along the erythroid / megakaryocytic, eosinophilic and granulocyte-macrophage pathways. From the various committed stem cells are derived intermediate stages and ultimately the morphologically recognizable precursors of the differentiated cells, such as proerythroblasts, myeloblasts, megakaryoblasts, monoblasts, and eosinophiloblasts, which in turn give rise to mature progeny.[10]

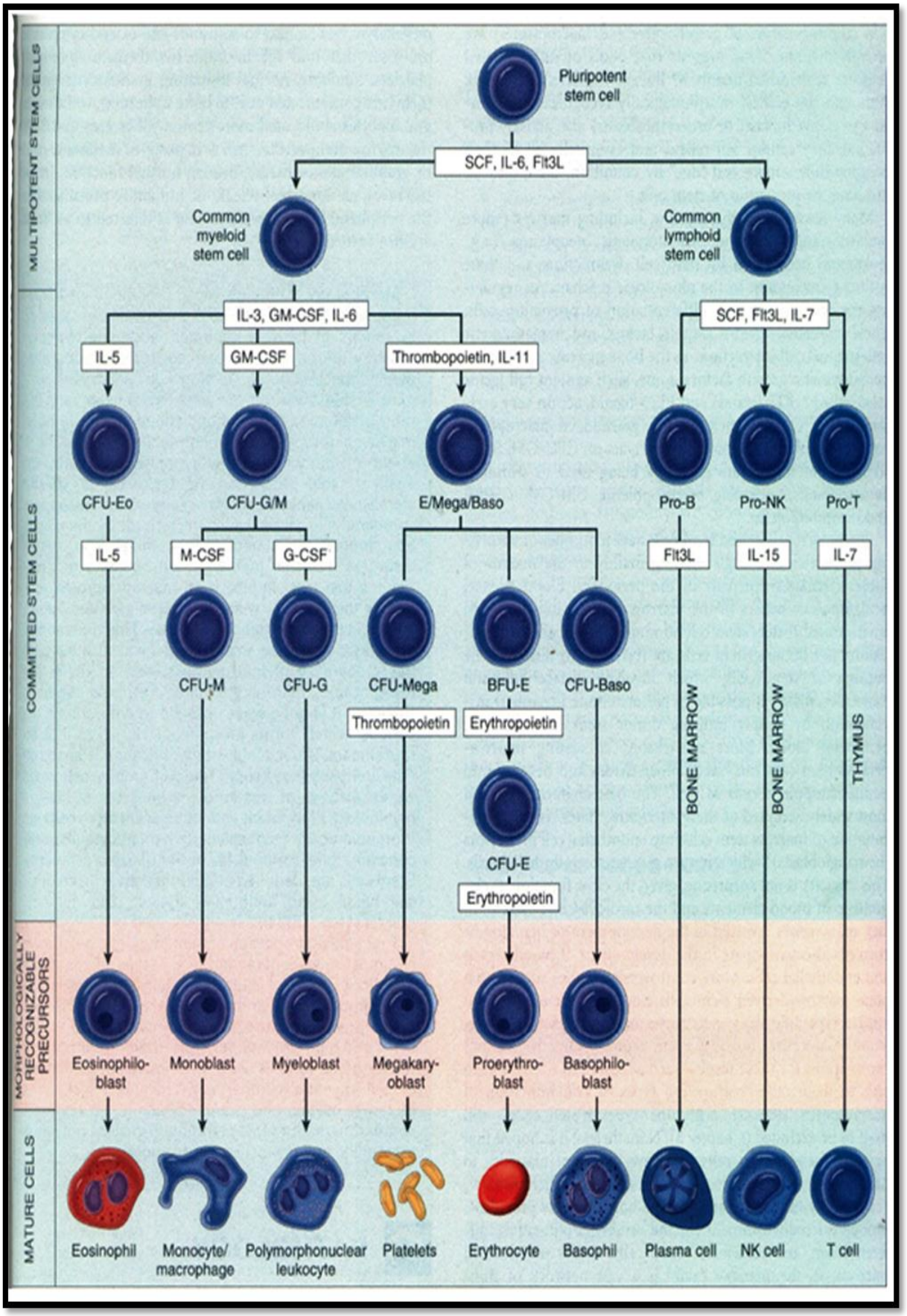


Figure 1: Origin and Differentiation of Haemopoetic Cells.

1. Regulation of Erythropoiesis[12]

Erythropoiesis, in postnatal life, takes place within the environment of the bone marrow mainly concentrated in the axial skeleton, ribs and pelvis.

In the steady state, the hematopoietic microenvironment is probably the most important controlling aspect of erythropoiesis, with local cytokine release from the bone marrow stroma and the binding of cytokines to the stromal matrix determining the rate of proliferation and differentiation.

Erythropoietin, which is produced predominantly by the kidneys in the adults, stimulates the conversion of Erythropoietin Responsive Cells (ERC) to pronormoblasts. Erythroid differentiation, at least in the later stages, seems to have relationship to macrophages, the so-called 'nurse cells'.

Within the haematopoietic sinusoids, the maturing precursor cells move towards the adventitial cells, which line the capillaries. Reticulocytes only escape into the circulation when they are sufficiently deformable to move between the adventitial cells.

The erythroid progenitor cells can be identified by the characteristics of the erythroid colonies, which generate in appropriate semi-solid media. The earliest recognisable committed progenitor for erythroid cells is the CFU-GEMM (Colony Forming Unit-Granulocytes Erythroid, Megakaryocyte and Macrophage).

The next identifiable progenitor cells are the Burst Forming Units-Erythroid (BFU-E). This BFU-E is divided into early and late compartments, depending upon the time taken to establish the bursts in vitro. Significant numbers of BFU-E is normally present in the peripheral blood.

The final progenitor cell is identified as CFU-E. The Epo-R (erythropoietin receptor) is expressed on the surfaces of all the committed erythroid cells in the progenitor compartment with relatively low numbers on CFU-GEMM and early BFU-E, increasing numbers in the intermediate and late BFU-E and maximal expression in CFU-E.

The binding of erythropoietin to Epo-R prevents apoptosis in the CFU-E pool and the cell cycle progresses into Erythron which is the morphologically recognisable erythroid cell compartment within the bone marrow consisting of pronormoblasts, early normoblast, intermediate normoblast, late normoblast and finally, with the extrusion of the nucleus, gives rise to the final stage reticulocyte.

2. Regulation of granulopoiesis and monopoiesis[13]

Granulocytes and Monocytes are derived from a common bipotential stem cell (CFU-GM) which is derived from the CFU-GEMM.

1. CFU-GEMM $\xrightarrow{\text{IL-3 GM-CSF}}$ CFU-GM (Colony forming unit Granulocyte macrophage).
2. CFU-GM $\xrightarrow[\text{G-CSF}]{\text{IL-3, GM-CSF}}$ CFU-G (Granulocytes)
3. CFU-GM $\xrightarrow[\text{M-CSF}]{\text{IL-3, GM-CSM}}$ CFU-M (Monocytes / Macrophages)
4. CFU-GEMM - CFU-Eosinophil $\xrightarrow[\text{IL-5}]{\text{IL-3, GM-CSF,}}$ (Eosinophils)
5. CFU-GEMM - CFU-Basophil $\xrightarrow{\text{IL-3}}$ (Basophils)

GM-CSF-Colony Stimulating Factor – Granulocyte, Monocyte and Macrophage. The available data suggests that macrophages play a key role in regulating the production of their own precursors as well as granulocytes. They do this by producing both Colony Stimulating Factor (CSF) as well as Prostaglandin E (PGE), 18 an inhibitor of CFU-E.

Granulocytes contain some inhibitory substances which may participate in the regulation of production of monocyte and granulocytes in-vitro by providing a negative feedback between the mass of mature granulocytes and rate of production of new cells.

3. Regulation of Megakaryopoiesis[13]

Megakaryocytes are derived from pleuripotent stem cells, the earliest recognised platelet precursor being a burst forming unit denoted by BFU-Megakaryocyte. Under the influence of thrombopoietin (TPO) and cytokines such as Interleukin-3, and Interleukin-11, the BFU-Megakaryocyte develop into megakaryocyte colony forming units (CFU-Meg).

As the CFU-Megs mature, they develop the morphological and biochemical features of megakaryoblasts and then megakaryocytes. The fully mature megakaryocyte has proliferation of the characteristic platelet granules (alpha-granules and dense bodies) and membrane glycoprotein which are vital to the platelet's function.

Thrombopoietin is able to induce the complete sequence of maturation without the addition of any other factors and may be considered the master growth factor for thrombopoiesis, as erythropoietin is for the erythron.

4. Regulation of Lymphopoiesis[13]

The lymphoid stem cell is derived from pleuripotent stem cell and gives rise to T and B lymphocytes which are morphologically identical, but immunologically and functionally diverse.

Lymphopoiesis can be divided into antigen-independent lymphopoiesis and antigen dependent lymphopoiesis.

Antigen independent lymphopoiesis occurs in the primary lymphoid tissue (bone marrow, thymus, foetal liver, yolk sac). This type of lymphopoiesis begins with the committed lymphoid stem cell and results in the

formation of immunocompetent T and B lymphocytes (virgin lymphocytes).

Antigen dependent lymphopoiesis occurs in secondary lymphoid organs (adult bone marrow, spleen, lymph nodes, gut associated lymphoid tissue), and it begins with the antigenic stimulation of the immunocompetent T and B lymphocytes. This type of lymphopoiesis results in the formation of effector T and B lymphocytes which mediate immunity through the production of lymphokines by T-lymphocytes and antibodies by B-lymphocytes.

Origin and Nature of Stromal Cells

A common mesenchymal precursor cell gives rise to endothelial, fibroblastic and adipogenic marrow stromal cells. This multipotential precursor cell also generates osteoblasts and chondroblasts for bone and cartilage formation.[14]

In adult life, stromal cell and haemopoietic cell development in normal narrow is from different committed precursor cells. The only normal stromal components to be derived from the multipotential haemopoietic precursors are resident tissue macrophages. However, during embryonic development, a precursor cell can be identified which has the capacity to differentiate along either haemopoietic or angiogenic pathways. This cell, the haemangioblast is important in morphogenesis of embryonic vasculature as well as in haemopoiesis. It disappears as definitive haemopoiesis moves from structures, associated with the yolk sac to the liver; at this time, haemopoietic stem cells of adult type become predominant.[14]

New evidence about the extreme plasticity of stem cells isolated from several organs suggests that these functions are strongly influenced by local environmental controls rather than by irreversible loss of capacity to generate daughter cells of many divergent types.[14]

These findings seem likely to lead to development of new therapeutic strategies for stem cell transplantation (with or without manipulation in vitro) or induction in vivo desired cell functions.[14]

Cellularity of the Marrow

The marrow cellularity is expressed as the ratio of the volume of haemopoietic cells to the total volume of the marrow space (cells plus fat and other stromal elements).[11]

Cellularity varies with the age of the subject and the site.[32]

Marrow cellularity is best judged by histological sections of biopsy or aspirated particles but should also be estimated from particles that are present in marrow films.[11]

The most reliable assessment of overall haemopoietic cellularity is based on the biopsy specimen. A visual estimation of the percentage of marrow space occupied by haemopoietic elements plus stroma is the typical parameter used to assess cellularity. Erythroid cellularity can be estimated visually by looking for erythroid aggregates which are clusters of darkly staining cells scattered throughout the marrow cavity, adequacy of megakaryocyte number also fairly readily evident at low power by the frequency of these large multilobulated cells. Nuclear chromatin pattern and cytoplasmic granulations are easily visualized. The eosinophilic granulocytes show brilliant large red granules. The specific granules of maturing neutrophilic granulocytes are easily seen and the granulocytic series is identifiable at all developmental stages.[15]

The myeloid/erythroid ratio is the ratio of total granulocytes to total normoblasts. In newborns and infancy, it is somewhat higher than in later childhood or adult life. In adults, the range is broad, varying from about 1.2:1 to 4:1.[11]

The numbers of megakaryocytes is estimated more reliably in sections than in marrow films.[11]

Evaluation of the Biopsy Speciments

In good histological preparations, the cell distribution and maturation abnormalities can be quite reliably determined. In addition to more reliable detection of the presence of lymphomas or metastatic tumor, the histological pattern can often be diagnostic of the type of neoplasm. In some conditions, such as myelofibrosis and hairy cell leukemia the bone marrow cannot be aspirated and biopsy is necessary to establish the diagnosis.[11]

Trephine biopsy sections can be used for immunohistochemistry, in situ hybridization, and PCR (polymerase chain reaction) in detection of various haematological malignancies and metastatic deposits.[14]

Table 1: Hematology reference values in normal adults.[16]

Test	Men	Women
Hemoglobin	12-18 g/dl	11-16 g/dl
Hematocrit	41.5-50.4%	36-45%
Red cell count	4.5-5.9 x 10^6/µl	4.5-5.1 x 10^6/µl
White cell count	4.4-11.3x10^3/µl	4.4-11.3x10^3/µl
MCV	80-96 fl	80-96 fl
MCH	27.5-33.2 pg	27.5-33.2 pg
MCHC	33.4-35.5 g/dl	33.4-35.5 g/dl
Platelet count	150-450x10^3/µl	150-450x10^3/µl
Reticulocyte count	0.5-2.5%	0.5-2.5%
ESR	0-15 mm/hr	0-20 mm/hr
RDW	11.6-14.6%	11.6-14.6%

Table 2: Biochemichal reference values in normal adults.

