ETIOLOGICAL PROFILE OF PANCYTOPENIA

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DECLARATION

I hereby declare that the dissertation entitled "**ETIOLOGICAL PROFILE OF PANCYTOPENIA**" was done by me in the Department of Pathology at Coimbatore Medical College and Hospital , Coimbatore during the period from March 2010 to August 2011,under the guidance and supervision of **Dr.A.Dhanalaxmi,M.D.,** Associate professor ,Department of Pathology , Coimbatore Medical college , Coimbatore .This dissertation is submitted to the Tamilnadu Dr.M.G.R.Medical University , Chennai towards the partial fulfillment of the requirement for the award of M.D.,Degree in Pathology. I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

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CERTIFICATE

This is to certify that the dissertation entitled "**ETIOLOGICAL PROFILE OF PANCYTOPENIA**" is a record of bonafide work done by **Dr.S.RENUGA**, Post graduate student in the Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore, under the supervision of **Dr.M.MURTHY, M.D.,** Professor & Head, Department of Pathology, Coimbatore Medical College and Hospital, and under the guidance of **Dr.A.DHANALAKSHMI,M.D.,** Associate professor, Coimbatore Medical College and Hospital, in partial fulfillment of the regulations of the Tamilnadu Dr. M.G.R. Medical University towards the award of M.D.Degree (Branch III) in Pathology.

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All men like to think they can do it alone, but reality is, there is no substitute for support, encouragement or a fellow" **- Tim Allen**

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LIST OF ABBREVIATIONS

AIDS	-	Acquired immunodeficiency syndrome
BM	-	Bone marrow
BMB	-	Bone marrow biopsy
CFU – GM	-	Colony forming unit – Granulocyte – Macrophage
CFU-E	-	Colony forming unit-Erythroid
CFU-S	-	Colony forming unit-Spleen
CLL	-	Chronic lymphocytic leukemia
DNA	-	Deoxyribonucleic acid
HLA	-	Human leucocyte antigen
NHL	-	Non hodgkin lymphoma
PCR	-	Polymerase chain reaction
PS	-	Peripheral smear

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ABSTRACT

Introduction:

Pancytopenia is a relatively common haematological entity. It 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. The severity of pancytopenia and the underlying pathology determines the management and prognosis. Thus, identification of the correct cause will help in implementing appropriate therapy.

Objectives :

- To find out the incidence of pancytopenia in the clinical pathological setup.
- To find out the various causes of pancytopenia.
- To study the clinicopathological correlation
- To analyse the levels of vital hematological parameters during presentation.

Methods :

This is a prospective study to evaluate patients with pancytopenia. 50 patients of age group between 2 to 60 years presenting with cytopenias were evaluated in Hematology unit, Department of Pathology, Coimbatore Medical College, Coimbatore during period of March 2010 to August 2011.Patients on myelotoxic chemotherapy were excluded.

Results :

Among 50 cases studied, age of patients ranged from 5-60 years with a mean age of 39.5 years, and female predominance M:F was 1:1.2. Most of the patients presented with generalised weakness and fever. Commonest physical finding was

pallor followed by hepatomegaly and splenomegaly. Dimorphic anaemia was predominant blood picture. Bone marrow aspiration was conclusive in all cases. Commonest marrow finding was hypercellularity with megaloblastic erythropoiesis. The commonest cause for pancytopenia was megaloblastic anaemia (68%) followed by aplastic anaemia (14%), myelofibrosis (12%),subleukemic leukemia (4%), myelodysplastic syndrome(2%).The commonest association was with Grade 2 marrowfibrosis in reticulin stain.

Conclusion:

Pancytopenia should be suspected on clinical grounds when a patient presents with unexplained anaemia, prolonged fever and tendency to bleed. Hence, present study concludes that detailed primary haematological investigations along with bone marrow aspiration in cytopenic patients is helpful for understanding disease process, to diagnose or to rule out the causes of cytopenia. As a large proportion of pancytopenia is of reversible aetiology, early and accurate diagnosis may be life-saving.

Key words :

Pancytopenia; Megaloblastic anaemia; Bone marrow study.

INTRODUCTION

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

Manifestations of peripheral pancytopenia are due to a wide variety of disorders which primarily or secondarily affect the bone marrow[7]. The presenting symptoms are usually attributable to anaemia, thrombocytopenia and rarely leucopenia[3] .

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

Varying factors encompassing geographic distribution, genetic factors, nutritional status and the prevalence of infective disorder may cause variation in the incidence of disorders causing pancytopenia [28].

Careful assessment of the blood film is important if the reason for the pancytopenia is not apparent from the clinical history[56]. 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[5].

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[6].

The severity of pancytopenia and the underlying pathology determine the management and prognosis of these patients[5].

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[5,7].

This study was carried out with an aim to obtain further information so that it would help in the management of patients with pancytopenia. Hence the present study has been undertaken to evaluate the various causes of pancytopenia and to correlate the peripheral blood findings with bone marrow aspirate and trephine biopsy.

The extent of marrow fibrosis in relation to the underlying pathology was also assessed by reticulin silver stain in all cases[57]. The data obtained would help in planning the diagnostic and therapeutic approach in patients with pancytopenia.

AIM OF THE STUDY

To assess the etiological profile of pancytopenia and do a correlation between the clinical presentation and the various vital hematological parameters.

OBJECTIVES OF THE STUDY

- To find out the incidence of pancytopenia in the clinical pathological setup.
- To find out the various causes of pancytopenia.
- To study the clinicopathological correlation.
- To analyse the vital hematological parameters during presentation.

REVIEW OF LITERATURE

DEFINITION

Pancytopenia is defined as reduction of all the three formed elements of blood below the normal reference range[1].

HISTORY

Haemopoiesis, the production of blood cells is a fundamental concept in hematology. The work of Neumann and Bizzozero established the relationship between blood and the bone marrow in eighteenth century. In 1868, Neumann noted that bone marrow was an important organ for the formation of red blood cells[2].Among the various causes of pancytopenia, literature regarding aplastic anemia and fanconi anemia alone are much available.

The earliest case description of aplastic anemia given, was by Dr.Paul Enrilch in 1888. He described a young woman who died following an abrupt illness that manifested as severe anemia, bleeding, hyperpyrexia and a markedly hypocellular marrow[3]. In 1904, the term aplastic anaemia was introduced by Chaufford[3] Aplastic anaemia, a disease due to the absence of haemopoiesis has had a parallel history since the discovery of the function of bone marrow in the midnineteenth century.

Familial syndrome of pancytopenia and congenital physical abnormalities was first reported in 1927 by Guido Fanconi . Fanconi described three brothers, who had pancytopenia as well as physical abnormalities; he called their macrocytic anaemia "perniziosiforme"[1]. Naegeli suggested in 1931 that the term Fanconi anaemia be used for familial aplastic anaemia and congenital physical anomalies[1].Pancytopenia due to nutritional causes and environmental influences were accounted only in the late 20th century.

STUDIES ON PANCYTOPENIA

Various studies are available in literature, to delineate the causes of cytopenias as manifestations of various systemic disorders **.**

Various studies throughout the world have reported aplastic anaemia as the commonest cause of pancytopenia[4]. The International Aplastic Anemia and Agranulocytosis Study(IAAAS) conducted a prospective study between 1980 and 1984 in Europe and Israel which showed the overall incidence as 2 cases per 1 million people ;however the incidence is 3 fold in southeast asia[8].

Studies throughout India, revealed megaloblastic anaemia as the commonest cause of pancytopenia. A largest study in India conducted by Khunger et al which included 200 cases of pancytopenia concluded megaloblastic anemia as the commonest cause which accounted to 72%[7].In anotherlong study carried out for 6 years by Kumar et al of about 191cases megaloblastic anemia was detected in about 39% of cases[5].In a recent study by Thilak et al, megaloblastic anemia was proved to be the commonest cause and also revealed few interesting and rare causes of pancytopenia like drug induced agranulocytosis,waldenstroms macroglobulinemia etc[4].

By 1934, aplastic anaemia, although still not clearly defined, was described as a distinct clinical entity characterized by pancytopenia and thought to be the result of depressed bone marrow activity[1]. A study by Khunger ,Morley A et al, of lymphocytes from eleven patients with aplastic anaemia, suggested that in 7 patients the DNA was abnormal and ,it was hence concluded that in aplastic anaemia, DNA damage in stem cells may lead to a failure of proliferation[7]. In the late 1960s , Mathe et al was among the first to postulate an autoimmune basis for aplastic anemia [9,10].

Aplastic anemia being the most common cause of pancytopenia worldwide, many studies are available in the literature accounting for their etiology. Idiopathic aplastic anaemia accounts for more than 70%cases of pediatric anaemia and it is imperative to search for an etiology in all cases of aplastic anaemia before they are labelled as idiopathic[11].

Chloramphenicol, a broad spectrum antibiotic introduced in 1949 causes a dose dependent suppression of hemopoiesis, particularly erythropoesis, through its action on mitochondrial DNA[12]. Recovery from aplastic anaemia occurred four months after the discontinuation of suspected myelotoxic drugs and use of haematinics[13].

A child with hereditary spherocytosis who acquired human parvovirus B19 infection developed transient pancytopenia[14]. Seronegative hepatitis precedes the diagnosis of aplastic anemia in 3 to 5% of cases and is recognized as hepatitis associated aplastic anemia[15].

A Leukemia Research Fund(LRF) -UK based study puts the annual incidence of MDS as 3.6 per 100000[17] . One group has suggested a prevalance of 1 in 500 in those who presented with pancytopenia[18]. In a clinical study of primary myelodysplastic syndrome (MDS) in 33 children, it was noted that pancytopenia was the predominant presenting feature[19]. In a study of the haematological spectrum of myelodysplastic syndrome in 31 cases, pancytopenia constituted 16.1%[20].

The bone marrow microenvironment played an important role in haematopoiesis by providing humoral factors and thus played a very pivotal role in arriving at the diagnosis (Harigaya et al, 1981; Ohkawa & Harigaya, 1987; Sudo et al, 1989; Zhang et al, 2004).

Stromal microenvironment gave the clue for hematopoietic activity and, the stromal collagen graded as reticulin has been proved over centuries(Weiss & Chen, 1975; Watanabe, 1985; Cattoretti et al, 1993). Siegfried first used the term 'reticulin' in 1892.

CLINICAL FEATURES OF PANCYTOPENIA

The onset of the disease is insidious, manifestations depending on the severity of anaemia, leucopenia, and thrombocytopenia[74].

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, petechiae and ecchymotic patches over the skin, mucous membranes and conjunctiva[74].

Presence of splenomegaly and lymphadenopathy call for attention to the possibility of leukemia, lymphoma, myelofibrosis and storage diseases.

On the other hand, lack of these signs as well as lack of evidence of vitamin B12 or folate deficiency should suggest multiple myeloma or aplastic anaemia.
Finally, rare presentations include diarrhea, jaundice and weight loss[2].

HEMATOPOIESIS

This is the process of production of the formed elements of the blood; there are cords, islands or clusters of precursor cells between the sinusoids, through whose endothelial cells the erythrocytes, leukocytes and platelets enter the blood stream[3].

The formed elements of blood – red cells, granulocytes, monocytes, platelets and lymphocytes – have a common origin from pluripotent haematopoietic stem cells[31].The pluripotent stem cell compartment gives rise to the stem cells for both myeloid and lymphoid lines, which in turn produce progenitor cells of progressively restricted potential[3].

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[24]. In addition stem cells give rise to mast cells, macrophages and osteoclasts, but not to the marrow fibroblasts and osteoblasts, whose origin is in the mesenchyme[26].

The only normal stromal components to be derived from the multipotential haemopoietic precursors are resident tissue macrophages[27].

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[27].

ETIOLOGICAL CLASSIFICATION OF PANCYTOPENIA

A wide range of disorders result in pancytopenia. For the sake of clarity, aetiological factors have been divided into seven different groups[3,21].

A) APLASTIC ANAEMIA

1) FAMILIAL

a) Fanconi constitutional pancytopenia

b) Shwachman – Diamond syndrome (pancreatic deficiency in children)

c)Dyskeratosis Congenita

d)Congenital Amegakaryocytic thrombocytopenia.

2) ACQUIRED[22]

a) Idiopathic (autoimmune)

b) Drugs

- Analgesic
- Antiarrhythmic
- Anticonvulsants
- Antimicrobials
- Antimetabolites
- Alkylating agents
- Anticonvulsants
- Antithyroid
- Miscellaneous.

c) Toxins

- Benzene
- Organophosphates
- Chlorinated hydrocarbons.

d) Viruses

- Ebstein –Barr Vrus
- Hepatitis
- HIV

e) Paroxysmal nocturnal hemoglobinuria

f) Autoimmune /Connective tissue disorders

g) Pregnancy

h) Iatrogenic

B) DISORDERS INFILTRATING THE BONE MARROW

- Hairy cell leukemia
- Agnogenic myeloid metaplasia
- Marble bone disease
- Osteopetrosis
- Aleukemic leukemia
- Multiple myeloma
- Metastatic carcinoma
- Myelofibrosis
- Myelosclerosis

C) DISORDERS INVOLVING THE SPLEEN

- Hypersplenism
- Lymphoma – Hodgkins and Non hodgkins
- Storage disorders – Gaucher's, Niemann Pick's disease
- Infectious diseases – Kala azar, Miliary tuberculosis, Syphilis

Primary splenic panhematopenia.

D) VITAMIN B12 OR FOLATE DEFICIENCY

- Pernicious anemia
- Malabsorbtion
- Sprue

E) DISSEMINATED LUPUS ERYTHEMATOSUS

F) PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA

G) MISCELLANEOUS DISORDERS (WITH CELLULAR MARROW)

- Overwhelming infections
- Mycobacterial infection
- Alcohol
- Brucellosis
- Sarcoidosis
- Some refractory anaemias
- Pregnancy (some cases)
- Sideroblastic anaemia (rarely)

PATHOPHYSIOLOGY OF PANCYTOPENIA

Pancytopenia is the simultaneous presence of anaemia, leucopenia and thrombocytopenia and therefore it exists when there is a pathology which affects hematopoietic stem cells before they get differentiated [31].

Pancytopenia can be due to decrease in hemtaopoietic cell production in the bone marrow e.g. by infections, toxins, malignant cell infiltration or suppression or can have normocellular or even hypercellular marrow, without any abnormal cells, e.g. ineffective hematopoiesis and dysplasia, maturation arrest of all cell lines and peripheral sequestration of blood cells[32].