Test	Value
RBS	Up to 140.00 mg/dl
SGPT	Up to 34.00 U/L
SGOT	Up to 31.00 U/L
S. Bilirubin Total Direct Indirect	 0.20-1.00 mg/dl Up to 0.20 mg/dl 0.20-0.80 mg/dl
S. Urea	15.00-40.00 mg/dl
S. Creatinine	0.50-1.00 mg/dl
LDH	180-360 IU/L
S. Na+	136-145 mEq/L
S. K+	3.5-5.0 mEq/L

Table 3: Differential counts of bone marrow aspirate.[16]

	Observed Range (%)	Mean (%)
• **Neutrophilic Series (Total)**	49.2 – 65	53.6
Myeloblasts	0.2 – 1.5	0.9
Promyelocyte	2.1 – 4.1	3.3
Mylocyte	8.2 – 15.7	12.7
Metamyelocyte	9.6– 24.6	15.9
Band	9.5 – 15.3	12.4
Segmented	6.0 – 12.0	7.4
• **Eosinophilic Series (Total)**	1.2 – 5.3	3.1
Myelocyte	0.2 – 1.3	0.8
Metamyelocyte	0.4 – 2.2	1.2
Band	0.2 – 2.4	0.9
Segmented	0 – 1.3	0.5
• **Basophilic and Mast Cells**	0 – 0.2	< 0.1
• **Erythrocytic Series (Total)**	18.4 – 33.8	25.6
Pronormoblast	0.2 – 1.3	0.6
Basophilic	0.5 – 2.4	1.4
Polyghromatophilic	17.9 – 29.2	21.6
Orthrochromatic	0.4 – 4.6	2.0
• Lymphocyte	11.1 – 23.2	16.2
• Plasma cells	0.4 – 3.9	1.3
• Monocyte	0 – 0.8	0.3
• Megakaryocyte	0 – 0.4	<0.1
• Reticulum cells	0 – 0.9	0.3
• Myeloid to erythroid ratio	1.5 – 3.3	2.3

Clinical Features of Pancytopenia

The onset of the disease is insidious; manifestations depend on severity of anaemia, leucopenia, and thrombocytopenia.[17]

Initial presenting symptoms include mild progressive weakness and fatigue attributable to anaemia.

Also patients are predisposed to various infections because of neutropenia.

Haemorrhage from skin, nose, and gums is due to thrombocytopenia.

Physical examination reveals fever, pallor, purpura and echymotic patches over the skin, mucous membranes, and conjunctiva.[17]

Presence of splenomegaly and lymphadenopathy calls attention to the possibility of leukaemia, lymphomas, myelofibrosis and storage diseases.

On the other hand, lack of these signs and absence of evidence of vitamin B12 or folate deficiency should suggest multiple myeloma or aplastic anaemia.

Finally rare presentations include diarrhoea, jaundice and weight loss.[18]

Causes of Pancytopenia

A wide range of disorders result in pancytopenia. Etiological factors have been divided into seven different groups.[19]

(A) Aplastic Anaemia

1) Familial

a) Fanconi constitutional pancytopenia
b) Shwachman – Diamond syndrome (pancreatic deficiency in children)
c) Putative hereditary defect in cellular uptake of folate.

2) Acquired

a) Agents that regularly produce marrow hypoplasia and aplasia if a sufficient dose is given.

- Benzene
- Ionizing Radiation
- Sulphur Or Nitrogen Mustard
- Anti - metabolites
- Certain Antibiotics
- Other Toxic Agent

b) Agents occasionally associated with hypoplasia or aplasia of marrow.

- Antimicrobial agents
- Anticonvulsants
- Antithyroid drugs
- Analgesics
- Sedatives and Tranquilizers

c) Viral infections

- Hepatitis
- Epstein – Barr virus
- HIV (human immunodeficiency virus),
- Dengue

d) Mycobacterial infections
e) Miscellaneous causes- pregnancy, Simmond disease, and sclerosis of the thyroid.
f) Idiopathic

B) Disorders Infiltrating the Bone Marrow
- Aleukemic leukaemia
- Multiple myeloma
- Metastatic carcinoma
- Myelofibrosis
- Myelosclerosis
- Marble bone disease
- Osteopetrosis

C) Disorders Involving the Spleen
- Congestive splenomegaly
- Lymphomas – Hodgkins and Non Hodgkins
- Infiltrative disorder – Gaucher's disease, Niemann Pick's disease
- Infectious diseases – Kala-azar, Miliary tuberculosis, Syphilis

D) Vitamin B12 or Folate Deficiency
- Pernicious anaemia
- Sprue

E) Disseminated Lupus Erythematosus
F) Paroxysmal Nocturnal Haemoglobinuria

G) Miscellaneus Disorders (with Cellular Marrow)
- Over whelming infection
- Mycobacterial infection
- Brucellosis
- Sarcoidosis
- Some refractory anaemias
- Pregnancy (some cases)
- Sideroblastic anaemia (rarely)

Etiology of Pancytopenia[20,21]

I. Pancytopenia with hypocellular Bone marrow
- Acquired aplastic anaemia
- Inherited aplastic anaemia (Fanconi's anaemia and others)
- Some myelodysplasia syndromes
- Rare aleukemic leukaemia (acute myelogenous leukaemia)
- Some acute lymphoblastic leukaemias.
- Some Lymphomas of bone marrow.

II. Pancytopenia with cellular Bone marrow

A. Primary bone marrow diseases
- Myelodysplastic syndromes
- Paroxysmal Nocturnal Haemoglobinuria
- Myelofibrosis
- Some aleukemic leukaemias
- Myelophthisis
- Bone marrow lymphoma
- Hairy cell leukaemia.

B. Secondary to systemic diseases
- Systemic lupus Erythematosus
- Sjogren's syndrome
- Hypersplenism
- B12 and folate deficiency (familial defect)
- Over whelming infection
- Alcohol
- Brucellosis
- Sarcoidosis
- Tuberculosis and atypical mycobacteria

III. Hypocellular Bone marrow ± Cytopenia
- Q fever
- Legionnaire's disease
- Mycobacteria
- Tuberculosis
- Anorexia nervosa, starvation
- Hypothyroidism

Aplastic Anemia[21]

Haematopoiesis, a fundamental concept in haematology and Aplastic anaemia, a disease due to the absence of haematopoiesis, has had parallel histories since the discovery of the function of bone marrow in the mid-19th century.

Neumann and Bizzozero (1868) observed nucleated erythroid cells in the marrow and concluded that it was the site of continuously proliferating blood cells.

Paul Ehrlich (1888) correlated the absence of formed elements in the blood in pregnant women, to severe marrow hypoplasia at autopsy. The disease was named by Vaquez and Aubertin in 1904 "Pernicious anemia with yellow marrow" and emphasized its pathophysiology of failed hematopoiesis which they called anhematopoiesis. Cabot stressed the marrow's distinctive pathology and the need for its examination in the diagnosis. Santesson (1897) recognised toxic substances such as Benzol as a cause of aplastic anemia.

Smith (1919) reported pancytopenia as well-defined clinical entity associated with aplastic anemia. Rhoades and Miller (1938) showed that marrow in cases of aplastic anaemia vary in cellularity from aplasia to hypoplasia. Organic Arsenicals, gold compounds and radioactive compounds were reported to cause aplastic anaemia.

Numerous substances added to the list include sulphonamides (Meyer and Perlmutter 1942), Mepacrine (Custer 1946, Parmer 1948), Streptomycin (Corelli 1947) and Tridone.[21]

Adams E.B. (1951) reported pancytopenia associated with idiopathic aplastic anaemia in 27 cases. He also reported pancytopenia with aleukemic leukaemia in 3 patients.[22]

Daniel et al (1958), in their analysis of 50 cases of aplastic anaemia, reported 43 cases of idiopathic aplastic anaemia. Remaining 7 cases were attributed to Benzol, phenylbutazone, chloramphenicol and arsenic fruit spray.[23]

Retief HP. and Haynes A.D. (1971-1975)[24] reported 195 patients with pancytopenia. Classic aplastic anaemia was found in 22 patients, with no apparent etiology in 16, previous phenylbutazone ingestion in 2 and Fanconi's anaemia in 4 patients. Aplastic anaemia associated with various drugs has been described which include OKT3 Ibuprofen and Ciprofloxacin.[25]

International Agranulocytosis and Aplastic Anemia study (1986) confirmed the risk of Aplastic anemia with phenylbutazone use and identified even higher probabilities with other NSAIDs.[21]

Lorenz et al (1955) from Australia first described association of aplastic anaemia with viral hepatitis. Bierman HR and Nelson ER (1965) have reported Dengue type viral infections in association with hypoplastic marrow.[26]

Puedssi et al (1977)[27] reported four cases of aplastic anaemia associated with sub massive hepatic necrosis. These patients were HbsAg negative and the exact cause could not be determined. Dennis et al (1978)[28] reported the association of aplastic anaemia with type B viral hepatitis. These patients were positive for HbsAg. Non A Non B hepatitis associated with aplastic anaemia was reported by Jerome et al (1979).[20]

Rafel M. et al (1998)[29] found transient pancytopenia after Non A, Non B and Non C acute hepatitis preceding ALL.

Pancytopenia associated with infectious mononucleosis was reported by Kenneth et al.(1981),[30] which was due to suppression of haematopoiesis by activated T-cells.

Young N and Mortimer P. (1984)[31] reported Parvovirus associated with aplastic anaemia. Osaki et al (1999)[32] have reported severe aplastic anaemia with human parvovirus B19 infection in a patient without underlying disorder. Yarali N. et al (2000)[33] have described parvovirus B19 infection reminiscent of Myelodysplastic Syndrome in three children with chronic haemolytic anemia who presented with pancytopenia.

Pancytopenia has been reported following infection with Human Immunodeficiency Virus (HIV). Marrow hypocellularity has been a common finding while aplastic anaemia has been described rarely.

Aplastic anaemia is strongly associated with rare collagen vascular syndrome called eosinophilic fasciitis. Pancytopenia with marrow hypoplasia can also occur in Systemic Lupus Erythematosus (SLE). This may be due to folate deficiency secondary to haemolysis, infections and treatment with drugs and autoimmunity.

Pereira et al (1998)[34] have noticed global hypocellularity (47.6%), increased reticulin production (76.2%) and necrosis (19%) in 21 bone marrow specimens from 21 patients with SLE. They concluded that bone marrow might be a target organ in SLE with cytopenias.

Paroxysmal nocturnal hemoglobinuria is a clonal disorder arising from a somatic mutation in the haematopoietic stem cell. Erythrocytes, white cells and platelets are affected by the mutation, which renders their membranes highly susceptible to lysis by complement. This abnormal sensitivity is predominantly due to a deficiency in complement regulatory membrane proteins such as Decay Accelerating Factor (DAF) and CD59 which are covalently attached to the cell membrane by a glycosyl-phosphatidyl inositol anchor (GPI). But the molecular mechanism of the abnormal haemolysis is now being rapidly clarified, and the lack of GPI-anchored membrane proteins has been shown to have diagnostic value in PNH.[6]

Two other syndromes associated with aplastic anaemia are Dyskeratosis Congenita characterised by aplastic anaemia, reticulated hyperpigmentation, nail dystrophy and mucosal leukoplakia described by Steier N. et al (1972)[36] and Schwachmann-Diamond syndrome associated with pancreatic insufficiency, pancytopenia and hypoplastic marrow described by Schwachmann H et al (1964).

Patients with infection associated haemophagocytic syndrome (IAHS) have fever, severe constitutional symptoms and blood cytopenias (usually pancytopenia).

A viral etiology has been demonstrated in many cases but other infections may occasionally cause similar changes. Pancytopenia due to haemophagocytic syndrome as the presenting manifestation of tuberculosis was described by Basu S et al (2000)[37]

Haemophagocytic syndrome presenting with pancytopenia as a complication of visceral leishmaniasis was described by Gagnaire M et al (2000)[38] and in Typhoid fever reported by Udden MM et al (1986)[39] and Sood R.et al (1997)[40]

Fatal pancytopenia in falciparum malaria was reported by Arya TV and Prasad RN.[41] Yamakawa H et al (1989)[42] have reported a case of plasmodium vivax malaria complicated with pancytopenia due to hypoplasia of the bone marrow. Plasmodium vivax causing pancytopenia after allogenic blood stem transplantation in a patient with chronic myeloid leukaemia was reported by Raina V.et al (1998).[15]

Classification of Aplastic anaemia

I. Acquired

A) Idiopathic

B) Secondary

a) Irradiation

b) Drugs and Chemicals

- Regular effects

Cytotoxic agents/ benzene
- Idiosyncratic reactions
Chloramphenicol/NSAIDs/Antiepileptic's/gold/other drugs and chemicals
c) Viruses
- Epstein Barr Virus
- Hepatitis (Non A, Non B, Non C hepatitis)
- Parvovirus B19
- HIV (AIDS)
d) Immune disorders
- Eosinophilic fascitis
- Hypoimmunoglobulinemia
- Thymoma / Thymic carcinoma
- Graft versus host disease in immunodeficiency
e) Paroxysmal nocturnal haemoglobinuria
f) Pregnancy

II. Inherited
- Fanconi's anaemia
- Dyskeratosis Congenita
- Schwachman-Diamond Syndrome
- Reticular dysgenesis
- Amegakaryocytic thrombocytopenia
- Familial aplastic anaemia
- Preleukaemia (Monosomy 7)
- Non-hematologic syndromes

Pathophysiology of aplastic anaemia[13]
Aplastic anemia, the paradigm of bone marrow failure syndromes is most simply defined as peripheral blood pancytopenia and a hypocellular marrow.

The etiologic hypothesis of aplastic anaemia has been referred to as the seed, soil, worm and fertiliser hypothesis. For a viable 'seed' (haematopoietic stem cell) to grow, it must be planted in good 'soil' (microenvironment of bone marrow) conducive to growth, protected from antagonists 'worms'(cellular or humoral immunosuppression of haematopoiesis) and nourished with 'fertilisers'(growth factors).

Some of the proposed causes of aplastic anaemia include.[43]
(1) Abnormalities of the haematopoietic stem cells
(2) Abnormal haematopoietic microenvironment
(3) Immune Mechanisms
(i) Decreases in haematopoietic factors produced by monocytes and lymphocytes
(ii) Damage by cytokines that suppress haematopoiesis
(iii) Suppression of haematopoiesis by cytotoxic T-cells (Killer T cells):
(iv) Suppression of haematopoiesis by natural killer (NK) cells
(v) Drugs and chemicals
(vi) Radiation
(vii) Viral infection
(viii) Paroxysmal nocturnal hemoglobinuria

The diagnosis of aplastic anaemia requires at least two of the following in addition to a hypocellular marrow.

(i) Haemoglobin < 9 g/dl
(ii) Platelet count < 100 x 10^9/L
(iii) Neutrophil count < 1.5 x 10^9 / L

The pathogenesis of aplastic anaemia remains unclear, but an autoimmune mechanism appears to be important. There may also be an as yet unidentified underlying genetic predisposition.

There is some association of HLA DR2, specially the DR15 split, with acquired aplastic anaemia.[44]

There is evidence of both quantitative and qualitative stem cell defect in aplastic anaemia and increased apoptosis of remaining early haemopoietic progenitor cells.[44] Not only do cytotoxic suppressor T lymphocyte release cytokines, such as interferon-α and tumor necrosis factor α (TNF-α), that are inhibitory to haemopoietic progenitor cells but TNF-α also upregulates Fas antigen expression on CD34+ cells, which may be one of the possible mechanisms for the reduced survival of aplastic anaemia marrow progenitor cells.[44]

Telomeres are complex structures located at the end of eukaryotic chromosomes and they have a role in preventing aberrant recombination at the chromosome ends. It is at least theoretically plausible that the accelerated terminal restriction fragment (TRF) loss in aplastic anaemia provides the background for the increased risk of transformation to MDS or acute leukaemia with cytogenetic anomalies. Also patients with acquired aplastic anaemia show increased TRF loss in leucocytes compared with age-matched control subjects, and the extent of the loss correlates with the duration of disease.[44]

Fanconi Anaemia
It is typified by pancytopenia and congenital defects in cutaneous, musculoskeletal and urogenital systems.[45]

In vitro, the cells of patients with Fanconi anaemia grow slowly and resist cell division, accumulating in G2. The haemopoietic defect in Fanconi anaemia is evident at the progenitor cell level. Colonies from bone marrow (CFU-GM, CFU-E, BFU-E) and blood (BFU-E) were all decreased in patients with Fanconi anaemia.[45]

Pancytopenia may be precipitated by chemical exposure or viral infection and whether measured cytogenetically or by cell proliferation, Fanconi anaemia cells in culture are extraordinarily sensitive to a wide range of physical and chemical agents that can damage DNA.[45]

Six of Fanconi anaemia genes have now been cloned; FANCA gene is most commonly mutated approximately in 70% of patients.[45]

Dyskeratosis Congenita

In the inherited disorder dyskeratosis congenita, in which aplastic anaemia usually develops in the second or third decade, the underlying genetic defect affect the telomerase complex, which has both RNA and protein components.[45]

In the X-linked form of the disease there are mutations in the gene DKCI, which codes for the protein dyskerin. In the autosomal dominant form there is a mutation that leads to a large deletion in telomerase RNA.[45] Stem cells from patients with both types have markedly short telomeres.

Ionizing Radiation

Bone marrow cells are affected by both high energy gamma rays as well as by adsorbed low energy alpha particles.

Acute radiation exposure in large doses leads to dose-related depressed marrow function. Bone marrow hypoplasia is observed at total body exposures between 1.0 and 2.5 Gy.[46]

Chronic radiation-induced aplasia also is dose dependent and radiation exposures greater than 4.4 Gy are associated with the development of aplasia.[46]

Large macromolecules such as DNA can be damaged directly by large amounts of radiant energy, which can rupture covalent bonds or indirectly by interaction with highly charged and reactive small molecule resulting from ionization of free radicals formed in solution.[46]

Acute radium poisoning, such as that affecting radium dial workers, is accompanied by striking changes in the blood. A high incidence of leukaemia and cancer has been observed in people exposed to ionizing radiation, and aplastic anaemia has been reported in a few survivors.[46]

The type and intensity of the radiation source and the distance and shielding of the subject are the major determinants of radiation injury.[46]

Drugs

Aplastic anaemia can be due to chemically diverse group of drugs.

Chloramphenicol is the most notorious drug documented to cause aplastic anaemia.[45] It is a prime example of drug that causes both dose related marrow suppression and idiosyncratic aplastic anaemia. In vitro, chloramphenicol inhibits the growth of both CFU-GM and CFU-E and also may inhibit the haemopoietic micro environment.[47]

Amphotericin B induced myelosuppression is mediated via release of TNF and INF.[48] In most cases; however, drug-related aplasia is idiosyncratic and occurs unpredictably in a minority of individuals.[46]

A genetic predisposition has been suggested for some cases of idiosyncratic drug induced aplasia.[46]

Chemicals

Benzene is a dangerous environmental contaminant found in organic solvents, coal tar derivatives, and petroleum products.[46]

It is concentrated in bone marrow fat, forms water-soluble intermediates, and damages DNA. It decreases the numbers of progenitors and damages stroma as well.

The risk of cytopenia is probably related to cumulative exposure.[46]

Other aromatic hydrocarbons found in insecticides and herbicides inhibit in vitro haemopoietic colony formation.[46]

Viruses

Hepatitis associated aplastic anaemia, causes bone marrow depression, when the patient is recovering. It is associated with diminished immune responsiveness, including decreased T cell number and function and lower serum immunoglobulins as compared with patients with idiopathic disease.[47]

Cytomegalo virus infect marrow stromal cells in vitro and inhibit their ability to produce growth factors. They also directly infect progenitor cells.[47]

Haematological changes are common in patients with AIDS. It can be nonspecific or specific bone marrow abnormalities, and one of them is bone marrow hypocellularity.[49]

Viral mediated alteration of the bone marrow microenvironment is the most likely mechanism responsible for haemopoietic suppression in AIDS patient. Both diminished production of factors that stimulate haemopoiesis and increased production of factors that inhibit haemopoiesis appear responsible for marrow suppression.[49]

Direct infection of either stem cell or progenitor cells does not have significant role in marrow failure in AIDS patient.[49]

Paroxysmal Nocturnal Haemoglobinuria

Aplastic anaemia and PNH are closely related syndromes.[50]

Haemopoietic progenitor number is severely decreased in patients with cytopenias and PNH, even when the marrow is cellular.[50]

The origin of the sensitive clone is probably an acquired genetic defect in a single enzyme system, responsible for the attachment of phosphor-inositol linked proteins to the cell surface; presumably, failure to normally express some cell surface proteins related to growth and proliferation leads to aplastic anaemia.[50]

Pregnancy

Bone marrow hypoplasia may be relatively common during pregnancy.

Oestrogens are related to aplasia of pregnancy and are suggested by the effect of large doses of these hormones on haemopoiesis in animals.[50]

Laboratory Features of Aplastic Anaemia

Blood findings

Patients with aplastic anaemia have varying degrees of pancytopenia.

Severe cases are characterized by low reticulocyte count. The anaemia may be normocytic or macrocytic and poikilocytes may be present. Platelets are of normal size. Thrombocytopenia usually develops initially, with subsequent onset of granulocytopenia and then anaemia.[51]

Plasma Findings

Plasma contains high levels of haemopoietic growth factors, including erythropoietin, thrombopoietin, and myeloid colony stimulating factors.[51]

Serum iron values are usually high.[51]

Marrow Findings

Morphology

The bone marrow may be difficult to aspirate with the result being a dry tap. In majority of patients a hypocellular aspirate is obtained with the fragments being composed largely of fat and the cell trials also being hypocellular. M:E ratio may be increased, normal or decreased. Dyserythropoiesis may be seen. Dysplastic changes in granulocytes are less common, megakaryocytes are often infrequent in the aspirate.[51]

Lymphocytes, plasma cells, macrophages, and mast cells may be prominent, reflecting a lack of other cells rather than an increase in these elements.[51]

Marrow biopsy is essential to confirm the overall hypocellularity because a poor yield of cells occasionally is obtained from marrow aspirate from patients with other disorders, especially if fibrosis is present.[51]

Marrow in trephine biopsy is usually hypocellular with marked reduction of haemopoietic cells. They are mainly replaced by fat but there is variable infiltrate composed of lymphocytes, plasma cells, macrophages, mast cells and sometimes eosinophils.[51]

Walls of the sinusoids may be disrupted and there may be edema and haemorrhage. Residual erythroid cells show dysplastic features. Iron stores are increased. In severe aplastic anaemia, as defined by the international aplastic anaemia study group, less than 25 percent cellularity or less than 50 percent cellularity with less than 30 percent haemopoietic cells is seen in the marrow.[51]

Progenitor Cell Growth

In vitro CFU-GM and BFU-E colony assays reveal a marked reduction in progenitor cells.[51]

Improvement in colony growth after incubation with anti T cell monoclonal antibodies may predict improvement after immunosuppressive therapy; however, this has not been a universal finding.[51]

Cytogenetic Studies

Cytogenetic analysis may be difficult because of low cellularity; thus, multiple aspirates may be required to provide sufficient cells for study. The results of analysis are normal in aplastic anaemia.[51]

Clonal cytogenetic abnormalities in otherwise apparent aplastic anaemia are indicative of an underlying hypoproliferative clonal myeloid disease.[51]

Image Studies

MRI (Magnetic resonance imaging) can be used to distinguish between marrow fat and haemopoietic cells. This may be a more useful overall estimate of marrow haemopoietic cell density than morphologic technique and may help to differentiate hypoplastic myelogenous leukemia from aplastic anaemia.[51]

Table 4: Diesease sevearity of aplastic anemia.

Severe AA	BM cellularity <25% or 25-50% with <30% residual haemopoietic cells. Two out of three of the following Neutrophils <0.5 x 10^9/L Platelets <20 x 10^9/L Reticulocytes <20 x 10^9/L
Very severe AA	As for severe AA but neutrophils <0.2 x 10^9/L.
Non severe AA	Patients not fulfilling the criteria for severe or very severe AA with a hypocellular marrow, with two out of three of the following; Neutrophils <1.5 x 10^9/L, platelets < 100 x 10^9/L, haemoglobin <10g/dL.

TEST	RATIONALE
Complete blood cell count and differential	Define severity of aplasia
Morphology	Define abnormal cells (eg: blasts or storage cells) vit B12 deficiency
Reticulocyte count	Define severity. Differentiate production V/S destruction
Bone marrow biopsy	To assess cellularity To assess architecture (eg – granuloma, fibrosis, haemophagocytosis, and infiltrative or metastatic disease)
Bone marrow aspirate	
Morphology	Malignant V/S benign disease Storage disease Haemophagocytosis Congenital disorder
Cytogenetics	Myelodysplasia
Culture	Infectious agents (eg: tuberculosis, or virus)
Other	DNA antigen – based viral tests
Peripheral blood studies-aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, bilirubin	To evaluate for hepatitis
Blood urea nitrogen, creatinine	To assess for chronic renal failure.
Serologic Testing	To evaluate for HIV, Epstein-Barr virus, Hepatitis, other virus
Ham test / fluorescence – activated cell sorter	To evaluate for PNH
Chromosomal breakage studies (diepoxybutane / mitomycin C)	To assess for Fanconi anaemia
Autoimmune disease evaluation	Evidence of collagen vascular disease
Histocompatibility testing	To establish potential transplant donor pool.

Myelodysplastic Syndrome (MDS)

The myelodysplastic syndromes are a group of clonal haemopoietic stem cell disorders characterized by dysplasia and ineffective haemopoiesis leading to cytopenias affecting one or more of the cell lineages.[14]

Most cases undergo clonal evolution and transformation to acute myeloid leukaemia.[14]

In low grade myelodysplasia (refractory anaemia and refractory anaemia with ringed sideroblasts) the bone marrow usually shows varying degree of hyperplasia with dyserythropoiesis. The granulocytic precursors and megakaryocytes do not usually have morphological evidence of dysplasia.[14]

These findings are relatively non-specific and can be seen in a variety of non-neoplastic conditions such as vitamin B12 and folate deficiency or as a result of chemotherapeutic agents. Thereby it is crucial to interpret the morphological feature in the light of all available clinical and haematological information.[14]

In high grade myelodysplasia, dyserythropoiesis is manifested principally by alteration in the nucleus including budding, internuclear bridging, karyorrhexis, multinuclearity, and megaloblastoid changes; cytoplasmic features including ring sideroblasts, vacuolization, and PAS (periodic acid Schiff) positivity, either diffuse or granular.[52]

Dysgranulopoiesis is characterized by small size, nuclear hypolobation (pseudo Pelger-Huet), and hypersegmentation, hypogranularity and pseudo Chediak- Higashi granules.[52]

Megakaryocyte dysplasia is characterized by hypolobulated micromegakaryocyte, nonlobulated nuclei in megakaryocytes of all sizes, and multiple, widely separated nuclei.[52]

Trephine biopsies are more useful, the presence of small clusters or aggregates of myeloblasts and promyelocytes (5-8 cells) in marrow biopsies localized in the central portion of the marrow away from the vascular structures and endostial surface of the bone trabeculae in MDS is referred to as ALIP.[52]

Abnormal localization of immature precursors (ALIP) is a reliable diagnostic feature of Myelodysplastic syndrome.

The presence of three or more foci in a section is considered as ALIP positive, and is frequently present in cases of RAEB and also indicates rapid evolution to acute leukaemia.[52]

Leukemia

A case of severe aplastic anaemia was reported preceding acute lymphoblastic leukaemia.[53]

In a study of 29 patients, who presented with bone marrow failure involving all cell lines and who had morphologic findings diagnostic of acute leukaemia, on bone marrow examination, marrow was hypocellular.[54]

Subleukemic Leukemia

The total white cell count in acute leukaemia ranges between subnormal to markedly elevated values. In about 25% of patients the total white cell count at the onset is reduced ranging between 1-4 x 10^3/L.

In subleukaemic patients blast cells may be present in very small numbers in peripheral blood. Buffy coat smear will help in detecting blasts under these circumstances.[55]

Peripheral smear shows anaemia with moderate anisopoikilocytosis.

Neutrophils show hypogranulation and Pelger – Huet like anomaly. Immature white and red cells are absent or present only in small numbers at onset, but appear in the course of the illness. Blast cells predominate.[55] Bone marrow examination provides the diagnosis.[55]

Megaloblastic Anaemia

The commonest cause of pancytopenia, reported from various studies throughout the world has been Aplastic anaemia.[56] This is in sharp contrast to Indian studies, which revealed Megaloblastic anaemia to be the commonest cause.