In other situations, however, the marrow may be normally cellular or even hypercellular and no abnormal cells may be present. The mechanisms leading to pancytopenia in these conditions may be due to ineffective haemopoiesis with cell death in the marrow, formation of defective cells that are rapidly removed from the circulation, sequestration or destruction of cells by the action of antibodies, and

trapping of normal cells in a hypertrophied and overactive reticuloendothelial system[21].

APLASTIC ANAEMIA

The word aplastic is derived from the Greek 'a' and 'plasso' meaning "without form". Despite the potentially misleading term anaemia, patients with aplastic anaemia fail to form blood cells of all three lineages[1].

Potential mechanisms responsible for acquired marrow cell failure include[22]

1) Direct toxicity to haemopoietic stem cell
2) A defect in the stromal microenvironment of the marrow required for haemopoietic cell development.
3) Impaired production or release of essential haemopoietic growth factors
4) Cellular or humoral immune suppression of marrow progenitor cells.

So there are two major groups of bone marrow failure[33]

- The aplastic anaemias, in which the failure lies in the pluripotent stem cell.
- The single – cell cytopenias, in which the failure lies in one of the committed cell lines. However there is overlap between these two groups.

Aplastic anaemia is defined by pancytopenia with a hypocellular bone marrow in the absence of an abnormal infiltrate and with no increase in reticulin[34]. Abnormal cells are not found in either the peripheral blood or in the marrow. The diagnosis is based on the absence of cells, not the presence of any characteristic feature[33].

The diagnosis of aplastic anaemia requires at least two of the following in addition to a hypocellular marrow[34]

i. Haemoglobin < 10 g/dl

ii. Platelet count < 50×10^9/L

iii. Neutrophil count < 1.5×10^9 / 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[33]. There is evidence of both quantitative and qualitative stem cell defect in aplastic anaemia and increased apoptosis of remaining early haemopoietic progenitor cells[33].

The sera of patients with aplastic anaemia is examined in a short term liquid culture system of human bone marrow which permits CFU-C proliferation. When aplastic serum was added to adherent cell-depleted liquid cultures, the CFU-C stimulating property is lost, but increased megakaryopoiesis still occurs[35]. Not only do cytotoxic suppressor T lymphocyte release cytokines, such as interferon-γ 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[36].

Both lymphokine induced expression of the Fas receptor on CD34 progenitor cells; triggering of the Fas receptor by its ligand initiates a fatal process of apoptosis. Apoptosis of hematopoietic cells in AA is suggested by the findings of high Fas receptor expression[37].

B) Aplastic anaemia secondary to drugs and physical agents

1. Ionising Radiation

Ionizing radiation is directly toxic to the bone marrow stem/progenitor cells and high doses (>1.5Gy to the whole body) can lead to severe pancytopenia within 2 to 4 weeks after exposure. LD_{50} has been estimated at about 4.5Gy ,and a dose of 10Gy or greater is thought to have 100% mortality[38].

Large macromolecules such as DNA can be damaged directly by large amounts of radiant energy, which can rupture covalent bonds directly or indirectly by interaction with highly charged and reactive small molecule resulting from ionization of free radicals formed in solution[1]. Radiation –induced bone marrow failure is dose dependent and is a consequence of direct toxicity to stem and progenitor cells[3.]

2. Drug associated

Association between drug exposure and aplastic anemia can be divided into two classes. Cytotoxic drugs which causes dose dependent marrow suppression include acute cancer chemotherapy drugs and idiosyncratic drugs in which the occurrence of pancytopenia is unexpected and rare[37].

Chloramphenicol is the most notorious drug documented to cause Aplastic Anaemia[40]. It is a prime example of a drug that causes both dose related marrow suppression and idiosyncratic aplastic anaemia[41].

3. Chemicals

Benzene is a dangerous environmental contaminant found in organic solvents, coal tar derivatives, and petroleum products[1].

The lipophilic properties of benzene , the constantly proliferative nature of red marrow ,and the singular juxtaposition of the marrow stem cell compartments to the vast marrow fat depot suggest a rational hypothesis[42].

The hematologic effect of chronic poisoning of arsenic is pancytopenia and megaloblastoid dyserythropoiesis. This effect is attributed to inhibition of DNA synthesis, impaired absorption and utilization of folic acid[43].

C) Aplastic anemia secondary to infections

Acute infection with Epstein-Barr Virus (EBV) is often associated with peripheral blood cytopenia. Rarely acute EBV infection can be complicated by the development of aplastic anaemia [44]. Hepatitis associated aplastic anaemia is a variant of aplastic anaemia in which aplastic anaemia follows an acute attack of hepatitis[45]. The mechanisms of virus –induced acute marrow failure are highly diverse.They include selective invasion and lysis of erythroid precursors(pavoviruses),infection of stromal cells(cytomegaloviruses) and elicitation of cytotoxic immune response(Epstein-Barr Virus)[46,47].

Virus associated cytopenia can be directly due to infection and cytolysis of hematopoietic cells or indirectly through the elaboration of inhibitory cytokines and in some cases it may result from idiosyncratic immune response directed against stem cells[3].

Direct infection of either stem cell or progenitor cells does not have significant role in marrow failure in AIDS patients[42].

D) Pancytopenia associated with Paroxysmal Nocturnal Hemoglobinuria

Paroxysmal Nocturnal Haemoglobinuria (PNH) often develops in patients with aplastic anaemia. PNH clone was detected in the bone marrow of patients with aplastic anaemia and pancytopenia before affected cells were evident in the peripheral blood. Flow cytometry with monoclonal antibodies against decay accelerating factor(DAF) and CD59 were used for the detection of the clone[48].PNH results from the expansion of an abnormal hematopoietic stem cells that harbors a somatic mutation of the X-linked gene PIGA .Small to moderate PNH clones are found in up to 70% of patients with aplastic anemia.

Typically,<20% GPI-AP –deficient granulocytes are detected in aplastic anemia patients at diagnosis,but patients may also have larger clones[40]. Aplastic anaemia and PNH are closely related syndromes[49].

FANCONI ANAEMIA

Fanconi anemia is an inherited chromosomal instability syndrome with a variable clinical presentation that includes congenital anomalies, progressive pancytopenia, and cancer susceptibility[40] .

In vitro, the cells of patients with Fanconi anaemia grow slowly and resist cell division, accumulating in G2. The haemopoietic defect in Fanconi anemia is evident at the progenitor cell level[40]. Fanconi anaemia is an inherited chromosomal instability syndrome with a variable clinical presentation that includes congenital anomalies,progressive pancytopenia, and cancer susceptibility[50].

The diagnostic hallmark of FA is increased chromosomal breakage in response to DNA-damaging agents such as Mitomycin C (MMC) or Diepoxybutane

(DEB)[39]. Physical anomalies and pancytopenia are not essential for diagnosis[37,51].The chromosomal breakage test is usually performed on metaphase spreads of peripheral blood lymphocytes treated with MMC or DEB.A total of 50 cells in metaphase are analysed for chromosomal breakage ,including the formation of radicals-a hallmark of this disease[52].

DYSKERATOSIS CONGENITA

Classic DC is an inherited disease characterized by the mucocutaneous triad of abnormal skin pigmentation ,nail dystrophy and mucosal leuoplakia[54].

In the inherited disorder Dyskeratosis Congenita, in which aplastic anaemia usually develops in the second or third decade, the underlying genetic defects affect the telomerase complex, which has both RNA and protein components[40].

In the X-linked form of the disease, a mutation is linked to Xq28 and the gene named DKCI, which codes for the protein dyskerin. In the autosomal dominant form, there is a Mutation found to reside in TERC gene and in few cases TERT that leads to a large deletion in telomerase RNA. Stem cells from patients with both types have markedly short telomeres[3].

BLOOD FINDINGS OF APLASTIC ANEMIA

Patients with aplastic anemia have varying degrees of pancytopenia. The reticulocyte count usually is less than 1% and may be zero despite the high levels of erythropoietin . Macrocytes may be present. The total leukocyte count and platelet counts are low[54].

The differential white cell count reveals a decrease in neutrophil and monocytes. Thrombocytopenia usually develops initially, with subsequent onset of granulocytopenia and then anaemia[54].

MARROW FINDINGS OF APLASTIC ANEMIA

Morphology

Both a bone marrow aspirate and trephine biopsy are required. Fragments should be readily obtained from the aspirate. The bone marrow is hypocellular with prominent fat and variable amounts of residual hematopoietic cells. Erythropoiesis is reduced or absent; dyserythropoiesis is very common and often marked[34].

Megakaryocytes and granulocytic cells are reduced or absent; dysplastic megakaryocytes and granulocytic cells are not seen in aplastic anemia. Lymphocytes, plasma cells, macrophages, and mast cells may be prominent, reflecting a lack of other cells rather than an increase in these elements[54].

In the early stages of the disease, prominent hemophagocytosis by macrophages occurs as well as background eosinophilic staining representing interstitial edema is seen. The trephine is needed to assess overall cellularity, the morphology of residual hematopoietic cells and to exclude an abnormal infiltrate[34].

The trephine is hypocellular throughout but is sometimes patchy with hypocellular and cellular areas. Reticulin is not increased and abnormal cells are not present[34].

In severe aplastic anemia, as defined by the International Aplastic Anemia 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[79].

TABLE 1 : DEFINITION OF DISEASE SEVERITY OF APLASTIC ANAEMIA (AA)[56].Camitta criteria (camitta et al .1975)

SEVERE APLASTIC ANAEMIA	BM cellularity <25% or 25-50% with <30% residual haemopoietic cells. Two out of three of the following Neutrophils $<0.5 \times 10^9$/L Platelets $<20 \times 10^9$/L Reticulocytes $<20 \times 10^9$/L
VERY SEVERE AA	As for severe AA but neutrophils $<0.2 \times 10^9$/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 $<1.5 \times 10^9$/L, platelets $< 100 \times 10^9$/L, haemoglobin <10g/dL.

MEGALOBLASTIC ANAEMIA

Megaloblastic anemia is anemia that results from inhibition of DNA synthesis in red blood cell production. When DNA synthesis is impaired, the cell cycle cannot progress from the G2 growth stage to the mitosis (M) stage. This leads to continuing cell growth without division, which presents as macrocytosis.

The defect in red cell DNA synthesis is most often due to hypovitaminosis, specifically a deficiency of vitamin B_{12} and/or folic acid. The retarded DNA synthesis results in unbalanced cell growth. The RNA synthesis remains unimpaired, while cell division is restricted. Megaloblastic anemia not due to hypovitaminosis

may be caused by antimetabolites that poison DNA production directly, such as some chemotherapeutic or antimicrobial agents. 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[57].

The morphlologic hallmark is nuclear-cytoplasmic dissociation which is best appreciated in precursor cells in the bone marrow aspirate. Megaloblastic nuclei are larger than normoblastic nuclei, and their chromatin appears abnormally dispersed due to its retarded condensation and hence termed as sieve like chromatin[57].

Macro-ovalocytes are especially characterisic of megaloblastic anemia but are not specific. Eventually, poiklocytosis becomes more pronounced with tear drop cells, and nucleated red cells , Howell-Jolly bodies ,and even cabot rings appear in the blood in severe megaloblastosis. 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[57].

MORPHOLOGY IN MEGALOBLASTIC ANEMIA[58]
PERIPHERAL SMEAR

- Increased mean corpuscular volume (MCV) with macroovalocytes (up to 14μm)which is variably associated with anisocytosis and poikilocytosis.
- Nuclear hypersegmentation and polmorphonuclear neutrophils(PMN)(one PMN with 6 lobes or >5% with 5 lobes)
- Thrombocytopenia (mild to moderate)
- Leukoerythroblastic morphology(from extramedullary hematopoeisis)

BONE MARROW ASPIRATE

- General increase in cellularity of all three major hematopoietic elements.
- Abnormal erythropoiesis-orthonormoblastic megaloblasts
- Abnormal leukopoiesis - giant metamyelocytes, band forms(pathognomonic) and hypersegmented PMNs.

Abnormal megakaryopoiesis – pseudohyperdiploidy

SUBLEUKEMIC LEUKEMIA

The total white cell count in acute leukemia 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 10^9/L[59].

In subleukemic patients blast cells may be present in very small numbers in peripheral blood. Buffy coat smear will help in detecting blasts under these circumstances[59].

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[59].

Bone marrow examination provides the diagnosis[59].

MYELODYSPLASTIC SYNDROME (MDS)

The myelodysplastic syndromes are a heterogeneous group of clonal stem cell disorders characterized by cytopenias due to impaired blood cell production, a hypercellular and dysplastic bone marrow, and an increased risk of leukemic transformation[27].

Pathology behind MDS is being explained on the basis of a stem cell disorder; Immunological abnormalities and apoptosis. The presence of trilineage dysplasia and cytogenetic abnormalities provides the evidence for a multipotent stem/progenitor cell origin. Immunological role is particularly apparent in cases of hypo plastic MDS those shares a number of features common with aplastic anemia notably clinical presentation with macrocytosis and varying level of dyserythropoiesis. The mechanism of cytopenia is being described on the basis of ineffective hematopoiesis and increased apoptosis[58]. Most cases undergo clonal evolution and transformation to acute myeloid Leukemia[27]. Clinical, haematological and histomorphological profile of MDS was studied in 37 cases. Primary MDS was seen in all age groups.The commonest presentation was Pallor and the commonest subgroup was Refractory Anaemia with Excess Blast (RAEB).

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[27].

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[27].

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 ringed sideroblasts, vacuolization, and PAS positivity, either diffuse or granular[60].

Dysgranulopoiesis is characterized by small size, nuclear hypolobation (pseudoPelger-Huet), and hypersegmentation, hypogranularity and pseudo Chediak-Higashi granules[60]. Megakaryocyte dysplasia is characterized by hypolobulated micromegakaryocyte, nonlobulated nuclei in megakaryocytes of all sizes, and multiple, widely separated nuclei[60].

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 endosteal surface of the bone trabeculae in MDS is referred to as ALIP[60].

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 leukemia[60].

MYELOFIBROSIS

Primary marrow fibrosis is a clonal myeloproliferative neoplasm of the pluripotent haematopoietic stem cell in which the proliferation of multiple cell lineages is accompanied by progressive bone marrow fibrosis characterized by splenomegaly, leucoerythroblastic picture, bone marrow fibrosis and extramedullary haematopoiesis[61].