In a study of 65 pancytopenic patients, Megaloblastic anaemia was detected in 25.4% cases. In another study of 191 pancytopenic cases Megaloblastic anaemia was detected in 39% of cases.[57]

In a study of 50 cases of pancytopenia, bone marrow examination revealed megaloblastic anaemia to be commonest cause, aplastic/hypoplastic anaemia constituted rest of cases.[58]

A study of 77 cases of pancytopenia showed, most common cause of pancytopenia is megaloblastic anaemia. It also revealed few interesting and rare causes of pancytopenia like drug induced agranulocytosis, Waldenstrom's macroglobulinemia.[59]

The megaloblastic anaemia is a group of disorders characterized by the presence of distinctive morphological appearances, of the developing red cells in the bone marrow. The cause is usually deficiency of either vitamin B12 (Cobalamin) or Folate, but megaloblastic anaemia may arise because of abnormal metabolism of these vitamins or because of failure in DNA synthesis not related to Cobalamin or Folate.[60]

The retarded DNA synthesis results in unbalanced cell growth. The RNA synthesis remains unimpaired, while cell division is restricted. As a result, cytoplasmic contents, especially haemoglobin, are synthesized in excessive amounts during the delay between cell divisions. An enlarged cell is the end product of such a process.[61]

The anaemia is macrocytic with an elevated MCV and is characterized by macro-ovalocytes and often extreme degrees of anisopoikilocytosis.[50]

Basophilic stippling, multiple Howell jolly bodies, nucleated red cells and even megaloblasts may be seen. Leukopenia is present. Granulocytes have increased number of lobes. Thrombocytopenia is usually encountered and, on rare occasions, is sufficiently severe to be responsible for bleeding.[61]

The bone marrow is hypercellular. The myeloid:erythroid ratio is normal or reduced and there is accumulation of primitive cells due to selective death of more mature forms. Erythropoiesis is characterized by the presence of megaloblasts. It is ineffective so that early erythroid cells are over-represented in comparison with mature cells; macrophages are increased.[60]

Granulopoiesis is also hyperplastic, giant metamyelocytes are usually present. Myelocytes and promyelocytes are also increased in size. Megakaryocyte may be normal or decreased and are hypersegmented.[60]

Serum Cobalamin and red cell Folate assay provides additional evidence for firm diagnosis and allows identification of specific vitamin deficiency.[60]

Multiple Myeloma

It is a bone marrow based, multifocal plasma cell neoplasm characterized by a serum monoclonal protein and skeletal destruction with osteolytic lesions, pathological fractures, bone pain, hypercalcemia and anaemia.[62]

The myeloma cells may be morphologically fairly normal or may be moderately or severely dysplastic, common cytological features include marked pleomorphism, increased size of cells, a high nucleo-cytoplasmic ratio, multinuclearity, nuclear lobulation, uniform cytoplasmic basophilia without a distinct golgi zone, presence of mitotic figures and cytoplasmic and nuclear inclusions.[62]

The cytoplasm of myeloma cells contain abundant endocytoplasmic reticulum, condensed or crystallized cytoplasmic immunoglobulin producing a variety of morphologically distinctive findings, including, multiple pale bluish – white grape like accumulation (Mott cells, Morula cells), cherry red refractive round bodies (Russell bodies), vermilion staining glycogen rich IgA (Flame cells) and crystalline rods.[62]

Peripheral smear in majority of patients show anaemia, which is either normocytic, normochromic or less often, macrocytic. There is increased rouleaux formation and increased background basophilic staining due to the presence in the blood of the paraprotein.[62]

The blood film is occasionally leuko-erythroblastic and it is often possible to find a small number of plasma cells or plasmacytoid lymphocytes.[62]

On biopsy it is characterized by excess of marrow plasma cells, seen in large foci, nodules or sheets. In general when 30% of the marrow volume is comprised of

plasma cells, a diagnosis of plasma cell myeloma is considered. In histological sections of marrow the myeloma mass may occasionally be associated with prominent osteoclastic activity.[62]

Interleukin-6 (IL-6) is over produced in the bone marrow of patients with multiple myeloma and circulates in the peripheral blood. IL-6 appears to play critical role as an osteoclast activating factor mediating the bone effects of Interleukin -1 and Tumor necrosis factor.[50]

Furthermore, interleukin β (IL β) was shown to be the cytokine supporting the bone resorbing activity present in the bone marrow of patients with multiple myeloma.[50]

Marrow destruction by tumor plasma cells results in anaemia, leucopenia and thrombocytopenia.[50]

Myelofibrosis

It is a clonal myeloproliferative disease characterized by the proliferation of mainly megakaryocytic and granulocytic elements in the bone marrow, associated with reactive deposition of bone marrow connective tissue and with extramedullary haemopoiesis. It is a descriptive term referring to the deposition of excessive collagen in the bone marrow. There is a stepwise evolution of the disease characterized by prefibrotic and fibrotic stage.[63]

Marrow aspiration reveals dry tap, although occasionally normal or even hyperplastic fragments are obtained. Trephine biopsy is a reliable diagnostic procedure.[63]

Smears from successful aspirates may show no abnormality, but usually there is neutrophilic and megakaryocytic hyperplasia. The megakaryocytes are often morphologically abnormal. Micro megakaryocytes and macro megakaryocytes are often observed, and there is nuclear – cytoplasmic asynchrony.[63]

Erythroid precursors may be normal or increased. Granulocytes may show hyper or hypolobulation, acquired Pelger-Huet anomaly, and nucleo-cytoplasmic asynchrony.[63]

Bone marrow biopsy is necessary to demonstrate fibrosis. Histological evidence of osteosclerosis may be present. Bone marrow sinusoids are expanded, and there is intravascular haemopoiesis. Increased number of mast cells may be observed in biopsy adjacent to fibrosis.[63]

Table 5: Morphological finding in myelofibrosis.

PREFIBROTIC STAGE	FIBROTIC STAGE
BLOOD	**BLOOD**
• No or mild leukoerythroblastosis • No or minimal RBC poikilocytosis • Few if any dacrocytes (Tear drop cells)	• Leukoerythroblastosis • Prominent RBC poikilocytosis with dacrocytes (Tear drop cells)
BONE MARROW	**BONE MARROW**
• Hypercellular • Neutrophilic proliferation • Megakaryocytic proliferation and atypia (clustering of megakaryocyte, abnormally lobulated megakaryocytic nuclei, naked megakaryocytic nuclei). • Minimal or absent reticulin fibrosis	• Reticulin and/or collagen fibrosis • Decreased cellularity. • Dilated marrow sinuses with intra luminal haemopoiesis • Prominent megakaryocytic proliferation and atypia (clustering of megakaryocytes, abnormally lobulated megakaryocytic nuclei, naked megakaryocytic nuclei). • New bone formation (osteosclerosis).

As the disease evolves, haemopoiesis frequently becomes ineffective and blood cell counts fall leading to pancytopenia. Products of cells are released in the marrow, including the platelet derived growth factor from megakaryocytes and stimulate deposition of reticulin and fibrous tissue.[63]

Malignant Lymphoma

Neoplastic proliferation of cells of the lymphoid series can give rise to solid tissue tumors, the malignant lymphoma. The two major categories of malignant lymphoma are Hodgkin lymphoma and Non Hodgkin lymphoma.[50]

Pancytopenia is rare but when present it may be as a result of hypesplenism, marrow involvement or most commonly, therapy related.[50]

NHL varies widely in its rapidity of onset and spread, but there are fewer tendencies to be confined to the axial lymph nodes and involvement of nasopharynx, tonsil, inguinal and mesenteric structure is very common, as involvement of the bone marrow.[50]

Most of the patients have normal haematological parameters early in the course of their illness. As progression occurs, the haemoglobin level falls and there may be thrombocytopenia and neutropenia.[50]

Metastatic Carcinoma

Patients with cancer frequently have anaemia, with or without other associated cytopenias. Cancer related anaemia can be a direct result of tumor invasion of the bone marrow, or indirect result of tumor therapy or systemic symptomatology, or an incidental finding resulting from other pathology in the patient.[64]

Cancer can have a major impact on bone marrow function. Anaemia in these patients is frequently multifactorial and arriving at a single diagnosis can be difficult. Direct laboratory evaluation and examination of the bone marrow can provide important diagnostic clues in many cases.[64]

Cytopenias as a direct result of the malignancy

Malignant tumors especially haemopoietic malignancies can be the primary cause of one or more cytopenias. The anaemia that is present is usually normocytic or macrocytic (with secondary folate deficiency) and associated with a reticulo-cytopenia and clonal erythropoiesis. While the diagnosis is often suspected after examination of the peripheral smear, confirmation requires evaluation of bone marrow aspirate smears and biopsy sections.[64]

Other haemopoietic neoplasms can also directly cause anaemia by suppression of the normal bone marrow. Acute leukaemia, regardless of whether it produces a hypercellular or hypocellular infiltrate, directly suppresses normal erythropoiesis and causes anaemia of marrow failure.[64]

Cytopenias indirectly resulting from malignancy

The majority of anaemic cancer patients will present with cytopenias arising as a result of tumor. Autoimmune haemolytic anaemia has been described in association with a number of tumors, although it is more frequently noted in patients with CLL (Chronic Lymphocytic Leukaemia). Another anaemia related to red cell destruction in patients with cancer is characterized by microangiopathy. Most cases of cancer associated microangiopathic haemolytic anaemia are seen in patients with known tumors, although occasionally, this anaemia may be the presenting feature of the tumor. Gastric carcinoma is the most frequently coexisting cancer; followed by breast cancer and lung cancer.[64]

One of the more common anaemias noted in patients with cancer is characterized by a normal red cell size, a low reticulocyte count, and an apparent increase in bone marrow iron storage. This process has been called the "anaemia of chronic disease" and is associated with the elaboration of inflammatory cytokines in the host. When anaemia of chronic disease is seen in cancer patients, a wide variety of marrow histology can be seen. Frequently the marrow is normocellular, with normal M:E ratio and an increase in histiocyte storage iron. However, variable degree of myeloid hypoplasia may result in an overall marrow hypocellularity and a decrease in M:E ratio. Occasionally erythroid hyperplasia is present.[64]

These abnormalities may be identified even in the absence of tumor invasion of the marrow and may reflect a systemic response to the presence of the malignancy.[64]

Hypersplenism

Hypersplenism is a clinical syndrome; it does not imply a specific causal mechanism. It has the following characteristic features.

1) Enlargement of spleen.
2) Reduction in one or more of the cell lines in the peripheral blood.
3) Normal or hyperplastic cellularity of the bone marrow, often with orderly maturation of earlier stages but paucity of more mature cells.
4) Premature release of cells in the peripheral blood, resulting in reticulocytosis and/or large immature platelets.
5) Increased splenic red cell pool, decreased red cell survival and increased splenic pooling of platelets with shortening of their life span.[65]

Hypersplenism can occur as a primary event due to an unknown pathogenic stimulus. Some of the important causes of secondary hypersplenism are haematological malignancies, storage disease, infections like malaria, typhoid, brucellosis, leishmaniasis, collagen vascular diseases, congestive splenomegaly and splenic tumors.[65]

The pathogenesis of hypersplenism is explained as follows:

1. **Anaemia:** Sequestration and hemodilution combine to produce the anaemia of hypersplenism. An expansion of the plasma volume accompanies hypersplenism and the degree of expansion is proportional to the size of the spleen.
2. **Neutropenia:** The neutropenia of hypersplenism is caused by an increase in the marginated granulocyte pool, which is located in the spleen.
3. **Thrombocytopenia:** Increased splenic platelet pooling. A massively enlarged spleen can hold ninety percent of the total platelet mass.[50]

Causes of Hypersplenism[66]

- Portal hypertension with congestive splenomegaly
- Lymphomas
- Sarcoidosis
- Felty's syndrome
- Lipid storage disease – Gaucher's disease
- Kala-azar,
- Chronic Malaria,
- Tropical splenomegaly
- Bacterial infections – Tuberculosis, brucellosis
- Thalassemia
- Chronic lymphocytic leukaemia
- Myelofibrosis
- Hairy cell leukaemia

Malaria

Italians in the 18th century named the disease 'mal' 'aria' meaning "foul air".[67]

It is a parasitic infection caused by obligate intracellular protozoa of the genus plasmodium.[67]

Anaemia is the most prominent haematological manifestation of malarial infection. It is most marked with plasmodium falciparum species, which invades erythrocytes of all ages. Cellular disruption and haemoglobin digestion lead directly to haemolysis.[67]

In plasmodium falciparum infection, ring forms predominate and finding numerous ring forms without mature stages is evidence for plasmodium falciparum infection, young rings being smaller. The presence of doubly infected cells and double chromatin dots in ring trophozoites occur more commonly in plasmodium falciparum. Gametocytes of plasmodium falciparum are readily identified by their characteristic sausage shape.[68]

An inadequate bone marrow response to anaemia is seen, with relative reticulocytopenia. Leucocyte number may be slightly increased or normal, but leucopenia as a result of splenomegaly and impaired marrow function is characteristic. Thrombocytopenia is seen in nearly 70% of infections.[69]

The bone marrow reactions caused by plasmodium vivax are qualitatively similar to those caused by plasmodium falciparum not only in the red cell lineage but also in other cell lines, characterized by dyserythropoiesis and ineffective erythropoiesis.[68]

Haemophagocytic syndrome (HPS) can be associated with plasmodium vivax infection, can present as pancytopenia. It is a clinico-pathological entity characterized by benign proliferation of monocytes or macrophages showing phagocytosis of haemopoietic cells. HPS is commonly associated with haematological malignancies, autoimmune conditions and viral, bacterial or parasitic infection. The etiological role of plasmodium vivax infection is suggested by the absence of other associated disease and the total clinical and haematological recovery after Chloroquine treatment.[69]

Haemophagocytic syndrome is often considered as a T cell mediated disorder with inappropriate and/or excessive production of cytokines such as Tumor necrosis factor-α, Interferon-γ and Macrophages-Colony-Stimulating Factor (M-CSF), resulting in macrophage activation. Strikingly high levels of these cytokines have been observed in patients with malaria.[69]

Disseminated Tuberculosis

Tuberculosis continues to be the important communicable disease in the world. The typical and varied spectrum of clinical presentation of tuberculosis poses a diagnostic and therapeutic challenge to the physicians.[70]

Various haematological presentations include normocytic normochromic anaemia, leucopenia, neutropenia, lymphocytopenia, monocytopenia, leucocytosis and monocytosis.[71]

Pancytopenia is a rare haematological finding in disseminated tuberculosis and its degree is influenced more by the duration of infection than its severity; the various postulated mechanisms for pancytopenia include:[72]

- Hypersplenism
- Histiocytic hyperplasia and indiscriminate phagocytosis of blood cells by histiocytes in bone marrow.
- Maturation arrest.
- Infiltration of the bone marrow by caseating or non-caseating tubercular granulomas; they replace marrow cells or suppress cells through release of interferon and lymphotoxin.[72]

Granulomas are found on bone marrow biopsy in 15-40% of patients with disseminated tuberculosis. Tuberculous granulomas usually contain Langhans type giant cells and caseation is present in approximately half the cases with marrow involvement.[73]

Acid fast bacilli cannot be demonstrated in most of the cases, and when seen they are usually scanty. Presence of bone marrow plasmacytosis in patients with tuberculosis is not uncommon.[73]

Storage Diseases

In various inherited diseases the deficiency of an enzyme leads to accumulation of a metabolite in body cells, often in macrophages. The morphologically abnormal bone marrow macrophages containing an excess of the relevant metabolite are referred to as storage cells.[73]

Both bone marrow aspirates and trephine biopsies are useful in the detection of storage diseases. Peripheral blood cells may show related abnormalities.[74]

Gaucher's Disease

It is an inherited condition in which glucocerebrosides accumulate in macrophages including those in the liver, spleen and bone marrow.[74]

There are usually no specific peripheral blood features, although very occasionally Gaucher's cells may be seen in the peripheral blood, particularly after splenectomy.[74]

Pancytopenia develops slowly, as a consequence of hypersplenism.

Gaucher cells are large, round or oval cells with a small, usually eccentric nucleus and voluminous weakly basophilic cytoplasm with a wrinkled or fibrillar or onion-skin pattern.[74]

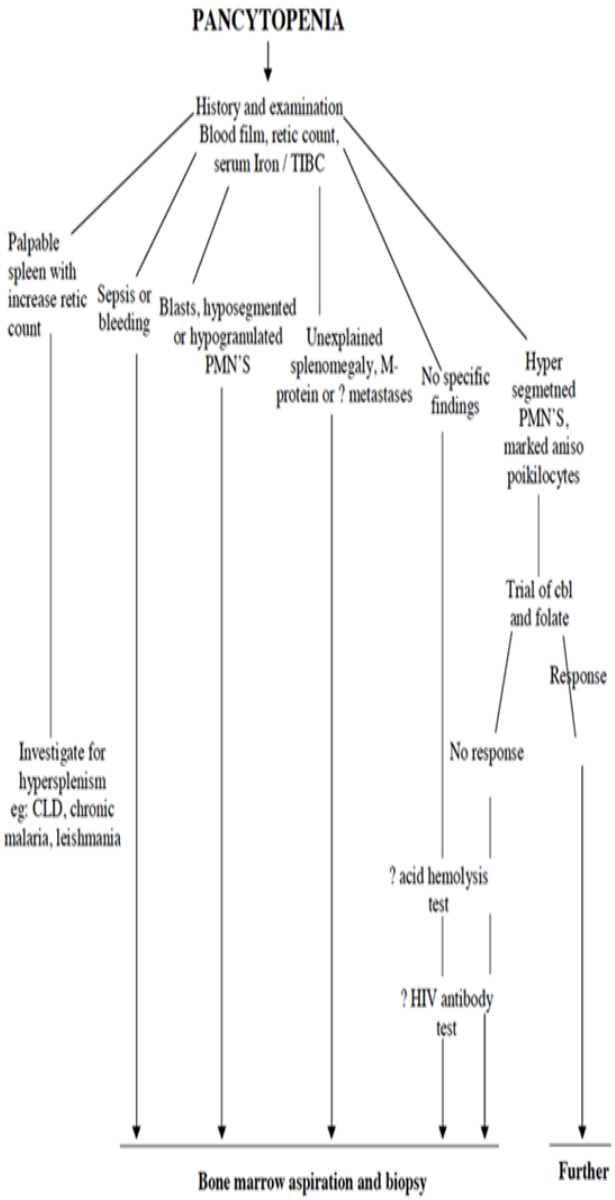
They may be isolated or appear in clumps or sheets, sometimes replacing large areas of the marrow. There may be an increase in reticulin and collagen deposition.[74]

Niemann-pick Disease
Inherited condition caused by reduced spingomyelinase activity characterized by the presence of foamy lipid containing macrophages in the bone marrow and other tissue.[74]

Anaemia and various cytopenias may occur as a consequence of hypersplenism.[74]

Foamy macrophages are large cells with multivacuolated cytoplasma and a nucleus in the centre. They stain pale blue with Romanowsky stains.[74]

Fig-II : DIAGNOSTIC APPROACH TO THE PANCYTOPENIC PATIENT[18]



PMN = polymorphonuclear neutrophil, TIBC = Total iron binding Capacity, Cbl = Cobalamin, HIV = human immunodeficiency virus

MATERIALS AND METHODS

- The study was conducted in Department of General Medicine, Government Medical College, Bhavnagar after taking permission from IRB (HEC).
- Study Design: Prospective
- Duration: 1 year (March 2015 to February 2016)
- Number of patients: 50
- Patient were subjected to following tests: Complete hemogram with thin peripheral smear, RBC indices, reticulocyte count, serum bilirubin total, direct and indirect, alanine transaminase (ALT), aspartate transaminase (AST), total serum proteins, albumin and globulin levels, serum electrolytes (namely sodium and potassium) and blood urea, serum creatinine, random blood sugar, HbsAg, HCV, HIV, chest X-ray, Ultrasonography abdomen and Bone marrow aspiration as per indication.

❖ **Inclusion Criteria**

1) Age >12 years
2) Patients giving written consent for study
3) Patients showing parameters Hemoglobin <9gm/dl, total leucocyte count<$4x10^9$/L and platelet count <$1.5x10^9$ /L.

❖ **Exclusion Criteria**

1) Patient with age <12years
2) Patients not giving consent for participation.
3) Patients who received blood and blood products in past 4 months.
4) Patients on radiotherapy and chemotherapy.

RESULTS

50 patients who presented with pancytopenia were studied.
Following results were recorded and analyzed.

1. Gender
There was a male preponderance (M:F = 1.7:1),

Table 1: Gender distribution of cases of pancytopenia in Present study.

Gender	Male	Female
Present Study	32	18

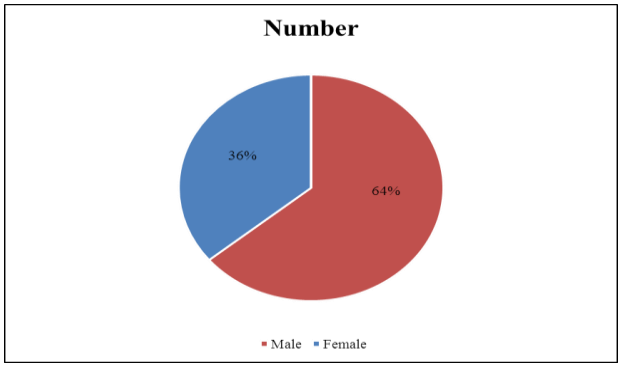


Chart 1: Gender distribution of cases of pancytopenia in present study

2. Age

Most common age group was 21-30 years in present study containing 17 cases among 50. Followed by 31-40 having 14 cases. It was observed less frequent in extremes of age groups 13- 20 years and 51 – 60 years.

Table 2: Sex and age distributions of cases of pancytopenia in present study.

Age Group (in years)	Total	Frequency (males)	Frequency (females)
13-20	4	3	1
21-30	17	11	6
31-40	14	9	5
41-50	10	7	3
51-60	5	4	1

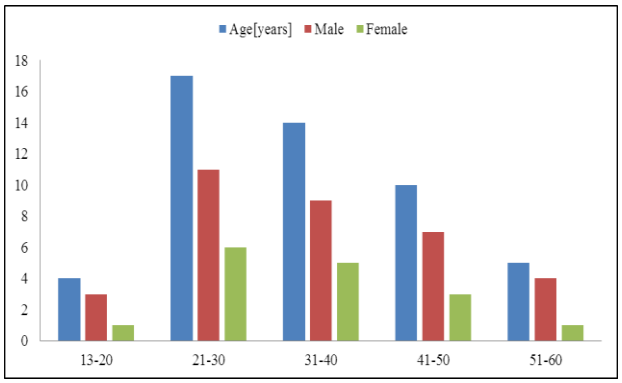


Chart 2: Sex and age distribution of cases of pancytopenia in present study

3. Chief complaints

- Chief complaints like generalized weakness (88%) common presenting feature, followed by bleeding, fever and dyspnea.

Table 3: Chief complaints in cases of pancytopenia in present study.

Clinical features	Cases	%
Generalized weakness	44	88%
Bleeding	17	34%
Fever	15	30%
Dyspnea	14	28%

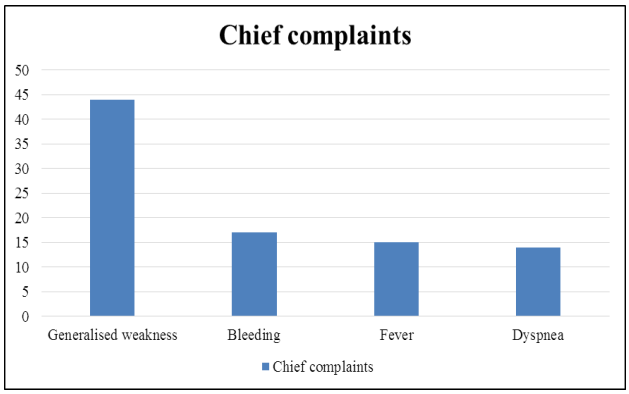


Chart 3: Chief complaints in cases of pancytopenia in present study.

4. Clinical findings

- Pallor was noted in 47 cases among 50 cases (94%). Followed by splenomegaly (40%) and hepatomegaly (30%).

Table 4: Clinical Findings in cases of pancytopenia in present study.

Clinical findings	Cases	%
Pallor	47	94%
Splenomegaly	20	40%
Hepatomegaly	15	30%
Lymphadenopathy	8	16%
Purpura	8	16%
Oedema	6	12%

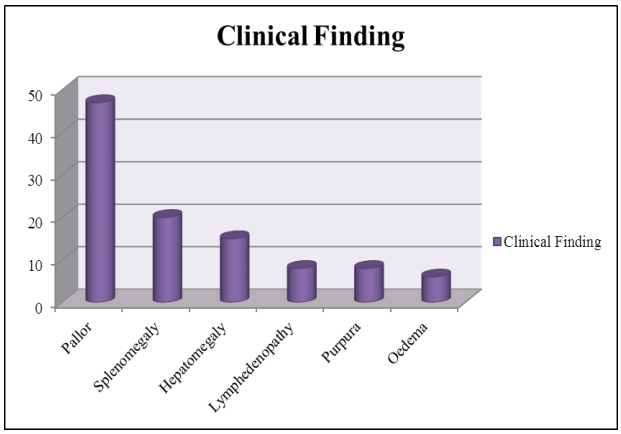


Chart 4: Clinical findings in cases of pancytopenia in present study.

5. Etiologies

In present study Megaloblastic anemia was the commonest cause constituting (58%) of the cases. Aplastic anemia (12%) was second common cause.

Table 5: Etiologies of pancytopenia in present study.

Etiology	Cases	%
Megaloblastic anemia	29	58%
Aplastic anemia	6	12%
Cirrhosis of liver	4	8%
Leukemia	3	6%
Dengue	2	4%
Myelodysplastic syndrome[MDS]	2	4%
Malaria	2	4%
Paroxysmal nocturnal hemoglobinuria[PNH]	1	2%
Acquired immunodeficiency syndrome[AIDS]	1	2%

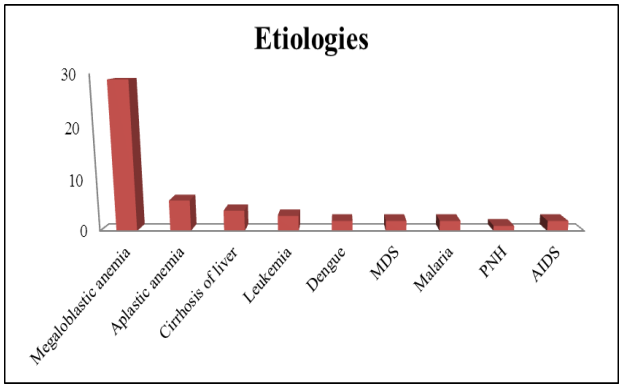


Chart 5: Etiologies of cases of pancytopenia in present study.

6. Vital hematological parameters in cases of pancytopenia

- **Hemoglobin percentage**

Hemoglobin percentage varied from 1.8- 9.0 gm/dl. Most of patients had hemoglobin percentage between 6-9 gm/dl lowest value of 1.8 gm/dl was seen in a case of aplastic anemia.

Table 6: Values of hemoglobin in cases of pancytopenia in present study.

Hemoglobin[gm/dl]	Cases	%
0-3	1	2%
3-6	12	24%
6-9	37	74%

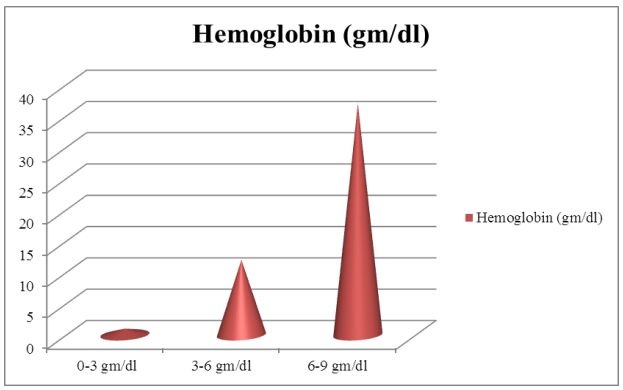


Chart 6: Values of hemoglobin in cases pancytopenia in present study.