In the early, so-called ‘cellular phase’ of CIMF, there may be little or no increase in bone marrow reticulin fibres. When an increase in bone marrow reticulin staining does occur, it is usually accompanied by an increase in bone marrow megakaryocytes, often morphologically atypical, as well as alterations in cellular and extracellular levels of cytokines with fibrogenic potential[61].

The fibrosis in this disease is thought to represent a 'reactive process mediated by cytokines that are produced by the cellular components of the clonal proliferation' (Tefferi, 2000). There is a stepwise evolution of the disease characterized by prefibrotic and fibrotic stage[62].

Attempts at bone marrow aspiration often yields a dry tap or a hemodilute sample and so bone marrow trephine biopsy is essential to make a diagnosis. Initial stages are characterized by an increase in the bone marrow cellularity in association with disorganization of marrow architecture and the presence of abnormal large megakaryocytes often occurring in clusters. Bone marrow fibrosis becomes increasingly dominant and progressively replaces hematopoeisis[61].

Smears from successful aspirates may show no abnormality, but usually there is neutrophilic and megakaryocytic hyperplasia. The megakaryocytes are often morphologically abnormal. Micromegakaryocytes and macromegakaryocytes are often observed, and there is nuclear – cytoplasmic asynchrony[62].

Erythroid precursors may be normal or increased. Granulocytes may show hyper or hypolobulation, acquired Pelger-Huet anomaly, and nucleo-cytoplasmic asynchrony[62].

Bone marrow biopsy is necessary to demonstrate fibrosis.Intrasinusoidal hematopoiesis can be seen at this stage. Histologically, late stages of fibrosis can cause thickening of trabecula and extensive deposition of osteoid. Increased number of mast cells may be observed in biopsy adjacent to fibrosis[62].

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[62].

Diagnostic criteria of myelofibrosis depends on the following factors ; Reticulin grade ≥3(on a 0-4scale), presence or absence of mutation in JAK2,palpable spleen, unexplained anemia,tear drop cells, leukoerythroblastic blood film,histological evidence of extramedullary hematopoeisis[61].

TABLE 2 : MORPHOLOGICAL FINDINGS IN MYELOFIBROSIS[62]

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

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[63].

The myeloma cells may be morphologically fairly normal or may be moderately or severely dysplastic, common cytological features include marked pleomorphism, increased cell size, 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[63].

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 accumulations (Mott cells, Morula cells), cherry red refractive round bodies (Russell bodies), vermilion staining glycogen rich IgA (Flame cells) and crystalline rods[63].

Peripheral smear in majority of patients shows anaemia, which is either normocytic, normochromic or, less often, macrocytic. There is increased rouleaux formation and increased background basophilic staining due to the presence of paraprotein in the blood [63].

The blood film is occasionally leukoerythroblastic and it is often possible to find a small number of plasma cells or plasmacytoid lymphocytes[63].

On biopsy ,it is characterized by an 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[63].

Marrow destruction by tumor plasma cells results in anaemia, leucopenia and Thrombocytopenia[49].

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].

HYPERSPLENISM

Hypersplenism is a clinical syndrome; it does not imply a specific causal mechanism. It has the following characteristic features[65].

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.

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].

MALARIA

Italians in the 18th century named the disease 'mal' 'aria' meaning "foul air"[51].

It is a parasitic infection caused by obligate intracellular protozoa of the genus Plasmodium[51].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[66].

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[67].

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].

DISSEMINATED TUBERCULOSIS

Tuberculosis continues to be an 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[69]. Various haematological presentations include normocytic normochromic anaemia, leucopenia, neutropenia, lymphocytopenia, monocytopenia, leucocytosis and monocytosis[70].

Pancytopenia is a rare haematological finding in disseminated tuberculosis and its degree is influenced more by the duration of infection than its severity[71].

Granulomas are found on bone marrow biopsy in 15-40% of patients with disseminated tuberculosis.

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[72].

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[58].

Since splenic enlargement and marrow infiltration frequently lead to anemia , thrombocytopenia and leucopenia, both bone marrow aspirates and trephine biopsies are useful in the detection of storage diseases. Related abnormalities are noted in peripheral smears also[58].

Gaucher's Disease

It is an inherited condition in which glucocerebrosides accumulate in macrophages including those in the liver, spleen and bone marrow[73].

There are usually no specific peripheral blood features, although very occasionally Gaucher's cells may be seen in the peripheral blood, particularly after splenectomy[73].

Gaucher cells are large, round or oval cells with a small, usually eccentric nucleus and voluminous weakly basophilic cytoplasm with a wrinkled fibrillar or onion-skin pattern[73].

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 tissues[73]. Anaemia and various cytopenias may occur as a consequence of Hypersplenism[73].

ANATOMY OF BONE MARROW

The term 'bone marrow' refers to the tissue occupying the cavities under the cortex within the honeycomb of trabecular bone[23]. The bone marrow provides a unique microenvironment for the orderly proliferation, differentiation, and release of blood cells[24].

The hematopoietic tissue is localized in the extra vascular compartment.Erythropoietic islands and megakaryocytes are associated with the marrow sinusoids in the central regions of the marrow cavities,early myeloid precursors lie close to the endosteal surfaces,while the more mature forms of the granulocytic series are also found in the central intertrabecular areas[23].

Normal megakaryocytes lie next to sinusoids and extend cytoplasmic processes that bud off into the blood stream to produce platelets[24].

It is estimated that the weight of the marrow in an adult is 1500-3700g, about 1000g of which is red marrow[23]. The marrow can undergo complete transformation in a few days and occasionally even in a 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[25].

Bone marrow microenvironment

The bone marrow stromal microenvironment is composed of cells, structural fibrils and extracellular matrix. These elements provide a connective tissue structure for the bone marrow and physical support for the haematopoietic progenitor cells.. Bone marrow collagen is primarily composed of type I and type III collagen.

The term reticulin has been defined histochemically as the argyrophilic fibres identified by various silver staining methods. Electron microscopic examinations reveals that reticulin is composed mainly of individual fibrils or small bunches of fibrils of type III collagen surrounding a core of type I collagen fibrils, all embedded in a matrix of glycoproteins and glycosaminoglycans (Fleischmajer et al, 1992; Fakoya, 2002; Ushiki, 2002).

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)[25].There are marked changes in the cellular composition of the marrow depending on the age of the subject and the site of aspiration[28].

Marrow cellularity is best judged by histological sections of biopsy or aspirated particles but should also be estimated from particles that are present in the marrow films[25]. The myeloid / erythroid ratio is the ratio of total granulocytes to total normoblasts. In new borns and infancy, it is somewhat higher than in later childhood or adult life. In adults, the range is broad, varying from about 1.5:1 to 3:1[25].

The most reliable assessment of overall haemopoietic cellularity is based on the biopsy specimen.. 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 numbers is also fairly readily evident at low power by the frequency of these large multilobulated cells[29].

Normocellular indicates about equal proportions or somewhat more hematopoiesis than fat cells. Hypocellular indicates reduction in hematopoiesis and a corresponding increase in fat cells. Variable marrow cellularity indicates intertrabecular spaces alternating with hyper or normocellular ones. Hypercellular is used when fat is decreased[3].

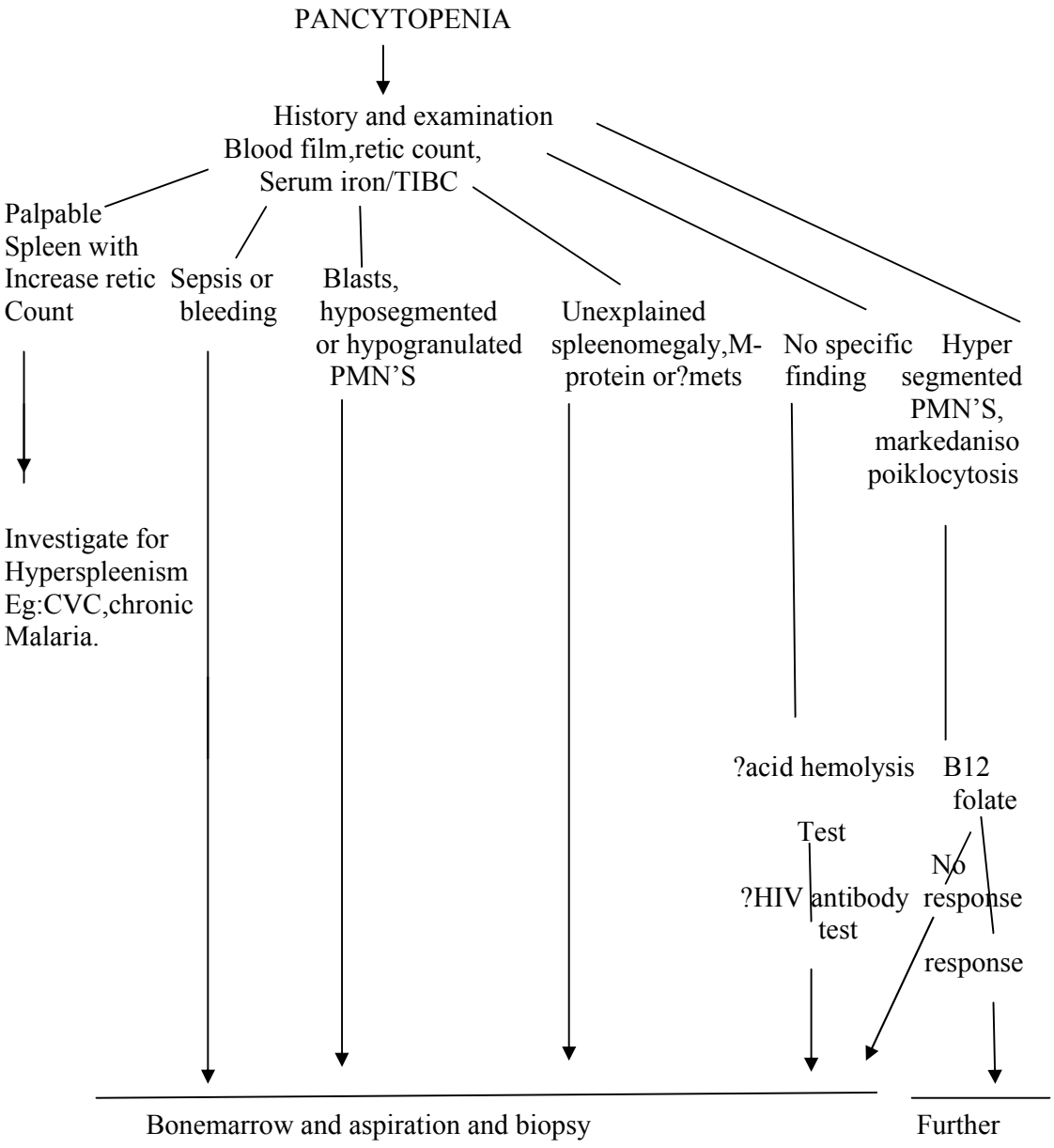
EVALUATION OF THE BIOPSY SPECIMENS

In a marrow smear regularly stained by Romanowsky method, 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 histologic pattern can often be diagnostic of the type of neoplasm[30].

In some conditions, such as myelofibrosis and hairy cell leukemia the bone marrow cannot be aspirated and biopsy is necessary to establish the diagnosis[25].

Trephine biopsy plays a vital role in histochemistry ,immunohistochemistry ,cytogenetics ,biochemical and electron microscopic studies[27].

DIAGNOSTIC APPROACH TO THE PANCYTOPENIC PATIENT[2]



PMN= Polymorhonuclear neutrophil,TIBC=total iron binding capacity,
HIV=human immunodeficiency virus, CVC = Chronic venous congestion

METHODOLOGY

The present prospective study on " Etiological Profile Of Pancytopenia-" was undertaken during the period of March 2010 to August 2011. Fifty patients from Government General Hospital attached to Coimbatore Medical College , Coimbatore formed the material of the study . The study samples were analyzed in Haematology Unit, Department of Pathology, Coimbatore Medical College, Coimbatore. Patient selection was based on clinical features and supported by laboratory evidences.

Bone marrow aspiration and trephine biopsy was subsequently carried out after obtaining written consent from the patient or the guardian.

Inclusion criteria

Presence of three of the following

- Haemoglobin < 9 g/dl
- TLC <4000/cumm and
- Platelet count <1,00,000 / cumm
- Patients whose bone marrow had diagnostic aspirate

Exclusion criteria :

- Patients on myelotoxic chemotherapy.
- Age <2years and >60 years.

A proforma was used to document demographic data, clinical presentation, dietary history, past history of anaemia, blood transfusions and drugs and treatment history refractory to treatment as given in the annexure-1. Details of physical examination were obtained from medical records of patients.

The study was conducted in a routine hematology laboratory at the same hospital. Three ml of blood sample was collected aseptically from each subject into tri-potassium ethylenediamine tetra-acetic acid (K3EDTA) anticoagulant bottle. This was thoroughly mixed for complete blood count (CBC) analysis. Blood sample was divided into 2 parts as follows: Two ml for manual method and one ml for automated method using hematology auto analyzer Sysmex KX-21. All manual samples were analyzed using standard hematological method as described by Dacie and Lewis [6]. All samples were analyzed within 30 minutes of collection.

The laboratory tests performed were:

1. CBC using Sysmex KX-21 automated analyser and the analysis was done following the manufacturer's operational guidelines.
2. In cases of very low counts and abnormal cells, a manual review of the instrument's results was performed using the improved Neubauer counting chamber using appropriate diluting fluids
3. A blood film was stained by the Leishman stain and evaluated for red cell morphology, platelet count and white cell morphology .
4. Reticulocyte count using 1% Brilliant Cresyl Blue for supravital staining.
5. Peripheral smear and bone marrow aspiration smear was stained by Leishman stain for all the cases and examined in detail.
6. Bone marrow trephine biopsy was done for each case,then proceeded with fixation, partial decalcification and stained with H&E.
7. Added to it reticulin stain was done and the extent of fibrosis was graded.

AUTOANALYSER[39]

PRINCIPLE

The hematological parameters were obtained from EDTA blood sample analyzed using SYSMEX –KX 21 autoanalyser. The principles of autoanlyser is as follows:

Principles of this analyser includes electric impedence, light scattering and radiofrequency conductivity. : This method detects the size of the blood cells by changes in direct-current resistance, and the density of the blood cell interior by changes in radio-frequency resistance. A blood sample is aspirated and measured, diluted to the specified ratio, and send to the applicable detector chamber. Inside the chamber is a tint hole called an "aperture," on both sides of which are electrodes. Between the electrodes flow the direct current and radio-frequency current.

Blood cells suspended in the diluted sample pass through the aperture, changing the direct-current resistance and radio-frequency resistance between the electrodes. The size of the blood cell is detected via changes in the direct-current resistance, and the density of the blood cell interior (size of the nucleus) is detected via changes in the radio frequency resistance, with such detections coming in the form of electrical pulses. Based on the size of these pulses, a two dimensional distribution (scatter gram) of the blood-cell size and internal density can be drawn.

Samples were analysed in autoanalyser and the values of 18 parameters was obtained in the printed format as given in annexure 3 table - (3).

PERIPHERAL SMEAR

From the available venous sample a tongue shaped peripheral smear was made in a glass slide. Following air drying smear was stained with leishmans stain as follows :

LEISHMANS STAIN

1. Slide is exposed to undiluted stain solution for 2min.
2. Without removing the stain from the horizontal slide, double the amount of buffer is carefully added ,and mixed by blowing gently and allowed for staining for 8-10mins.
3. The stain is flushed from the horizontal side with water but not more than 30secs.
4. The back of the slide is cleaned with gauze and allowed to air dry.

Examination of blood smear

Each blood smear is examined for adequacy, morphology and any immature forms in red cells, white cells and platelets. Thorough examination for the presence of any hemoparasites was also done.

Reticulocyte count

A small amount of 1% briliant cresyl blue stain was filtered into a test tube. Two drops of the filtrate and two drops of well-mixed blood specimen was transferred into a small test tube with the help of two separate Pasteur pipettes. New methylene blue stain and the blood specimen were mixed and the test tube was covered with a cork to prevent evaporation. The test tube was left undisturbed for 15 minutes at 37 ^{0}C.

After 15 minutes the contents of the tube were removed and one small drop of the mixture was transferred to a clean grease-free slide. A thin smear was prepared with the help of a spreader slide. Smear was air dried. The smear was first examined

under the low power objective for scanning and a thin portion of the smear where the red cells were evenly distributed was located. Reticulocytes were identified by the fine, deep violet filaments arranged in a network and fine dot like structure.

The slide is examined under the microscope using the oil immersion objective. To decrease the eye field a small circular piece of black paper in the centre of which has been cut a small sqaure with sides of about 4 mm in length was inserted into the eyepiece. A total of 1000 red blood cells were counted using the oil immersion field and during the count, number of reticulocytes were noted and the percentage was calculated using the formula

$$\text{Reticulocyte count}(\%) = \frac{\text{Number of reticulocytes counted}}{\text{Number of red cells counted}} \times 100$$

Normal value → 0.5 – 2.5% (reference Dacie & Lewis)

BONE MARROW ASPIRATION

Bone marrow aspiration was performed in all the patients using Salah needle after obtaining written consent for the procedure either from the patient or the guardian. The aspiration site was prepared, cleaned with an antiseptic (spirit and betadine), scrubbed and draped, exposing only the aspiration site. Skin and the area down to the periosteum was infiltrated with 2 ml of local anaesthetic (2% lignocaine) using 5 ml syringe.

The needle with the stylet in place was introduced into the site by gentle screwing motion, after adjusting the guard to appropriate length. The outer plate of bone was pierced with a gentle boring motion. As the marrow cavity was entered, sensation of giving in was experienced. Then the stylet was removed, and 0.2 ml of marrow material was aspirated with the help of 10 ml disposable syringe.

The aspirate was transferred to a set of slides and films were prepared by crushing the marrow particles. The needle was withdrawn and the puncture site was sealed with tincture benzoin swab. In cases of unsuccessful attempts of bone marrow aspiration, a repeat aspirate was done at the different site and only cases where diagnostic aspirate could be obtained have been included in the study.

Slides were dried and later stained with Leishman stain and marrow aspiration smears were examined for

i) Cellularity v) Megakaryopoiesis

ii) M:E ratio vi) Others – plasma cells, lymphocytes, mast cells

iii) Erythropoiesis vii) Parasites

iv) Myelopoiesis viii) Abnormal cells

(Comparative normal ranges of BM – DC is given in annexure 3 table -2)

BONE MARROW TREPHINE BIOPSY

Following aspiration, bone marrow biopsy is done in the same site using the Jamshidi needle and its immediately fixed in 10% formalin. The biopsy is processed to obtain paraffin wax blocks. Less than 4µm thick sections are cut and stained with hematoxylin and eosin stain and reticulin.

Stained slides were examined for the following:

I. Cellularity of bone marrow.

II. Bone marrow architecture.

III. Bone structure.

IV. Focal lesions

V. Marrow fibrosis analysed using Reticulin stain.

RETICULIN STAIN

Bone marrow biopsy sections can be stained for reticulin using a silver impregnation technique, such as Gomori's stain that probably identifies the glycoprotein matrix in which the collagen fibrils lie, rather than the collagen fibrils themselves.

The reticulin stain is based on the high content of hexose sugars in reticulin. Reticulin fibres have little natural affinity for silver solutions and must be pretreated to produce sensitized sites where silver will deposit.

1) Deparaffinize sections and bring to water.
2) Treat with 1%potassium permanganate solution -1min (the adjacent hydroxyl groups of the hexose sugars of glycoproteins are oxidized to aldehydes by potassium permanganate.)
3) Rinsed in tap water.
4) Bleached in 2%potassium metabisulfate solution-1 min,then rinsed in tap water.
5) Sensitized with ferric ammonium sulphate – 1 min and washed in distilled water.
6) Impregnated in silver solution (containing silver nitrate , KOH, and ammonia) for 1 min.
7) Washed in distilled water several times.
8) Reduced in 20% formalin solution for 3 minutes (local silver reaction is amplified to produce visible silver deposits). Then rinsed in tap water.
9) Toned in 0.2 % gold chloride for 10 minutes. Rinsed again in tap water.
10) Treated with 2 % potassium metabisulfite solution for 1 min. Rinsed again in tap water.

11) Treated with 2 % sodium thiosulfate solution for 1 min . Rinsed again in tap water.

12) The section is typically counterstained with eosin so the defined reticulin fibres appear black against a background of red tissue

Ideally, both a trichrome collagen stain and a reticulin stain should be performed and both the type and amount of fibrosis should be described using a clearly defined grading scale; however, in practice, collagen is almost never revealed by trichrome stain unless there is a marked increase in reticulin.

Grading of fibrosis was done using the Bauermister scale as 0-4.

Quantification of bone marrow reticulin and collagen (Bauermeister, 1971; Bain et al, 2001)

Modified Bauermeister scale

Grades	Morphology
0	No reticulin fibres demonstrable
1	Occasional fine individual fibres and foci of a fine fibre network
2	Fine fibre network throughout most of the section; no coarse fibres
3	Diffuse fibre network with scattered thick coarse fibres but no mature collagen (negative trichrome staining)
4	Diffuse, often coarse fibre network with areas of collagenization (positive trichrome staining)

OBSERVATION AND RESULTS

Out of 4370 cases enrolled in the Inpatient section of Hematology Unit in the Department of Pathology, Coimbatore Medical College and Hospital patients who presented with pancytopenia were analysed.A total of 50 cases were enrolled as per inclusion and exclusion criteria.

TABLE 3

PROCEDURE CARRIED OUT IN 50 PATIENTS

Sl.No.	Procedure	No. of patients
1	Baseline haematological procedure	50
2	Bone marrow aspiration	50
3	Bone marrow trephine	50
4	Reticulin stain	50

In the present study 50 patients were taken for the study of Baseline haematological procedure, Bone marrow aspiration, Bone marrow trephine and Reticulin stain (chart-1).

TABLE 4

DISTRIBUTION OF VARIOUS CAUSES OF PANCYTOPENIA

Sl. No	Causes	No. of cases	Percentage
1	Megaloblastic anemia	34	68
2	Aplastic anemia	7	14
3	Myelofibrosis	6	12
4	Subleukemic leukemia	2	4
5	Myelodysplastic syndrome	1	2
Total		50	100

In the present study, Megaloblastic anemia was the commonest cause constituting 68% followed by Aplastic anemia (14%), Myelofibrosis (12%), Subleukemic leukemia (4%) and Myelodysplastic syndrome (2%) (chart-2).

TABLE 5

INCIDENCE OF PANCYTOPENIA IN DIFFERENT AGE GROUPS

Sl. No.	Age group (years)	No. of cases	Percentage
1	2-10	3	6
2	11-20	5	10
3	21-30	9	18
4	31-40	4	8
5	41-50	15	30
6	51-60	14	28
Total		**50**	**100**

In the above age groups frequency table, it is observed that the incidence of pancytopenia is high (30%) in the age group of 41-50 followed by the age group of 51-60 (28%), 21-30 (18%), 11-20 (10%), 31-40 (8%) and 2-10 (6%) (chart-3).

TABLE 6

INCIDENCE OF PANCYTOPENIA IN DIFFERENT SEX GROUPS

Sl. No	Sex	No. of cases	Percentage
1	Male	23	46
2	Female	27	54
Total		50	100

From the above table, it is seen that the incidence of pancytopenia is comparatively high in women than men (chart-4)**.**

TABLE 7

AGE-WISE AND SEX-WISE DISTRIBUTION AMONG 50 PATIENTS, UNDER THE PRESENT STUDY

Sl.no.	Age group (years)	Females	Males	Total	Percentage
1	2-10	3	0	3	6
2	11-20	4	1	5	10
3	21-30	4	5	9	18
4	31-40	2	2	4	8
5	41-50	7	8	15	30
6	51-60	7	7	14	28
TOTAL		27	23	50	100

The average age of the women is 35 .5 years ,and the men is 39yrs with the standard deviation17.44 and 13.56 respectively . The coefficient of variation for the females is 49.11 while for the males is 34.79 . From this, it is evident that there is a vast variation in the age group of females (chart-5).

TABLE 8

PRESENTING COMPLAINTS AND PHYSICAL FINDINGS IN PANCYTOPENIA

Sl.no.	Presenting complaints &physical findings	No .of .patients	Percentage
1	Generalized weakness	50	100
2	Dyspnoea	23	46
3	Fever	23	46
4	Bleeding manifestation	1	2
5	Weight loss	5	10
6	Pallor	43	86
7	Splenomegaly	18	36
8	Hepatomegaly	20	40
9	Jaundice	4	8
10	Bony tenderness	1	2
11	Lymphadenopathy	1	2

For this study of pancytopenia, various physical complaints and findings were taken into account. All the patients were affected by the generalized weakness. Nearly 46% of the them had Dyspnoea and Fever ,nearly 86% of the cases had Pallor; 40% cases had hepatomegaly, 36% had splenomegaly, 10% of the cases had weight loss,

8% had jaundice and 2% had bony tenderness, lymphadenopathy and bleeding manifestations,each(chart-6).

TABLE 9

VITAL HAEMATOLOGICAL PARAMETERS IN CASES OF PANCYTOPENIA

Sl.no	Parameter	Range	No .of. cases	Percentage
1	Hemoglobin(gm%)	1.8-5	20	40
		5.1-8	24	48
		8.1-9.0	6	12
	Total		50	
2	Total leukocyte Count (cells/mm3)	500-1,000	3	6
		1,001-2,500	14	28
		2,501-3,900	33	66
	Total		50	
3	Reticulocyte Count(%)	<0.5	13	26
		0.6-1	24	48
		1.1-2	13	26
	Total		50	
4	Platelet count (cells/mm3)	10,000-50,000	22	44
		51,000-80,000	25	50
		81,000-95,000	3	6
Total			50	

Hemoglobin percentage:

Hemoglobin percentage varied from 1.8 % - 9.0g %. Most of the patients had Hemoglobin percentage between 5.1 – 8g %. While 40 % had Hemoglobin percentage between 1.8 – 5g %, 12 % had Hemoglobin percentage between 8.1 – 9.0g %.

Total leukocyte count:

Total leukocyte count ranged from 500 – 3,900 cells/mm^3. Almost two-third of the patients (66%) had leukocyte count in the range 2,501 – 3,900 cells/mm^3. Only 6 % of the patients had white cell count in the range of 500 – 1,000 cells/mm^3.

Reticulocyte count:

Reticulocyte count ranged from 0.5 – 2 %. Almost half of the patients (48 %) had reticulocyte count between 0.6 – 1 %. Among the remaining 52 % of the patients, half of them (26 %) had the reticulocyte count< 0.5% and the remaining 26 % of the patients had reticulocyte count in the range 1.1 – 2%.

Platelet count:

Platelet count varied from 10,000 – 95,000 cells/mm3. While 50 % of the patients had their Platelet count in the range 51,000 – 80,000 cells/mm3, another 44 % of the patients had their Platelet count in the range 10,000 – 50,000 cells/mm3. Only 6 % of the patients had platelets count in the range of 81,000 – 95,000 (chart-7).

TABLE 10

PERIPHERAL BLOOD PICTURE IN PANCYTOPENIC PATIENTS

Sl.No.	Procedure	No. of patients	Percentage
1	Dimorphic anaemia	23	46
2	Macrocytic anaemia	12	24
3	Normocytic normochromic	10	20
4	Normocytic hypochromic	5	10
	Total	50	100

In the present study of 50 patients pancytopenia, the incidence of Dimorphic anaemia was higher (46%) than Macrocytic anaemia (24%), Normocytic normochromic anemia(20%) and Normocytic hypochromic anemia(8%) (chart-8).

TABLE 11

CELLULARITY OF BONE MARROW

Sl.no	Type of cellularity	No.of patients	Percentage
1	Hypercellularity	38	76
2	Hypocellularity	11	22
3	Normocellularity	1	2
	Total	50	100

In the present study, Hypercellularity is the commonest association constituting 78% followed by Hypocellularity (20%) and Normocellularity (2%) (chart-9).

TABLE 12

CAUSES OF HYPERCELLULAR BONE MARROW ASSOCIATED WITH PANCYTOPENIA

sl.no	**Aetiology**	**No.of cases**	**Percentage**
1	Megaloblastic anemia	33	87
2	Sub leukemic leukemia	2	5
3	Cellular phase of MF	2	5
3	MDS	1	3
Total		38	100

In the present study ,out of the 40 patients with hypercellular bone marrow, Megaloblastic anemia was observed in 87%, Cellular phase of MF in 5%, Sub leukemic leukemia in 5% and MDS in 3% (chart-10).