- **Total leucocyte count**

Total leucocyte count ranged from 1710-4000 cells/cmm. Most of patients had total leucocyte count in range of 3000-4000cells/cmm. Lowest count of 1710 cells/cmm was seen in a case of aplastic anemia.

Table 7: Values of Total Leucocyte Count in cases of pancytopenia in present study.

Total leucocyte count[/cmm]	Cases	%
0-1000	0	0
1000-2000	6	12%
2000-3000	14	28%
3000-4000	30	60%

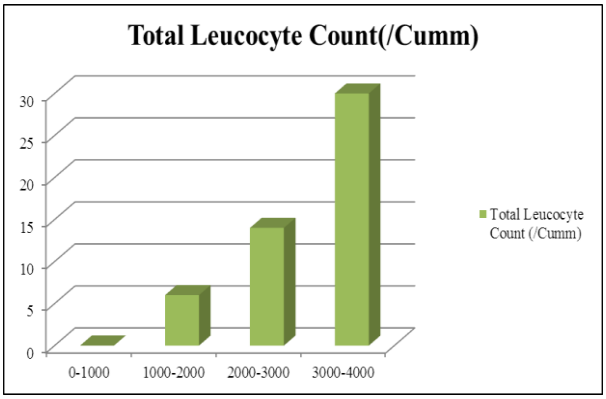


Chart 7: Values of Total Leucocyte Count in cases of pancytopenia in present study.

- **Platelet count**

Platelet count ranged from 0.08- 1.5 lakh/cmm. Most of patients had 1.0-1.5lakh/cmm. Lowest platelet count was 8000/cmm in a case of Megaloblastic anemia.

Table 8: Values of platelet count in cases of pancytopenia in present study.

Platelet count[lakh/cmm]	Cases	%
0-0.5	12	24%
0.5-1.0	13	26%
1.0-1.5	25	50%

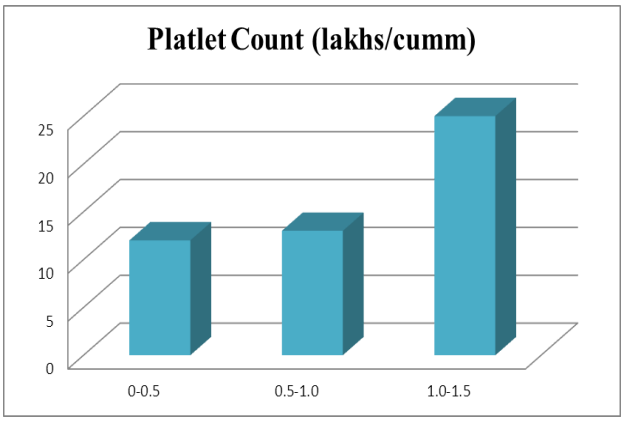


Chart 8: Values of platelet count in cases of pancytopenia in present study.

7. Peripheral blood picture in cases of pancytopenia

Macrocytosis was seen in majority of pancytopeniac cases

Table 9: Peripheral blood picture in cases of pancytopenia in present study.

Blood picture	Cases	%
Normocytic Normochromic	7	14%
Microcytic Hypochromic	2	4%
Macrocytic	38	76%
Dimorphic	3	6%

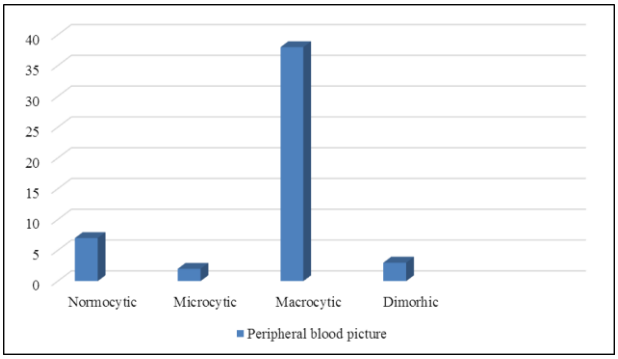


Chart 9: Peripheral blood picture in cases of pancytopenia in present study.

8. Cellularity of bone marrow

- Bone marrow aspiration has been performed in 41 patients. Hypercellular bone marrow was observed in 30 patients. Among them most common cause was Megaloblastic anemia.

Table 10: Cellularity of bone marrow in cases of pancytopenia in present study.

Bone marrow	Cases	%
Normocellular	5	28%
Hypercellular	30	60%
Hypocellular	6	12%

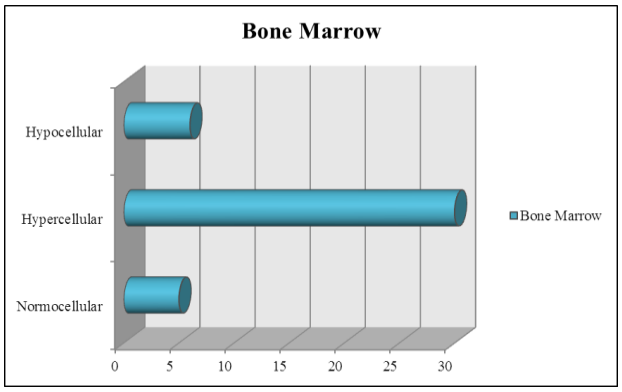


Chart 10: Cellularity of Bone marrow in cases of pancytopenia in present study.

The most common cause of patients having pancytopenia in this study is Megaloblastic anemia.

- **Megaloblastic Anemia**

✓ Clinical features

- The main symptoms were those of anemia like generalized weakness, fever and dyspnea.
- In clinical finding pallor was most common followed by splenomegaly and hepatomegaly.

✓ Bone marrow findings

- Bone marrow aspirations were satisfactory and hypercellular with normal or reversal of M:E ratio.
- Erythroid series of cells were hyperplastic with Megaloblastic erythropoiesis.
- Myelopoiesis revealed giant myelocytes and metamyelocytes.
- Megakaryopoiesis was normal (few cases showed atypical bizarre forms).

✓ Prevalence

- In this study 29 out of 50 patients having megaloblastic anemia. It is more prevalent in males. Commonest age group is 21-30 years containing 12 males and 8 females.

Table 11: Age-sex distribution of megaloblastic anemia in cases of pancytopenia in present study.

Age group(years)	Total cases	Male	Female
13-20	1	1	0
21-30	12	8	4
31-40	10	7	3
41-50	3	1	2
51-60	3	3	0

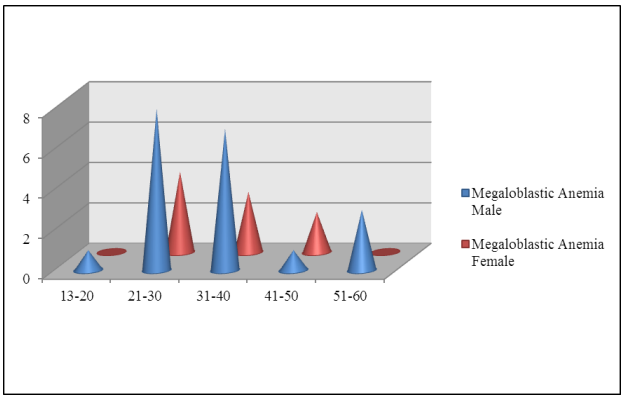


Chart 11: Age-sex distribution of megaloblastic anemia in cases of pancytopenia in present study.

- **Aplastic Anemia**

✓ 6 patients diagnosed as aplastic anemia having complaints of generalized weakness and dyspnea.

✓ Clinical finding showed pallor and splenomegaly most commonly.

✓ Patients having macrocytic anemia in peripheral smear and bone marrow aspiration were hypocellular with showing marked hypocellularity of hemopoietic elements and increase in the fat spaces.

- **Cirrhosis of Liver**

✓ 4 patients had diagnosis of cirrhosis of liver who presented with pancytopenia. patients presented with generalized weakness and bleeding. On clinical examination patients having oedema, pallor and splenomegaly. Their ultrasonographical finding suggestive of irregular margin, cirrhosis of liver and splenomegaly.

- **Malaria**

✓ Malarial infestation was seen in 2 cases in present study who presented with pancytopenia. Both presented with fever, chills, rigor, vomiting and headache.

- ✓ Clinical examination revealed pallor and hepatosplenomegaly.
- ✓ Peripheral smear showed normocytic hypochromic anaemia with marked anisopoikilocytosis, neutropenia, thrombocytopenia and gametocytes of Plasmodium vivax were seen.

- **Leukemia**

- ✓ In the present study leukemia of was seen in 3 cases in adults. 2 cases were seen in male, 1 case was seen in female. They presented with fever, generalized weakness and abdominal discomfort. Clinical examination revealed pallor and hepatosplenomegaly.
- ✓ One case presented with bleeding diathesis in the form of per vaginal bleeding. Lowest hemoglobin was seen in this case was 1.8 gm/dl. Peripheral smear showed normocytic normochromic anaemia with neutropenia and thrombocytopenia. Myeloblasts with Auer rod constituted 20%. BM was hypercellular in both cases.
- ✓ Erythroid and megakaryocytic series were reduced. Majority of cells were myeloblasts constituting 40% of cells in marrow.

- **Myelodysplastic Syndrome**

- ✓ 2 cases had been found having myelodysplastic syndrome. 1 was male and another was female. Bone marrow findings were hypercellular in both cases.

- **Other Causes of Pancytopenia**

- ✓ 2 patients of DENGUE fever presented with fever, bleeding, headache and retro orbital pain. They had been positive for dengue NS-1 antigen.
- ✓ 1 patient with diagnosis of PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) presented with pancytopenia.
- ✓ 1 patient with diagnosis of ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS) presented with pancytopenia. Patient was reactive for both HIV-1 and HIV-2.

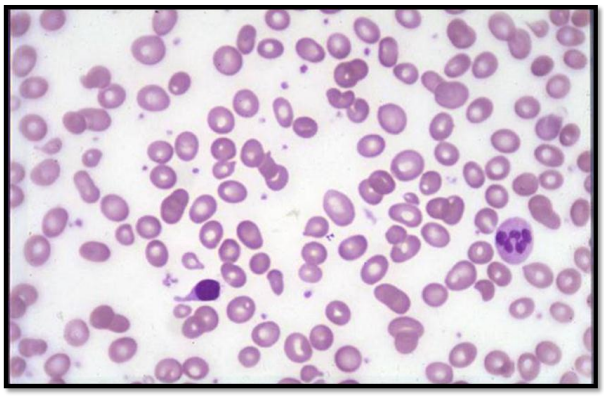


Fig. 1: Peripheral smear showing Macrocytic anemia with hypersegmented neutrophil (Field's stain 100xs).

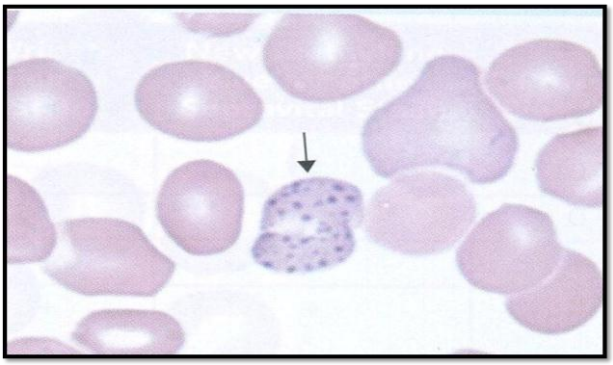


Fig. 2: Peripheral smear showing basophilic stippling in case of Megaloblastic anemia (Field's stain 100 xs).

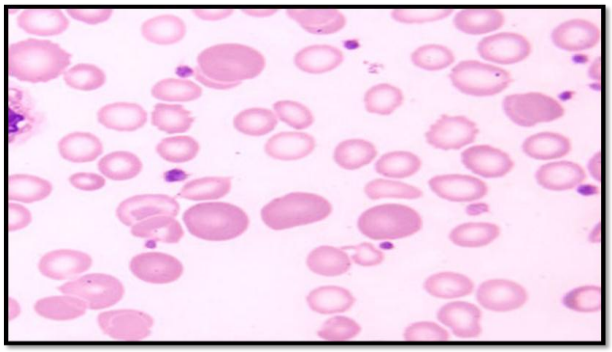


Fig. 3: Peripheral smear showing Dimorphic anemia with macrocytes and microcytes (Field's stain 100 xs).

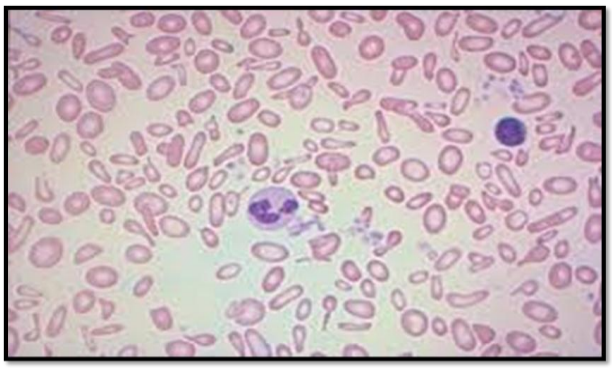


Fig. 4: Peripheral smear showing microcytic hypochromic anemia with pencil cells, tear drop cells and severe anisopoikilocytosis in Iron deficiency anemia (Field's stain 100 xs).

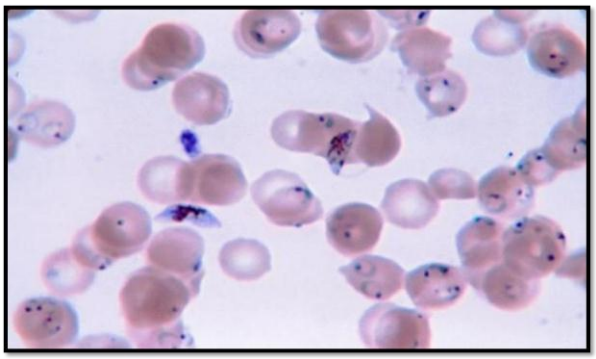


Fig. 5: Peripheral smear with ring forms and gametocytes (Field's stain 100xs).