TABLE 13

INCIDENCE OF MEGALOBLASTIC ANAEMIA IN DIFFERENT AGE GROUPS

Sl. No.	Age group (years)	No. of cases	Percentage
1	2-10	2	6
2	11-20	3	9
3	21-30	8	23.5
4	31-40	2	6
5	41-50	11	32
6	51-60	8	23.5
Total		34	100

Age incidence:

Megaloblastic anemia showed highest incidence (32%) in the age group of 41-50years , equal incidence (23.5%) in age groups of 21-30years and 51-60years followed by 9% in 11-20years,6% in the age group of 2-10years and 31-40years respectively (chart-11). Figures 5&6 depicts the bone marrow picture of magaloblastics anemia.

TABLE 14

GENDER DISTRIBUTION OF MEGALOBLASTIC ANEMIA

Sl.no.	Sex	No.of cases	Percentage
1	Male	16	47
2	Female	18	53
Total		34	100

Sex Incidence:

The incidence of Megaloblastic Anaemia is more or less same for men and women. Approximate male to female ratio is 1:1.1(chart-12).

TABLE 15

VITAL PARAMETERS IN CASES OF MEGALOBLASTIC ANAEMIA ASSOCIATED WITH PANCYTOPENIA(n=34)

Parameter	Range	No.of patients
Hemoglobin(gm%)	1.8-5	12
	5.1-8	14
	8.1-9.0	8
Total		34
Total leukocyte Count (cells/mm3)	500-1,000	3
	1,001-2,500	7
	2,501-3,900	24
Total		34
Reticulocyte Count(%)	<0.5	6
	0.6-1	22
	1.1-2	6
Total		34
Platelet count (cells/mm3)	10,000-50,000	15
	51,000-80,000	16
	81,000-95,000	4
Total		34

Hemoglobin percentage:

Hemoglobin percentage varied from 1.8 % - 9.0g %. Half of the patients had Hemoglobin percentage between 5.1 – 8g %. While 12 patients had Hemoglobin percentage between 1.8 – 5g%, 8 patients had Hemoglobin percentage between 8.1 – 9.0g %.

Total leukocyte count:

Total leukocyte count ranged from 500 – 3,900 cells/mm^3 . While 7 patients had leukocyte count in the range 1,001 – 2,500 cells/mm^3, 24 patients had white cell count in the range of 2,501 – 3,900 cells/mm3. Only 3 patients had white cell count in the range of 500 – 1,000.

Reticulocyte count:

Reticulocyte count ranged from 0.5 – 2 %. As much as 65% of the patients had Reticulocyte count between 0.6 – 1 %. Among the remaining 12 patients, 6 of them had the Reticulocyte count< 0.5 and the remaining 6 patients had the Reticulocyte count in the range 1.1 – 2.

Platelet count:

Platelet count varied from 10,000 – 95,000 cells/mm^3. While 15 patients had their Platelet count in the range 10,000 – 50,000 cells/mm^3, an equal number of the patients had their Platelet count in the range 51,000 – 80,000 cells/mm^3. Only 4 patients had white cell count in the range of 81,000 – 95,000(chart-13).

TABLE 16

RBC MORPHOLOGY IN MEGALOBLASTIC ANAEMIA

Sl.No.	Blood picture	No. of cases	Percentage
1	Dimorphic anaemia	14	41
2	Macrocytic anaemia	11	32
3	Normocytic normochromic	7	21
4	Normocytic hypochromic	2	6
Total		34	100

Majority of the cases (41 %) showed Dimorphic anaemia, 32% of the patients showed Macrocytic anaemia. While 21 % of the cases showed Normocytic normochromic anemia, 6 % showed Normocytic hypochromic anemia (chart-14).

TABLE 17

PERIPHERAL SMEAR PICTURE IN MEGALOBLASTIC ANEMIA

Sl.no	Morphology	No.of.patients	Percentage
1	Macrocytosis	34	100
2	Hypochromasia	22	65
3	Anisopoiklocytosis	34	100
4	Hypersegmentation of neutrophils	30	88
5	Leukoerythroblastosis	15	44

While all the 34 patients had Macrocytosis and Anisopoiklocytosis, as much as 88% had Hypersegmentation of neutrophils. Two-third of the patients had Hypochromasia (chart-15 & figure 4).

TABLE 18

INCIDENCE OF BONE MARROW HYPOPLASIA IN DIFFERENT AGE GROUPS

Sl.no	**Age group (years)**	**No.of cases**	**Percentage**
1	2--20	1	14
2	21-40	2	28
4	41-60	4	58
Total		**7**	**100**

Age Incidence:

Bone marrow hypoplasia showed its highest incidence (58%) in the age group 41-60 followed by the age groups 21-40 (28%), and 2-20 (14 %) (chart-16). Figures 7&8 depicts the bone marrow hypocellularity.

TABLE 19

INCIDENCE OF BONE MARROW HYPOPLASIA IN BOTH GENDERS

Sl.no.	**Gender**	**No.of cases**	**Percentage**
1	Male	3	42
2	Female	4	58
Total		**7**	**100**

Sex Incidence:

The incidence of Bone marrow hypoplasia is comparatively higher in women than men**,** approximate male to female ratio being 2:3(chart-17).

TABLE 20

VITAL HAEMATOLOGICAL PARAMETERS IN BONE MARROW HYPOPLASIA

Parameter	Range	No.of cases
Hemoglobin(gm%)	1.8-5	2
	5.1-8	4
	8.1-9.0	1
Total		7
Total leukocyte Count (cells/mm3)	500-1,000	0
	1,001-2,500	5
	2,501-3,900	2
Total		7
Reticulocyte Count(%)	0.5	1
	0.6-1	5
	1.1-2	1
Total		7
Platelet count (cells/mm3)	10,000-50,000	3
	51,000-80,000	3
	81,000-95,000	1
Total		7

Hemoglobin percentage:

Hemoglobin percentage varied from 1.8 % - 9.0g %. Half of the patients had Hemoglobin percentage between 5.1 – 8g %. While one-third of the patients had Hemoglobin percentage between 1.8 – 5g %, only one patient had Hemoglobin percentage between 8.1 – 9.0g %.

Total leukocyte count:

Total leukocyte count ranged from 500 – 3,900 cells/mm^3. While three fourth of the patients had leukocyte count in the range 1,001 – 2,500 cells/mm^3, one-fourth of the patients had white cell count in the range of 2,501 – 3,900 cells/mm^3. None of the patients had white cell count in the range of 500 – 1,000cells/mm^3.

Reticulocyte count:

Reticulocyte count ranged from 0.5 – 2 %. As much as 74% of the patients had reticulocyte count between 0.6 – 1 %. While 13 % of the patients had the reticulocyte count 0.5 and the remaining 13 % of the patients had the reticulocyte count in the range 1.1 – 2.

Platelet count:

Platelet count varied from 10,000 – 95,000 cells/mm^3. While 84% of the patients had their platelet count in the range 10,000 – 50,000 cells/mm^3 and 51,000 – 80,000 cells/mm^3 each with 42%. Only 1 patient had platelet count in the range of 81,000 – 95,000cells/mm^3(chart-18).

TABLE 21

PERIPHERAL BLOOD PICTURE IN BONE MARROW HYPOPLASIA

Sl.No.	Blood picture	No. of cases	Percentage
1	Dimorphic anaemia	**3**	**44**
2	Macrocytic anaemia	1	**12**
3	Normocytic hypochromic	3	**44**
Total		**7**	**100**

Among 7 cases 44% of the patient had dimorphic anemia, normocytic hypochromic anemia in another 44% of the patients and macrocytic anemia in 12% of cases (chart-19).

TABLE 22

GRADE OF MYELOFIBROSIS AS SEEN BY RETICULIN STAIN

Sl.No.	Grading	No. of cases	Percentage
1	Grade 0	10	20
2	Grade 1	14	28
3	Grade 2	22	44
4	Grade 3	4	8
5	Grade 4	0	0
Total		50	100

While 44 % of the patients had Grade 2, 28 % had Grade 1, 20 % had Grade 0, 8 % had Grade 3. None of the patients had Grade 4 myelofibrosis (chart-20). Varying grades of myelofibrosis are demonstrated by reticulin stain is given in figures 17, 18 and 19.

STATISTICAL ANALYSIS

TABLE 23

T-Test for vital hematological parameters and the clinical presentation

Group Statistics

	Fever	N	Mean	Std. Deviation	Std. Error Mean
TLC (cells/mm3)	Negative	27	2916.67	746.402	143.645
	Positive	23	2458.70	990.713	206.578

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
TLC (cells/mm3)	Equal variances assumed	4.125	.048	1.862	48	.069	457.971	246.004	-36.652	952.594
	Equal variances not assumed			1.820	40.422	.076	457.971	251.611	-50.389	966.331

Values were tabulated in T-Test as above , which analysed the association between the vital hematological parameters and the clinical presentation .Results concluded the statistically significant relationship between the following:

1. TLC count and occurrence of Fever.
2. Platelets count and occurrence of Bleeding.
3. Hb count and occurrence of Dyspnoea and Pallor.
4. TLC, platelets and Hb count and occurrence of Hepatomegaly.
5. TLC, platelets and Hb count and occurrence of Spleenomegaly.

TABLE 24

Correlation of bone marrow study with the final diagnosis.

CROSS TABLE

			Final diagnosis						
			AA	ALL-L2	AML-M2	MA	MDS	MF	Total
BMA	Dry	Count	0	0	0	0	0	3	3
		% within BMA	.0%	.0%	.0%	.0%	.0%	100.0%	100.0%
		% within Final diagnosis	.0%	.0%	.0%	.0%	.0%	50.0%	6.0%
	Hypocellularity	Count	7	0	0	0	0	1	8
		% within BMA	87.5%	.0%	.0%	.0%	.0%	12.5%	100.0%
		% within Final diagnosis	100.0%	.0%	.0%	.0%	.0%	16.7%	16.0%
	Hypercellularity	Count	0	1	1	34	1	0	37
		% within BMA	.0%	2.7%	2.7%	91.9%	2.7%	.0%	100.0%
		% within Final diagnosis	.0%	100.0%	100.0%	100.0%	100.0%	.0%	74.0%
	Normalcellularity	Count	0	0	0	0	0	2	2
		% within BMA	.0%	.0%	.0%	.0%	.0%	100.0%	100.0%
		% within Final diagnosis	.0%	.0%	.0%	.0%	.0%	33.3%	4.0%
Total		Count	7	1	1	34	1	6	50
		% within BMA	14.0%	2.0%	2.0%	68.0%	2.0%	12.0%	100.0%
		% within Final diagnosis	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	86.458[a]	15	.000
Likelihood Ratio	69.222	15	.000
N of Valid Cases	50		

a. 21 cells (87.5%) have expected count less than 5. The minimum expected count is .04.

On the basis of the statistical significance values provided by the Chi-Square Tables, we arrive at the following conclusions:

1. The count of MA,AA ,MF ,ALL,AML ,MDS in the **Final Diagnosis** are statistically related to the count of them in the **Peripheral Smear**.

2. The count of MA, AA, MF, ALL, AML, MDS in the **Final Diagnosis** are statistically related to the count of them in the **Bone Marrow Aspiration (BMA).**

3. The count of MA, AA, MF, ALL, AML, MDS in the **Final Diagnosis** are statistically related to the count of them in the **Bone Marrow Trephine(BMT)**.

The above statistical findings establish the strong correlation between the **peripheral blood findings** with **Bone Marrow Aspirate**..

LIST OF CHARTS

CHART 1

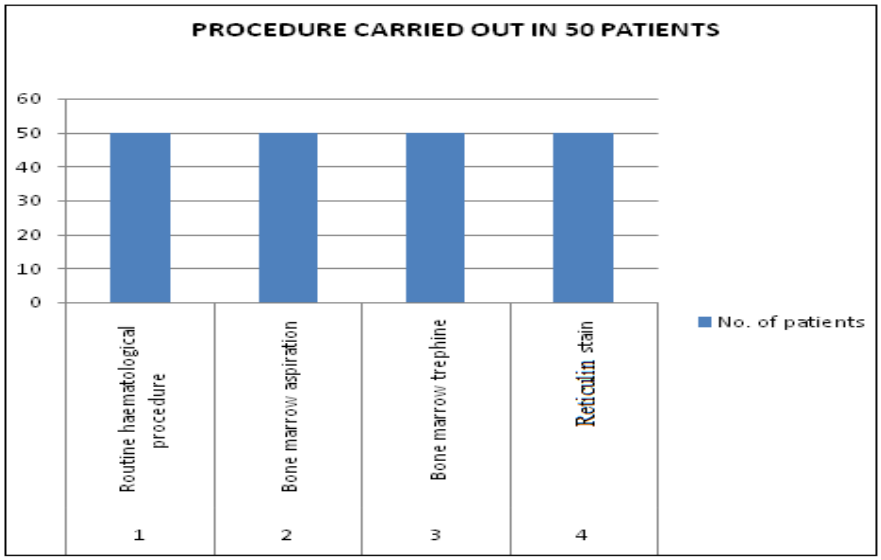
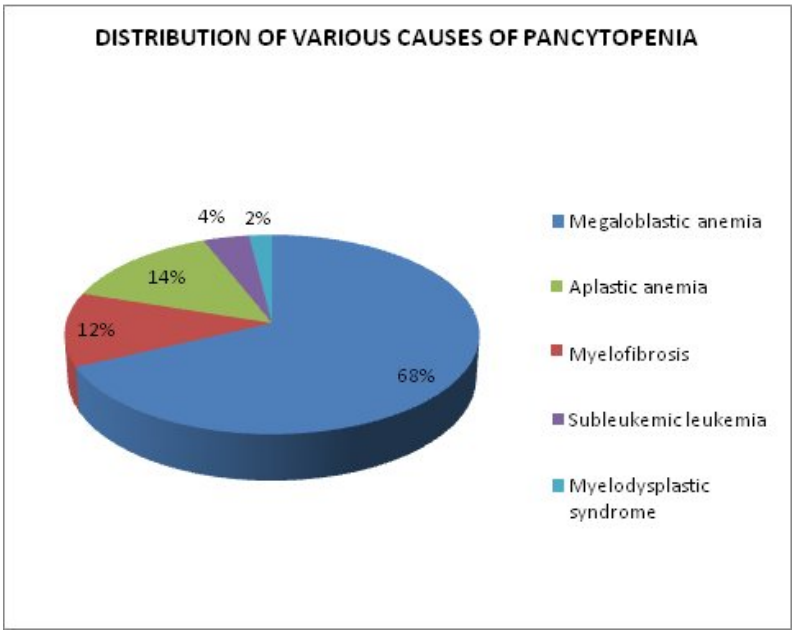


CHART 2



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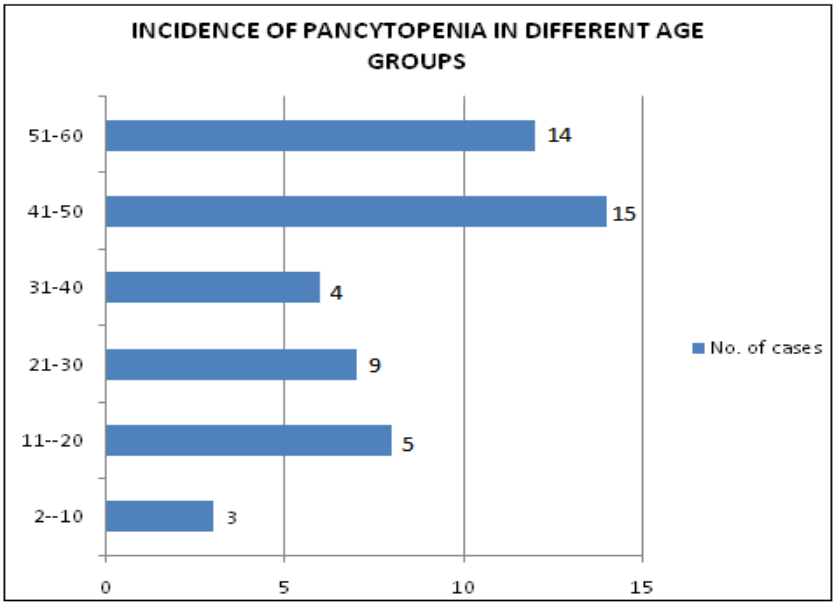

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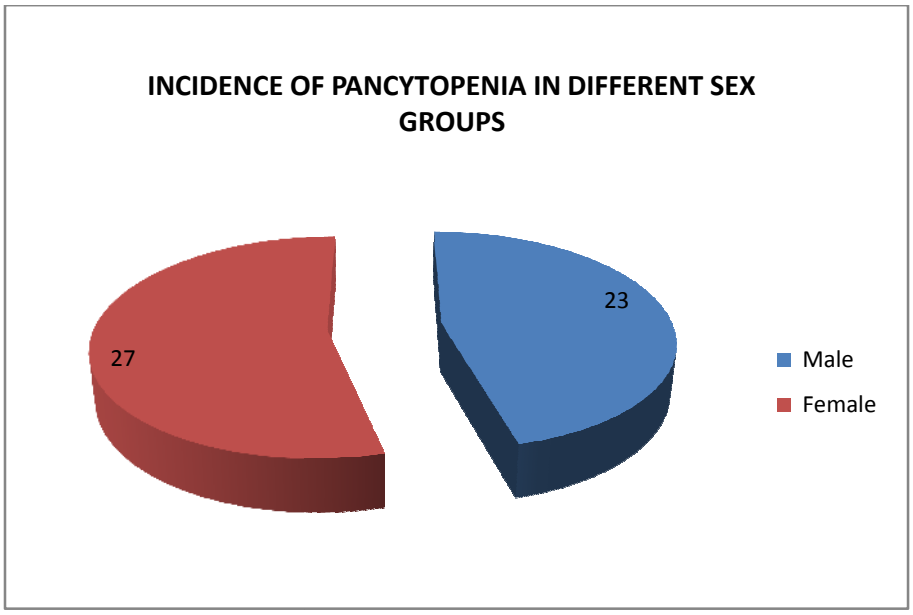

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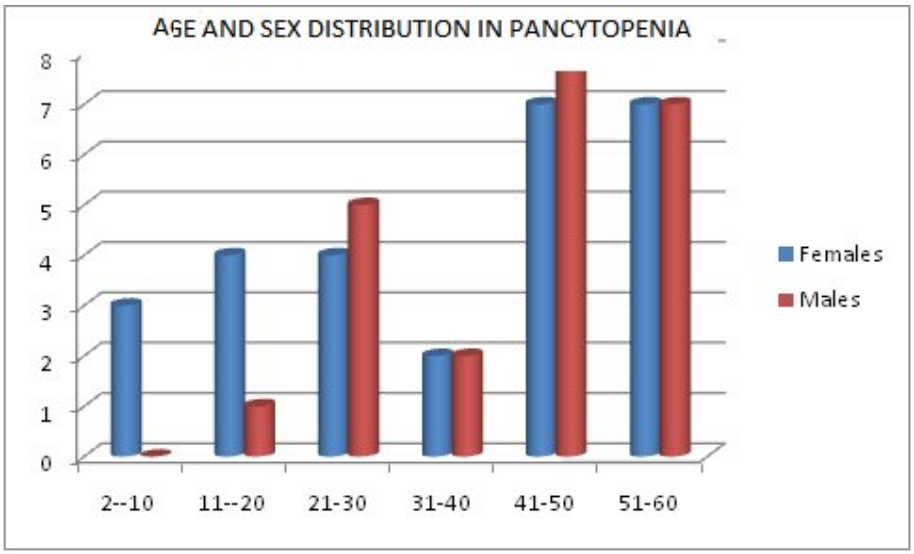

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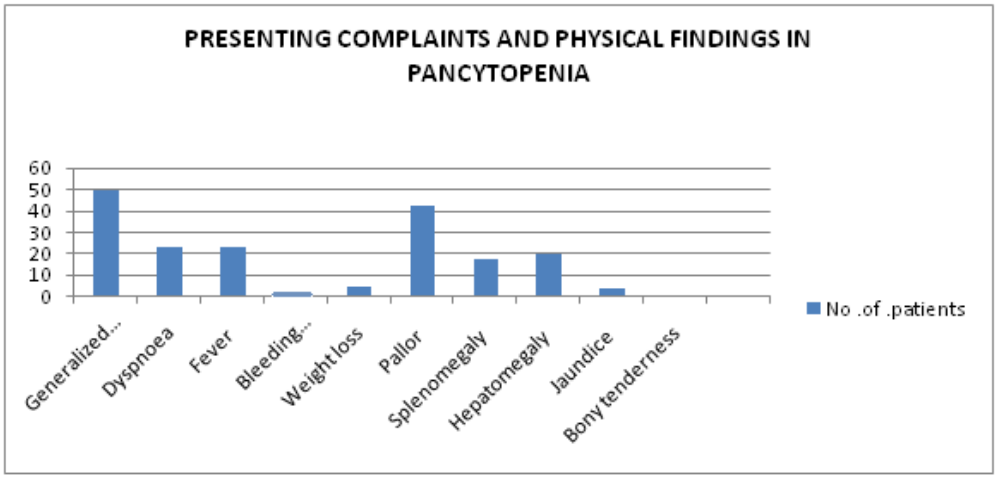

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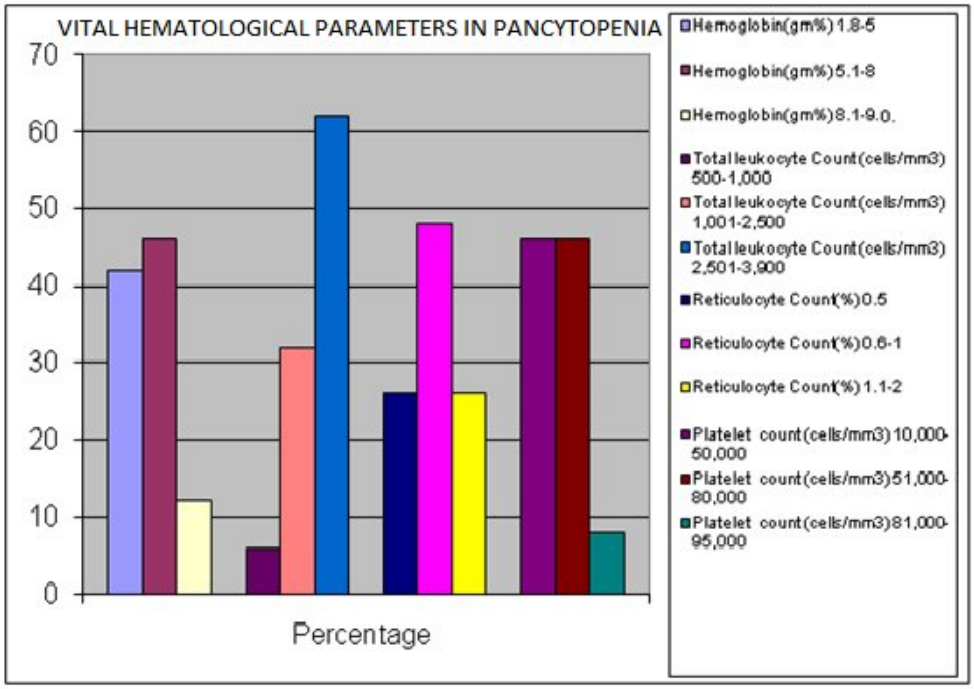

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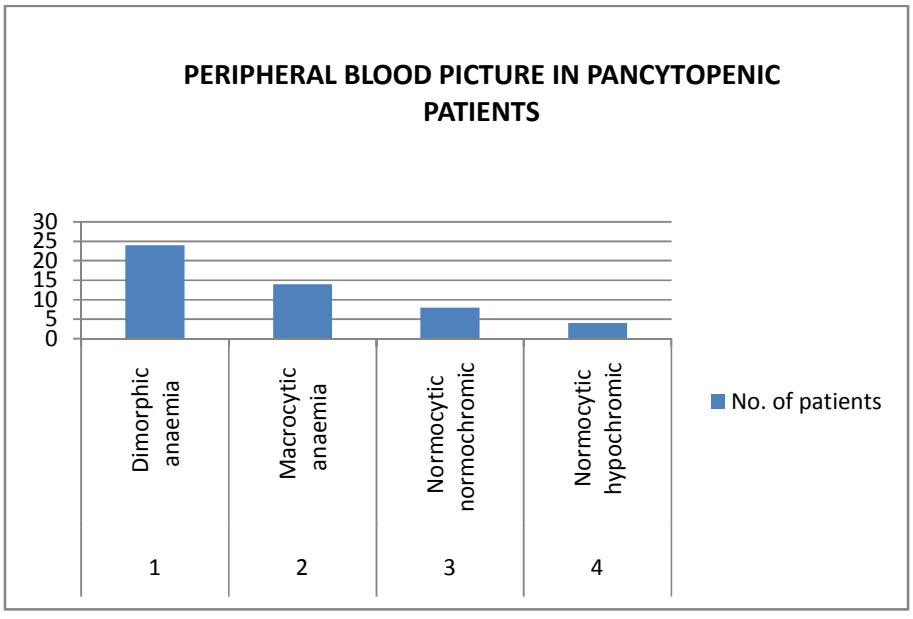

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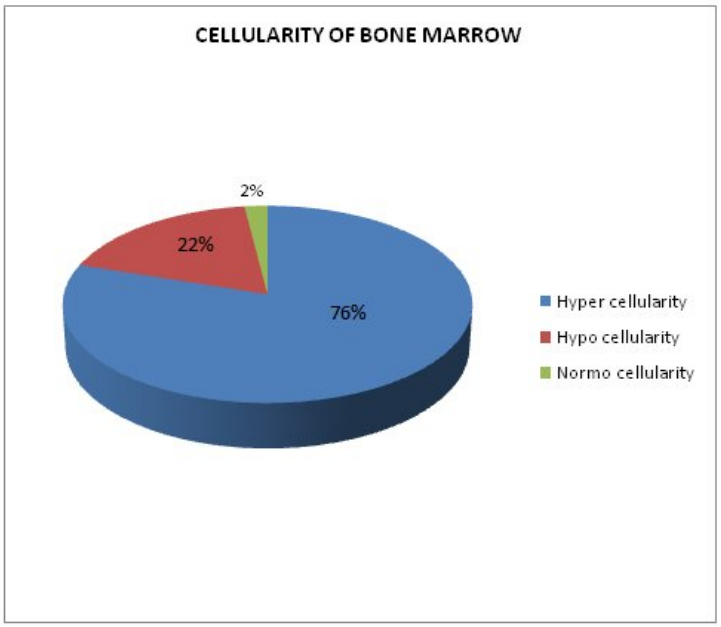

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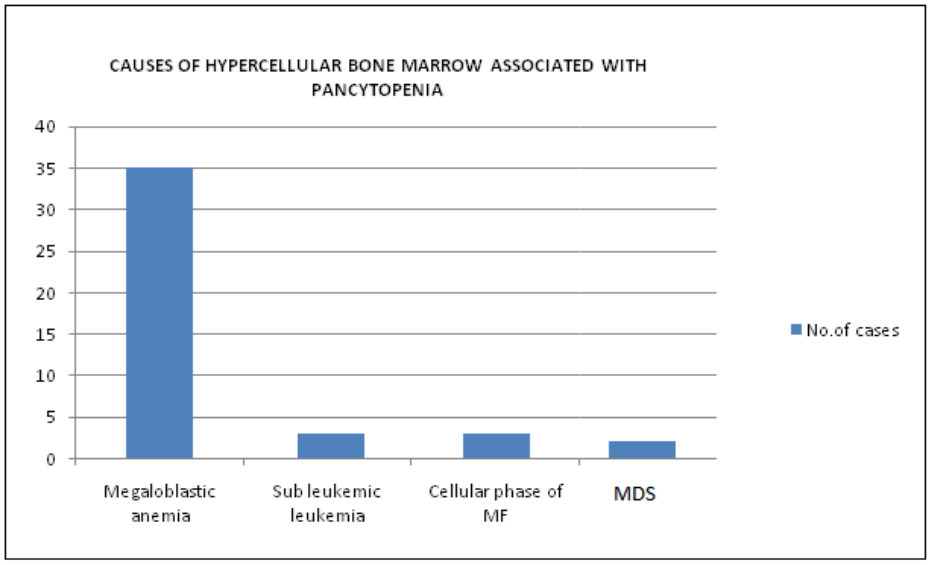