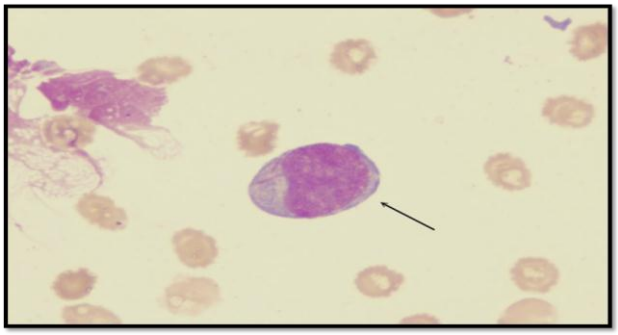


Fig. 6: Peripheral smear showing myeloblast with Auer rod in case of Acute Myeloid Leukemia (Field's stain 100 xs)

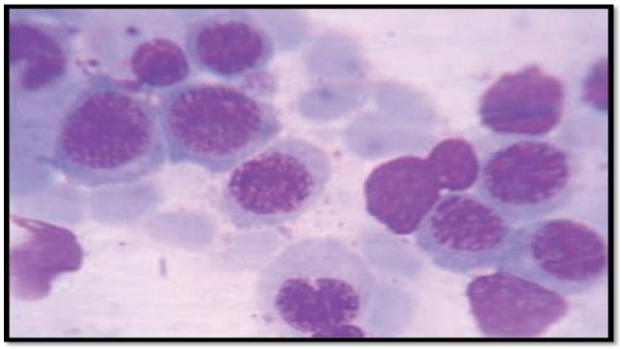


Fig. 7: Bone marrow aspiration showing erythroid hyperplasia with Megaloblastic erythropoiesis (sieve like nuclear chromatin) (Field's stain 100xs).

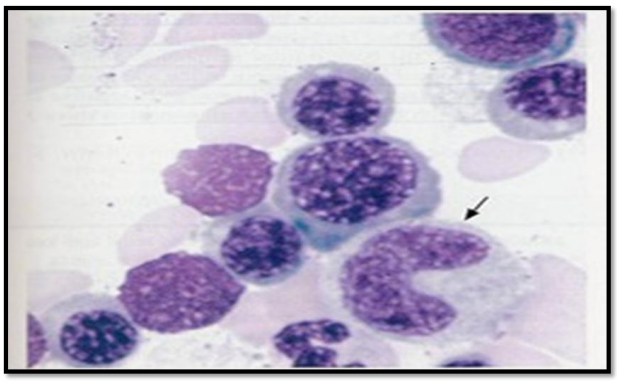


Fig. 8: Bone marrow aspiration showing giant metamyelocyte in case of Magaloblastic anemia (Field's stain 100 xs).

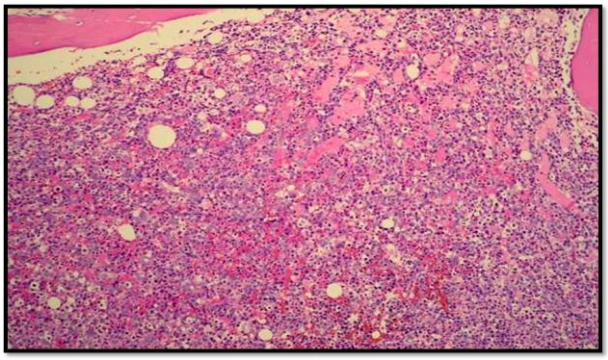


Fig. 9: Bone marrow biopsy with hypercellularity in case of Megaloblastic anemia (low power, HandE).

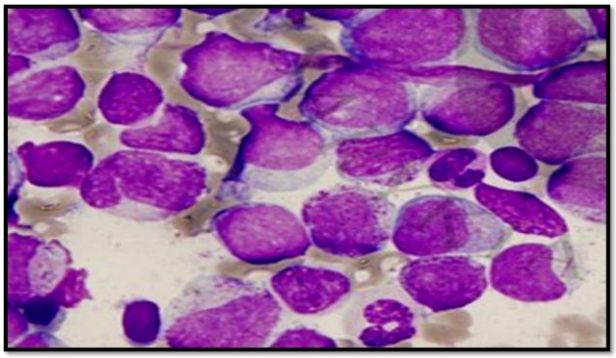


Fig. 10: Bone marrow aspiration showing myeloblasts in AML-M2 (Field's stain 100xs).

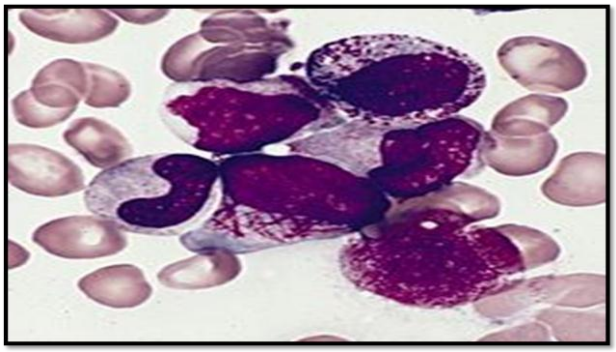


Fig. 11: Bone marrow aspiration showing Faggot cell in AML-M3 (Field's stain 100xs).

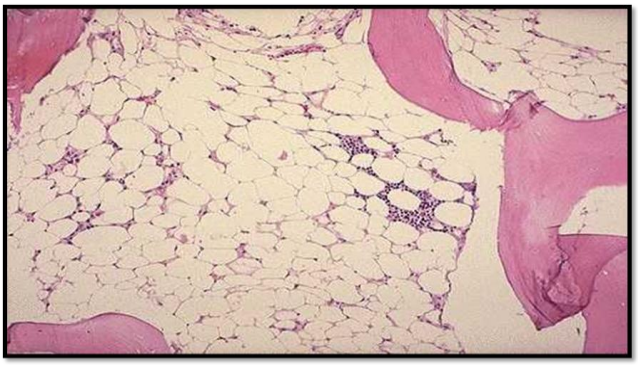


Fig. 12: Bone marrow biopsy showing hypocellularity with replacement of hemopoietic tissue by fat in case of aplastic anemia (low power, HandE).

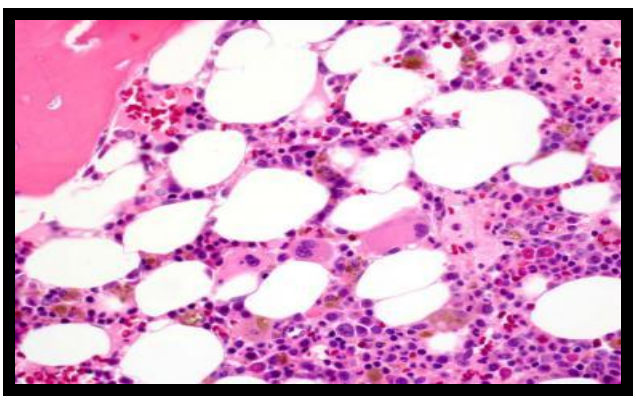


Fig. 13: Bone marrow biopsy showing hypocellularity with hemosiderin deposition due to transfusion in MDS.

Data			Complains				Physical Examination							Complete Hemogram					Serum Billirubin{gm/dl}			Special	Bone Marrow Aspiration	Diagnosis
Sr N.	AGE {years}	SEX	G.W.	Fever	Dyspnea	Bleeding	Pallor	Jaundice	Lymphadenopathy	Hepatomegaly	Splenomegaly	Purpura	Oedema	HB {gm/dl}	TC (/cmm)	Dc{%} (N/L/E/M/B)	PC/lac	Peripheral Smear	Total	Direct	Indirect			
1	26	F	Y	N	Y	N	Y	N	Y	N	Y	N	N	7.3	2500	72/22/5/1/0	0.98	MA	0.8	0.5	0.3		Hypercellular	Megaloblastic anemia
2	31	M	Y	Y	N	N	N	N	N	N	Y	N	N	9	3422	80/18/2/2/0	0.51	NN	1.8	1.1	0.7	Mp by card-positive (p. vivax)		Malaria
3	50	M	Y	Y	Y	N	Y	N	N	N	N	N	N	5.1	3980	74/20/3/3/0	0.68	MA	0.9	0.4	0.5		Hypercellular	Megaloblastic anemia
4	36	M	Y	N	N	N	Y	N	N	N	N	N	N	6.6	3800	76/22/2/0/0	1.1	MA	0.6	0.4	0.2		Hypercellular	Megaloblastic anemia
5	54	F	Y	N	Y	N	Y	N	Y	Y	Y	Y	N	4.9	2000	62/30/6/2/0	1.3	MC	1	0.5	0.5		Hypercellular	Leukaemia
6	39	F	Y	N	N	N	Y	N	N	N	N	N	N	7.8	2880	84/12/3/1/0	0.99	MA	1.3	0.8	0.5		Hypercellular	Megaloblastic anemia
7	50	M	Y	N	Y	Y	Y	N	Y	Y	Y	N	N	6.3	2380	78/18/3/1/0	0.62	NN	1.9	0.7	1.2		Normocellular	PNH
8	45	M	Y	N	Y	Y	Y	Y	N	N	Y	N	Y	4.8	3240	73/20/4/2/1	0.22	MA	11	2.7	8.3	USG-Cirrhosis of liver		Cirrhosis of liver
9	35	M	Y	N	N	N	Y	N	N	N	N	N	N	3.9	1200	79/18/2/1/0	1.2	MA	0.8	0.5	0.3		Hypercellular	Megaloblastic anemia
10	42	M	Y	Y	Y	Y	Y	Y	N	N	Y	N	Y	3.4	2430	83/14/2/1/0	0.96	MA	33.8	12.4	21.4	USG-Cirrhosis of liver		Cirrhosis of liver
11	34	F	Y	N	N	N	Y	N	N	Y	N	N	N	6.7	3740	68/27/2/3/0	1	DA	0.9	0.6	0.3		Hypocellular	Aplastic anemia
12	29	F	Y	N	Y	N	Y	N	N	N	N	N	N	7.6	2840	82/15/3/2/0	1.2	MA	0.6	0.2	0.4		Normocellular	Megaloblastic anemia
13	13	M	Y	Y	Y	Y	Y	N	Y	N	Y	Y	Y	1.8	2410	76/22/2/0/0	0.44	MC	0.7	0.3	0.4		Hypocellular	Aplastic anemia
14	24	M	Y	N	N	N	Y	N	N	Y	N	N	N	8.8	2870	84/12/2/2/0	0.94	MA	0.5	0.2	0.3		Hypercellular	Megaloblastic anemia
15	46	F	N	Y	N	N	Y	N	N	N	Y	N	N	8.7	4000	72/22/5/1/0	0.64	NN	0.6	0.3	0.3	Mp by card-positive(p. vivax)		Malaria
16	54	M	N	N	N	N	Y	N	N	N	N	N	N	9	3580	70/22/4/3/1	1.1	MA	1.1	0.8	0.3		Hypercellular	Megaloblastic anemia
17	28	M	Y	N	N	N	Y	N	N	N	N	N	N	8.6	3300	68/26/4/2/0	1.5	MA	0.4	0.3	0.1		Hypercellular	Megaloblastic anemia

18	18	F	N	Y	N	Y	Y	N	N	N	N	N	N	9	3850	62/24/12/2/0	0.14	MA	0.5	0.2	0.3		Hypercellular	MDS
19	22	M	Y	Y	N	Y	Y	N	N	N	N	N	N	4.6	2430	66/24/6/4/0	0.04	NN	0.3	0.1	0.2		Hypocellular	Aplastic anemia
20	38	M	N	N	N	N	Y	N	N	N	N	N	N	8	3860	72/22/2/3/0	1.46	MA	0.5	0.2	0.3		Hypercellular	Megaloblastic anemia
21	29	M	Y	N	N	N	Y	N	N	N	N	N	N	8.6	3000	69/17/1/3/0	1.3	MA	1.1	0.5	0.6		Hypercellular	Megaloblastic anemia
22	54	M	Y	Y	Y	N	Y	N	Y	Y	Y	Y	N	4.1	2430	63/20/3/4/0	0.84	NN	1.3	0.4	0.9		Hypercellular	Leukaemia
23	30	M	Y	Y	N	Y	Y	N	N	Y	Y	Y	N	6.9	2500	70/25/2/3/0	0.33	MA	0.9	0.4	0.5		Hypercellular	MDS
24	60	M	Y	Y	N	N	Y	N	N	Y	N	N	N	7.1	1710	62/22/10/5/1	1.02	MA	0.4	0.3	0.1		Hypercellular	Megaloblastic anemia
25	42	M	Y	N	N	Y	Y	Y	N	N	Y	N	Y	7.4	3920	82/15/3/2/0	0.89	MA	22.7	7.2	15.5	USG-Cirrhosis of liver		Cirrhosis of liver
26	27	F	Y	Y	N	N	Y	N	Y	Y	Y	N	N	6.8	3240	74/22/3/1/0	1.4	NN	1	0.6	0.4	HIV -Reactive		AIDS
27	37	F	N	N	N	Y	Y	N	N	N	N	Y	N	8.8	1280	73/21/1/6/0	0.5	MA	2.2	1.4	1.8		Normocellular	Megaloblastic anemia
28	46	F	Y	Y	N	N	N	N	N	Y	N	Y	N	9	2480	83/10/5/2/0	0.2	NN	1.1	0.3	0.8	DENGUE-Positive		Dengue
29	21	M	Y	N	N	N	Y	N	N	N	N	N	N	4.5	3960	66/24/4/6/0	1.4	MA	1.3	0.4	0.9		Hypercellular	Megaloblastic anemia
30	28	M	Y	N	N	N	Y	N	N	N	N	N	N	7.2	3170	72/22/2/3/0	1.5	MA	0.9	0.5	0.4		Hypercellular	Megaloblastic anemia
31	21	F	Y	N	N	N	Y	Y	N	N	Y	N	N	6.9	2340	61/30/7/2/0	0.13	MA	3.3	2.1	1.2		Normocellular	Megaloblastic anemia
32	29	M	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y	7.7	3650	73/22/3/2/0	0.86	MA	30.5	18.5	12	USG-Cirrhosis of liver		Cirrhosis of liver
33	44	M	Y	N	Y	Y	Y	N	N	Y	Y	Y	N	4.6	3120	60/27/2/1/0	0.34	DA	0.5	0.2	0.3		Hypocellular	Aplastic anemia
34	45	F	Y	N	N	N	Y	N	N	N	N	N	N	6.9	4000	67/24/6/3/0	1.32	MA	0.9	0.2	0.7		Hypercellular	Megaloblastic anemia
35	56	M	Y	N	Y	N	Y	N	N	Y	N	N	N	5.3	1800	73/20/5/2/0	0.87	MA	0.7	0.5	0.2		Hypercellular	Megaloblastic anemia
36	34	F	Y	N	N	Y	Y	N	N	N	Y	N	N	6.5	2140	78/16/4/2/0	0.18	MA	1.7	0.5	1.2		Hypercellular	Megaloblastic anemia
37	43	F	Y	N	N	N	Y	N	N	N	N	N	N	6.9	3780	62/24/8/6/0	1.26	MA	1.2	0.8	0.4		Hypercellular	Megaloblastic anemia
38	25	F	N	N	N	Y	N	Y	N	N	N	Y	N	9	1300	75/20/3/2/0	0.08	MA	2.6	0.4	2.2		Normocellular	Megaloblastic anemia
39	32	M	Y	N	N	N	Y	N	N	N	N	N	N	6.7	4000	62/22/10/5/1	1.36	MA	0.4	0.1	0.3		Hypercellular	Megaloblastic anemia
40	18	M	Y	N	N	N	Y	N	N	N	N	N	N	7.3	3500	65/20/9/5/1	1.25	MA	1	0.3	0.7		Hypercellular	Megaloblastic anemia
41	24	F	Y	Y	N	Y	N	N	Y	N	Y	N	N	9	3870	64/23/9/4/0	0.08	NN	1.2	0.5	0.7	DENGUE-Positive		Dengue
42	33	M	Y	N	N	N	Y	N	N	N	N	N	N	7.8	3480	67/21/10/2/	1.29	MA	0.8	0.4	0.4		Hypercellular	Megaloblastic