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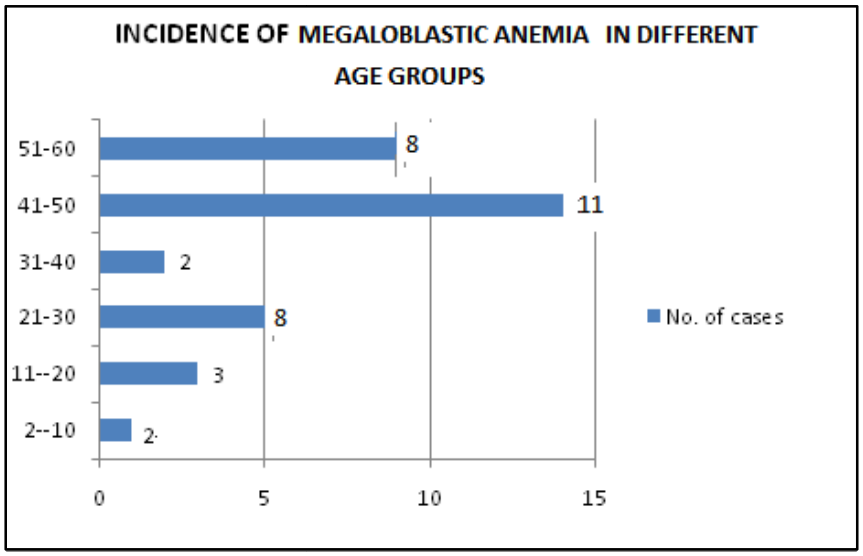

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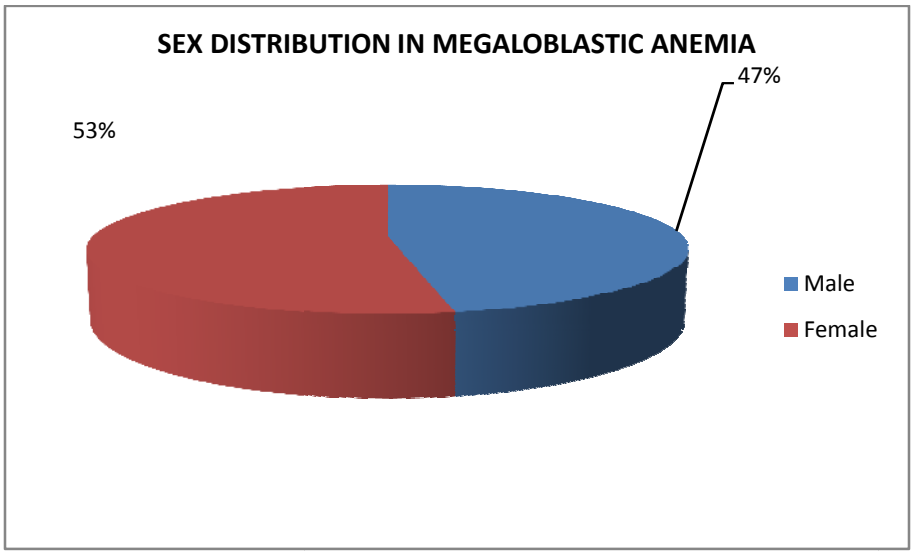

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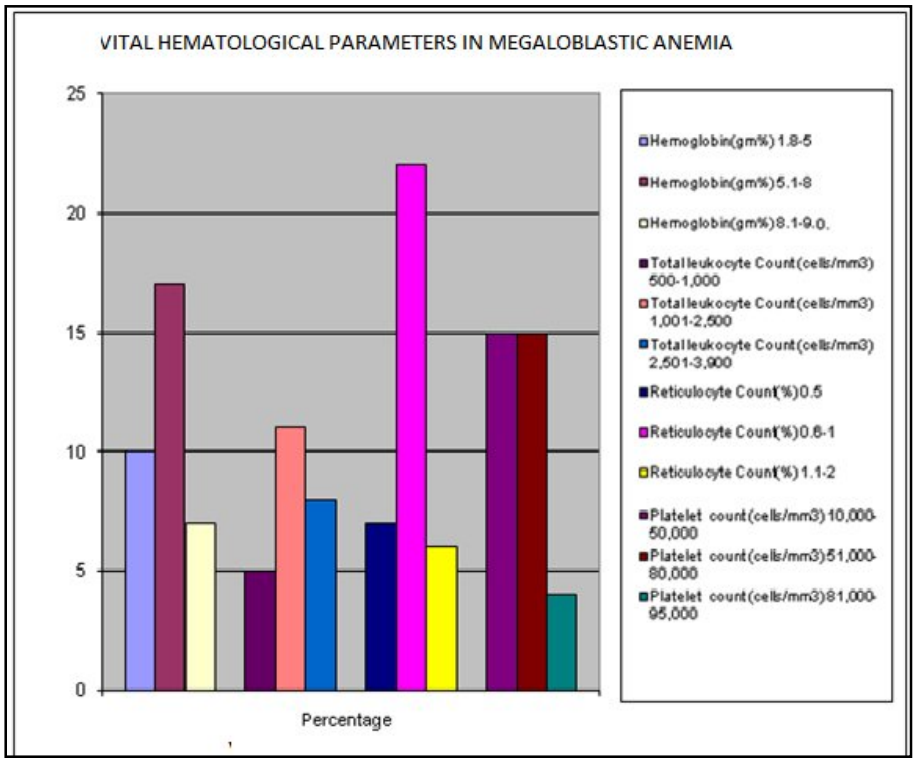

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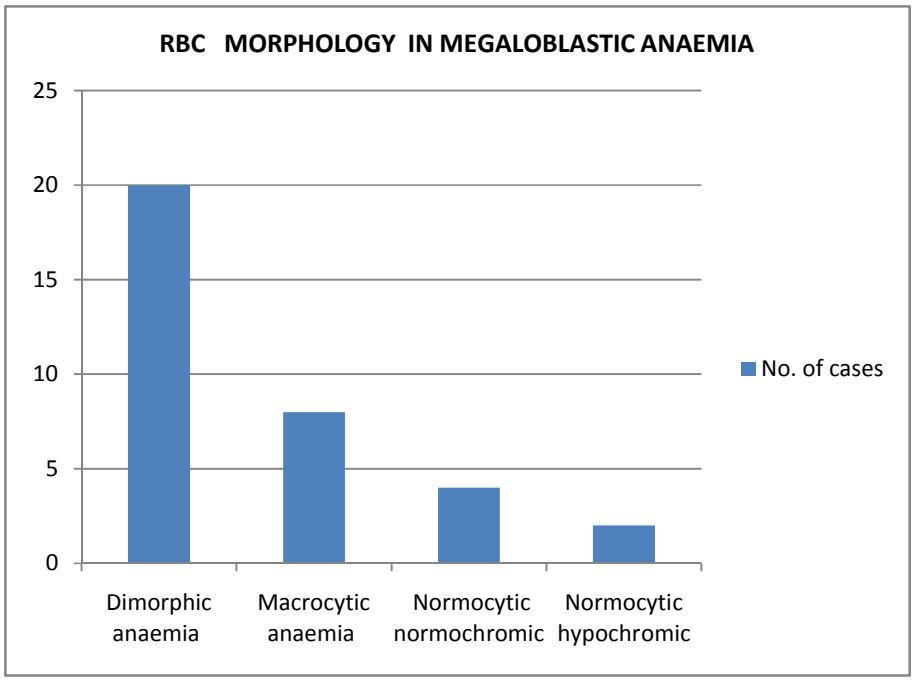

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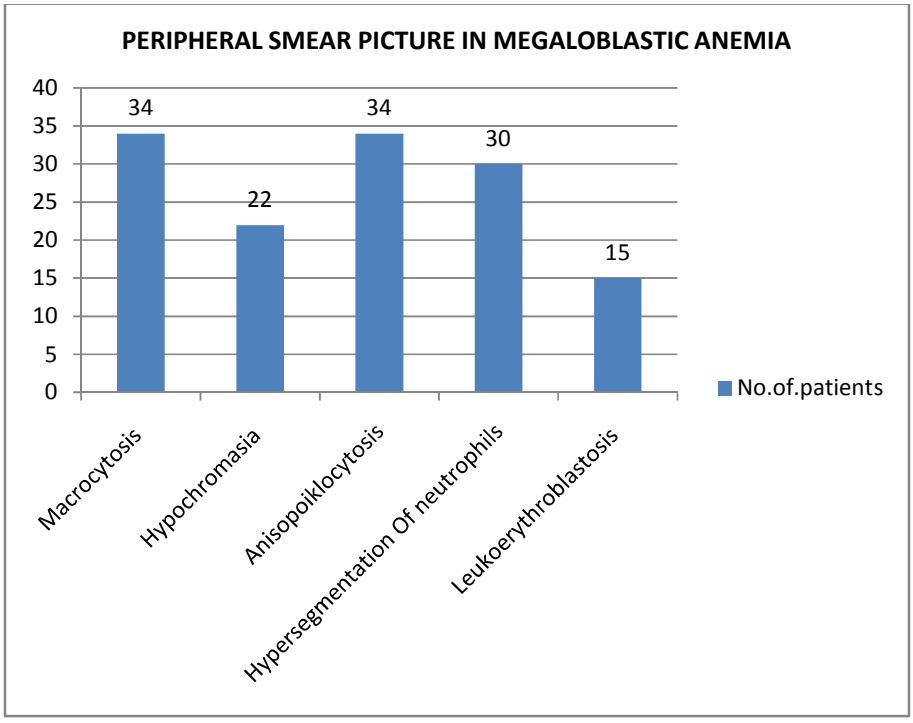

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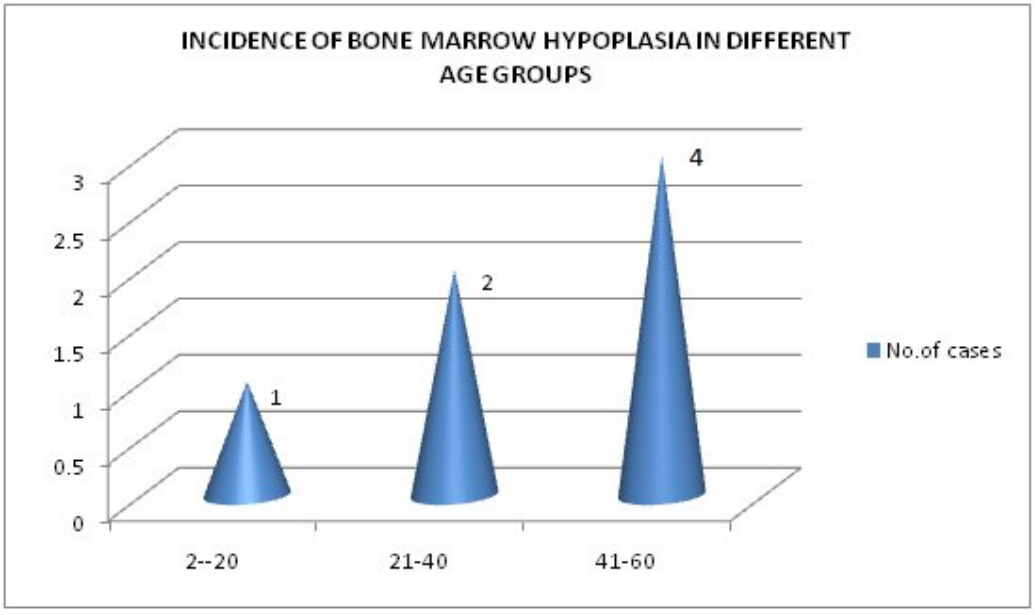

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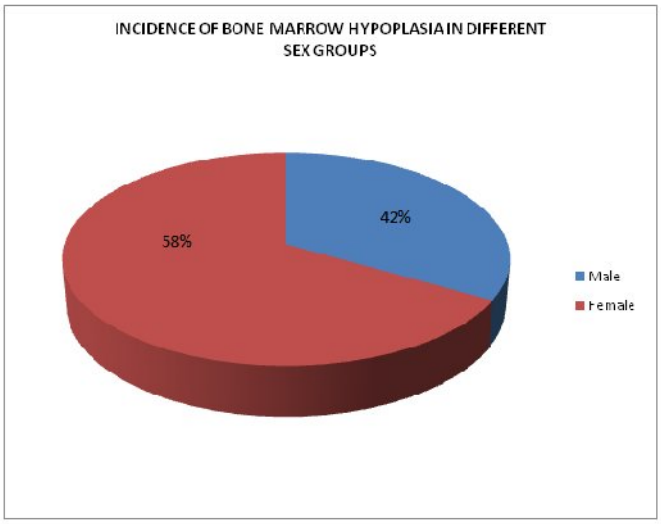

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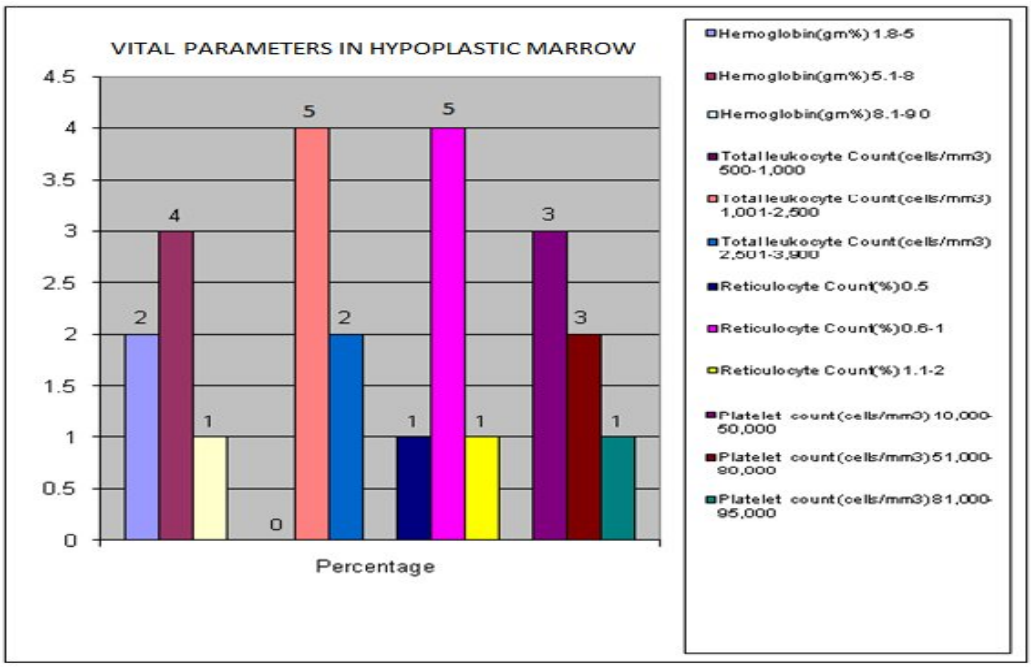