															0								anemia
43	42	M	Y	N	Y	Y	Y	N	N	Y	Y	N	Y	6.2	3460	61/30/7/2/0	0.84	DA	1.2	0.9	0.3		Hypocellular	Aplastic anemia
44	32	M	Y	N	N	N	Y	N	N	N	N	N	N	7.2	3890	66/24/4/6/0	1.14	MA	0.8	0.3	0.5		Hypercellular	Megaloblastic anemia
45	34	F	Y	Y	N	Y	Y	N	Y	Y	Y	N	N	8.7	3100	70/27/2/1/0	1.42	NN	1.3	0.8	0.5		Hypercellular	Leukaemia
46	29	M	Y	N	N	N	Y	N	N	N	N	N	N	6.6	2290	82/14/3/1/0	0.98	MA	0.6	0.4	0.2		Hypercellular	Megaloblastic anemia
47	21	M	N	N	N	N	Y	N	N	N	N	N	N	8.8	3140	78/16/4/2/0	1.35	MA	0.9	0.6	0.3		Hypercellular	Megaloblastic anemia
48	27	M	Y	N	N	N	Y	N	N	N	N	N	N	5.4	2460	66/23/8/3/0	1.12	MA	1.5	0.8	0.7		Hypercellular	Megaloblastic anemia
49	16	F	Y	N	Y	Y	Y	N	N	Y	Y	N	N	4.3	3620	75/20/3/2/0	0.18	DA	1.4	0.6	0.8		Hypocellular	Aplastic anemia
50	31	M	Y	N	N	N	Y	N	N	N	N	N	N	6.6	3230	73/20/5/2/0	1.32	MA	0.6	0.3	0.3		Normocellular	Megaloblastic anemia

Key to Master Chart

G.W.	–	Generalised weakness
Hb	–	Haemoglobin
TC	–	Total leucocyte count
DC	–	Differential leucocyte count
PC	–	Platelet count
DA	–	Dimorphic anaemia
NA	–	Nutritional anemia
MA	–	Megaloblastic anemia
PNH	–	Paroxysmal nocturnal hemoglobinuria
P.VIVAX	–	Plasmodium vivax
HIV	–	Human immunodeficiency virus
USG	–	Ultrasonography
MDS	–	Myelodysplastic syndrome
AIDS	–	Acquired immunodeficiency syndrome
Y	–	Yes
N	_	No

DISCUSSION

50 cases of pancytopenia were studied. Statistical data of age, sex, presenting complaints, systemic examination various causes of pancytopenia, peripheral smear, biochemical analysis and bone marrow aspiration (as and when required) were studied, and compared with those published in the literature.

Table 12: Age, sex distribution compared to other studies of pancytopenia.

Sr. No.	Authors	No of cases	M:F	Age range
1	Khunger JM et al[8] (2002)	200	1.2:1	2-70 years
2	Kumar R et al[4] (2001)	166	2.1:1	12-73 years
3	Khodke K et al[5] (2001)	50	1.3:1	3-69 years
4	Tilak V et al[6] (1999)	77	1.14:1	5-70 years
5	Present study	50	1.8:1	13-60 years

The age of the patients ranged from 13 to 60 years with a mean age of 33.5 years.

Cytopenias were observed more in males (64%) than females (36%) with M:F ratio of 1.8 : 1. Age and sex distribution was comparable with other studies of pancytopenia.

Table 13: Physical findings compared to other studies.

	Splenomegaly	Hepatomegaly	Lymphadenopathy	Total
Khunger JM et al[8] study	64(32%)	63(31.5%)	10(5%)	200
Tilak V et al[6] study	32(41.5%)	29(37.7%)	6(7.8%)	77
Present study	20(40%)	15(30%)	8(16%)	50

The most common presenting complain in the present study was generalized weakness (88%), bleeding(34%), fever (30%) and dyspnea (28%). The most common physical finding was pallor (94%) followed by splenomegaly (40%) and hepatomegaly (30%).

The presenting symptoms were usually attributed to anemia, or thrombocytopenia.

Leucopenia was an uncommon cause of the initial presentation of the patient, but can become the most serious threat to life during course of the disorder.

Physical findings were comparable with other studies, though lymphedenopathy was found in more cases in present study.

Table 14: Various causes of pancytopenia compared to other studies.

Causes	Khunger JM et al[8] (2002)	Kumar R et al[4] (2001)	Khodke et al[5] (2001)	Tilak V et al[6] (1999)	Present study
Aplastic anemia	28(14%)	49(29.5%)	7(14%)	6(7.8%)	6(12%)
Megaloblastic anemia	144(72%)	37(22.3%)	22(44%)	53(64.9)	29(58%)
Subleukemic leukemia	10(5%)	20(12.1%)	1(2%)	1(1.3%)	4(8%)
Lymphoma	2(1%)	10(6.2%)	-	2(2.6%)	-
Myelodysplastic syndrome	4(2%)	6(3.6%)	1(2%)	-	2(4%)
Marrow metastasis	-	2(1.2%)	-	-	-
Myelofibrosis	2(1%)	2(1.2%)	-	1(2%)	-
Malaria	2(1%)	5(3%)	-	3(3.9%)	2(4%)
Enteric fever	-	2(1.2%)	-	-	-
Malignant histiocytosis	-	1(0.6%)	-	-	-
Disseminated Tuberculosis	1(0.5%)	1(0.6%)	1(2%)	1(2%)	-
Multiple myeloma	2(1%)	-	2(4%)	1(2%)	-
Waldenstrom's macroglobulinemia	1(0.5%)	-	-	1(2%)	-
Acquired immuno-deficiency syndrome(AIDS)	-	-	1(2%)	-	1(2%)
Dengue	-	-	-	-	2(4%)
Cirrhosis of liver	-	-	-	-	4(8%)
Paroxysmal nocturnal hemoglobinuria(PNH)	-	-	-	-	1(2%)
Total	200	166	50	77	50

The variations in the frequency of various diagnostic entities causing pancytopenia has 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.

The commonest cause of pancytopenia, reported from various studies throughout the world has been Aplastic anemia.

This is sharp contrast with the results of our study where the commonest cause of pancytopenia is Megaloblastic anemia.

Similar findings were observed in other studies conducted in India.

This seems to reflect the higher prevalence of nutritional anemia in Indian subjects.

In the present study of 50 cases, 4 cases had Dimorphic anemia, macrocytic anemia was found in 38 cases, normocytic normochromic in 7 cases and microcytic hypochromic anemia constituted rest of the cases.

The incidence of Megaloblastic anaemia varied from 22.3 to 72% of all pancytopenic patients. Our incidence of Megaloblastic anaemia was 58%.

Incidence of 72% was reported by Khunger JM[8] et al and 68% by Tilak V et al.[6]

All the above studies done in India, stress the importance of Megaloblastic anemia being the major cause of pancytopenia.

It is a rapidly correctable disorder and should be promptly notified.

Bone marrow aspiration showed megaloblastic erythroid hyperplasia. Megaloblasts had the characteristic feature of sieved nuclear chromatin, asynchronous nuclear maturation, and bluish cytoplasm with cytoplasmic blebs.

Giant metamyelocytes and band forms were predominant in leucocytic series.

Although bone marrow aspiration study is uncommon in a suspected Megaloblastic anemia, if the diagnosis does not appear straight forward or if the patient requires urgent treatment and hematological assays are not available, bone marrow aspiration is indicated.

As facilities for estimating folic acid and vitamin B12 levels are not routinely available in most centers in India, the exact deficiency is usually not identified.

Incidence of aplastic anaemia varies from 7.8 to 29.5% among pancytopenic patients. Our incidence of Aplastic anemia was 12% which correlated with the studies done by Khodke K et al[5] and Khunger JM et al[8] whose incidence for the same was 14%.A higher incidence of 29.5% was reported by Kumar R et al[4]

Most of the cases of Aplastic anemia were idiopathic. Marrow aspirates in all were hypocellular with fragments composed largely of fat. Normoblastic erythropoiesis was seen with normal M:E ratio and there was mild increase in lymphocytes and plasma cells. Dyserythropoiesis was the feature in few cases.

Aplastic/hypoplastic anaemia/bone marrow failure can be inherited or acquired and can involve just one cell line or all the three cell lines.

The incidence of aplastic anaemia quoted from the west is much higher than that observed by us. This increased incidence may be related to environmental factor such as increased exposure to toxic chemicals.

Pancytopenia was the common feature in our study, this co-related with Kumar R et al[4] and Khunger JM et al[8] studies.

We encountered 4% case of Malaria in our study compared to Khunger JM et al[8] who has reported an incidence of 1%, Tilak V et al[6] reported 3.9% and Kumar R et al[4] reported 3% of cases.

We encountered 4% case of Myelodysplastic syndrome in our study compared to Khunger JM et al[8] who has reported an incidence of 2%, and Kumar R et al[4] reported 3.6% of cases.

We encountered 8% cases of cirrhosis of liver which is contrast to other studies. Because in our region more patients came to us from lower socioeconomic status; with history of alcoholism.

As in other studies cases of Storage disorder, Multiple myeloma and Lymphoma were not encountered in our study.

CONCLUSION AND SUMMARY

Pancytopenia is not an uncommon hematological problem encountered in clinical practice and should be suspected on clinical grounds when a patient presents with unexplained anemia, prolonged fever and tendency to bleed.

The physical findings and peripheral blood picture provides valuable information in the work of cytopenic patients.

Evaluation of peripheral blood film reveals the most probable cause of anemia, presence of nucleated RBC's

and/or immature myeloid cells may suggest marrow infiltration or primary haematologic disorder.

Bone marrow aspiration is an important diagnostic tool in hematology which helps to evaluate various cases of cytopenia. Bone marrow examination is accurate, reproducible, rapidly available information at an economical cost and with minimal discomfort to the patient.
Bone marrow aspiration is sufficient to make a diagnosis in cases of nutritional anaemias and initial diagnosis of leukemia.

Megaloblastic anaemia was the commonest cause which indicates the high prevalence of nutritional anemia in our region.

The other common causes were Aplastic anemia and cirrhosis of liver.

Present study concludes that detailed primary hematological investigations along with bone marrow aspiration in cytopenic patients is helpful for understanding of the disease process, to diagnose or to rule out the causes of cytopenia and helpful in planning further investigations and management of cytopenic patients.

- This is a Clinico-Hematological study on Pancytopenia over a period of 1 year in the Department of Medicine, Government Medical College and Sir Takhatsinhji General Hospital, Bhavnagar, Gujarat.
- 50 patients in age group of 13-60 years presenting with pancytopenia were evaluated.
- A combined evaluation of physical findings, primary hematological investigations, biochemical investigations and bone marrow aspiration (as and when required) were done in cytopenic patients.
- The age of the patients ranged from 13 to 60 years with a mean age of 33.5 years. Males accounted for 64% and females accounted for 36% with M:F ratio of 1.8 : 1.
- Commonest presenting complaint was generalized weakness and bleeding.
- Commonest physical finding was pallor followed by splenomegaly and hepatomegaly.
- Megaloblastic anaemia (58%) was the commonest cause of cytopenia, followed by Aplastic anaemia (12%).
- Lowest hemoglobin percentage was 1.8 gm/dl and noted in a case of Aplastic anemia.
- Lowest total leucocyte count was 1710 cells/cmm and noted in a case of Aplastic anemia.
- Lowest platelet count of 8000 cells/cmm was noted in a case of Megaloblastic anemia.
- Macrocytic anemia was predominant blood picture in cytopenic patients.
- Hypercellular marrow was noted in 60% and the common cause was Megaloblastic anemia, followed by cirrhosis of liver.
- Aplastic anemia and Leukemia were seen in 6 and 3 patients respectively.
- Two case of Malaria (P. vivax) was noted.
- Hypocellular marrow was noted in 11 patients and commonest cause was hypoplastic/aplastic anemia.
- Lymphocytosis and plasmacytosis was the predominant feature in hypoplastic / aplastic anaemia.
- The probable etiologic factor in hypoplastic / aplastic anaemia was idiopathic.
- 2 cases of Dengue, 1 case of Acquired immunodeficiency syndrome, 2 cases of Myelodysplastic syndrome and 1 case of Paroxysmal nocturnal hemoglobinuria were noted.

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