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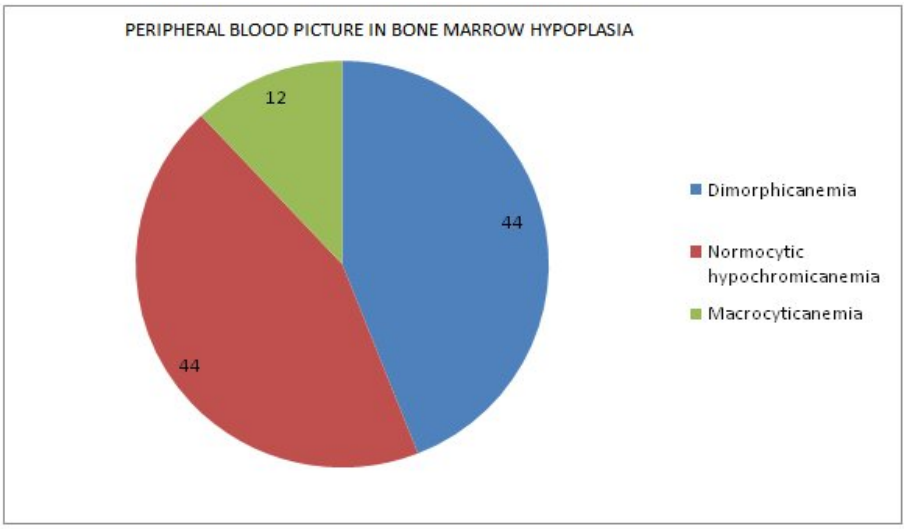

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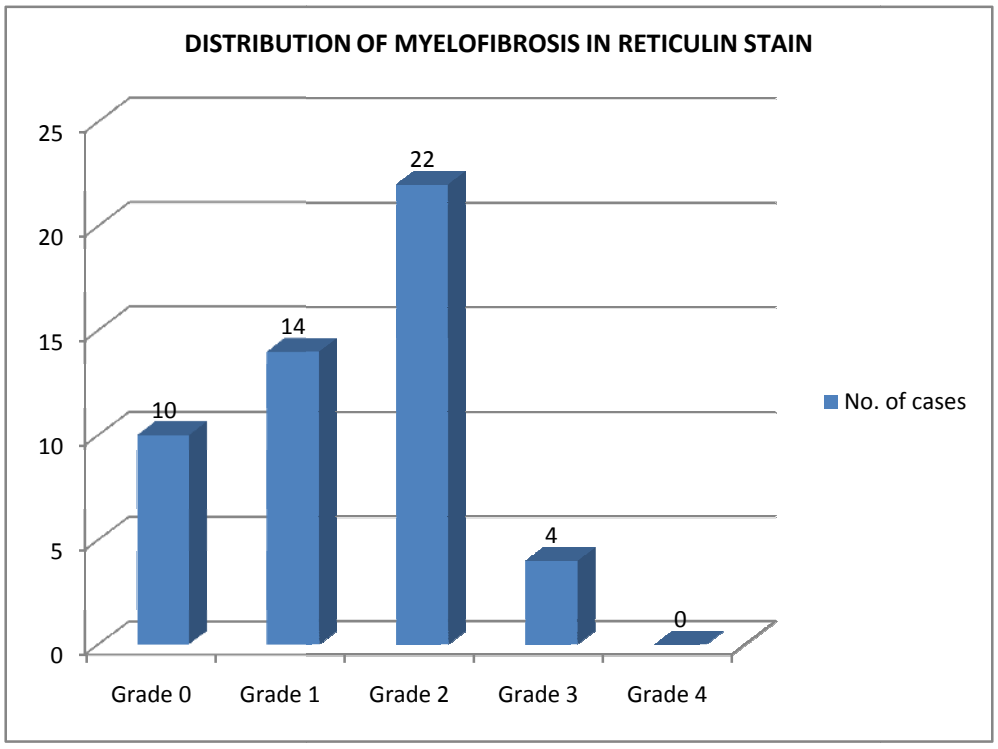

ERYTHROID HYPERPLASIA

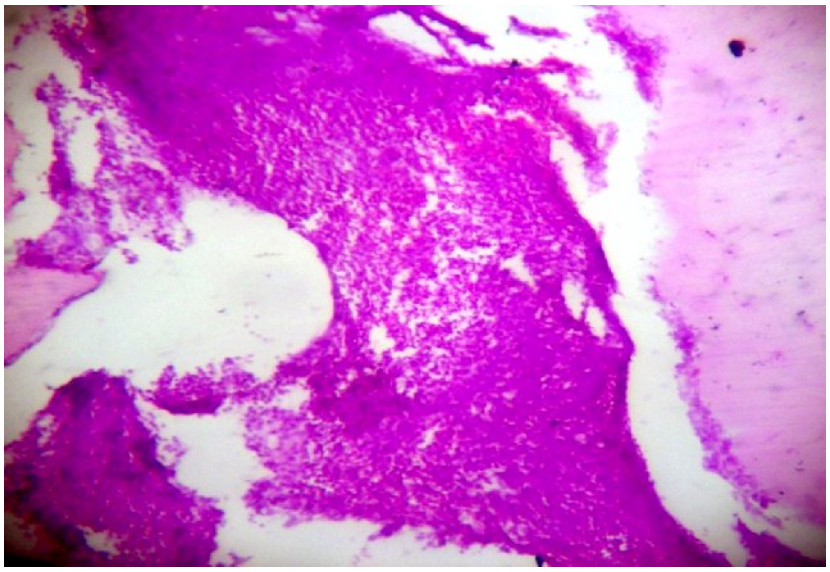

Figure 1:Bone marrow trephine biopsy showing erythroid hyperplasia(100x in H&E).

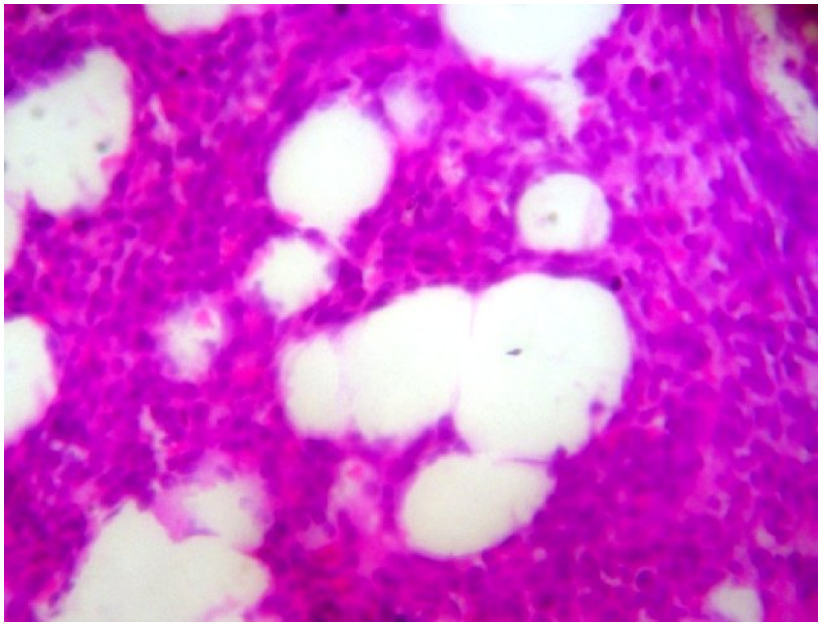

Figure 2:Bone marrow trephine biopsy showing erythroid hyperplasia(400x in H&E).

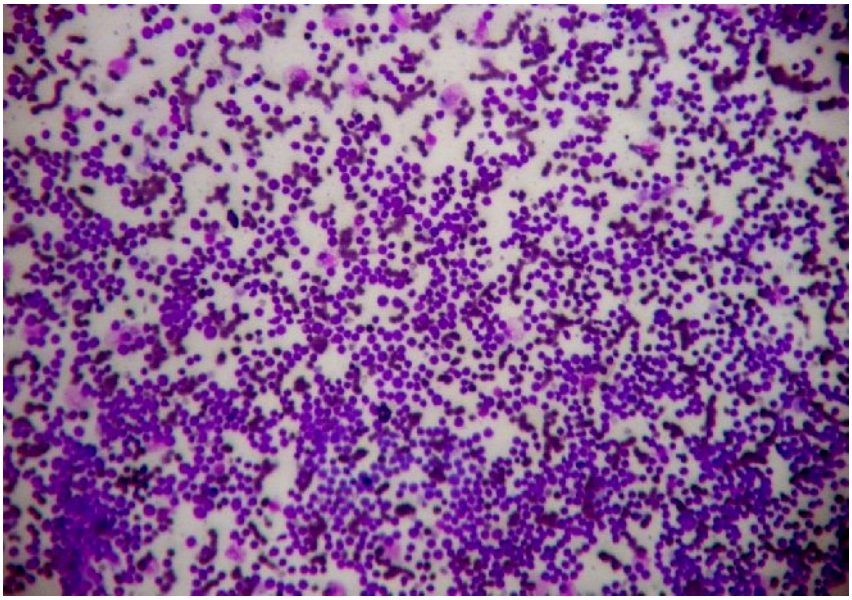

Figure 3: Bone marrow aspiration showing erythroid hyperplasia(400x in Leishman's).

MEGALOBLASTIC ANEMIA

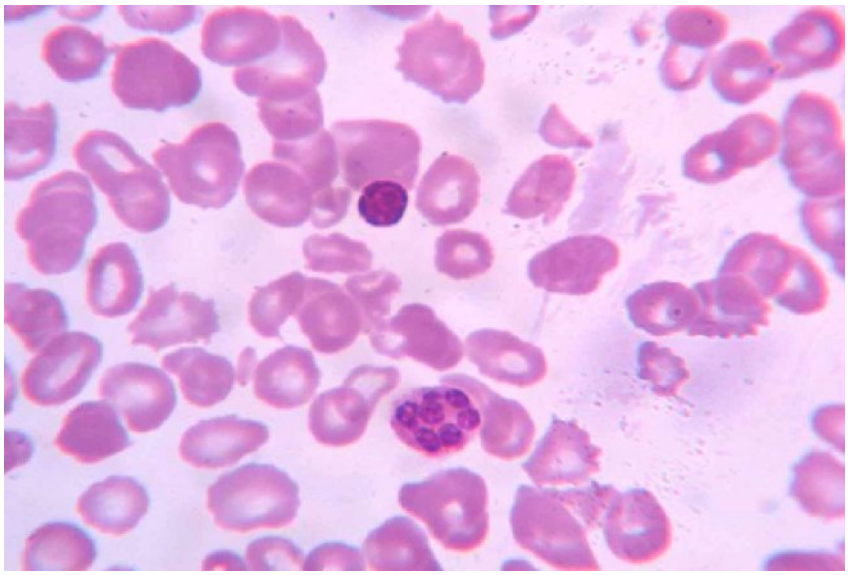

Figure 4:PS showing macroovalocytes and hypersegmented neutrophil(1000x in Leishman's).

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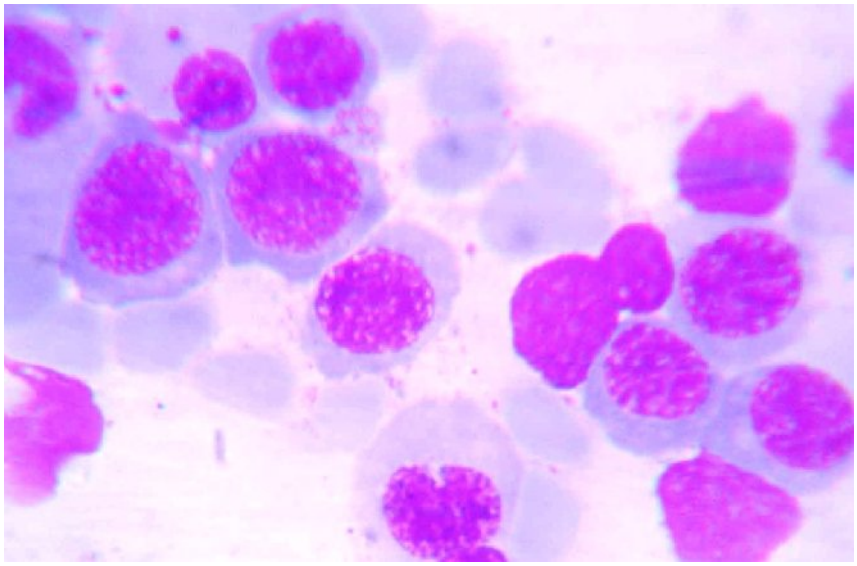

Figure 5:Bonemarrow showing Megaloblast with Royalblue cytoplasm and sieve like chromatin. (1000x in Leishman's).

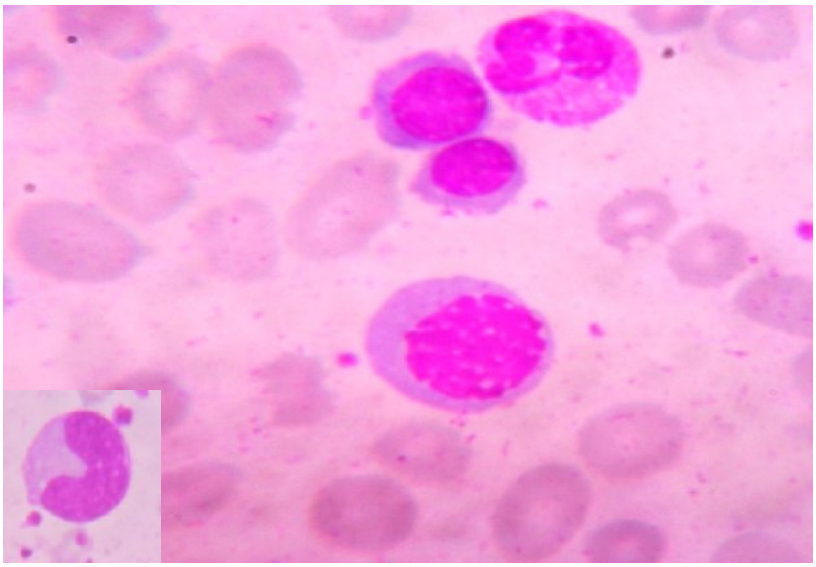

Figure 6:Bonemarrow showing Megaloblastic picture with dyserythropoesis(1000x in Leishman's).
Inset showing giant metamyelocyte .

HYPOPLASTIC/APLASTIC ANEMIA

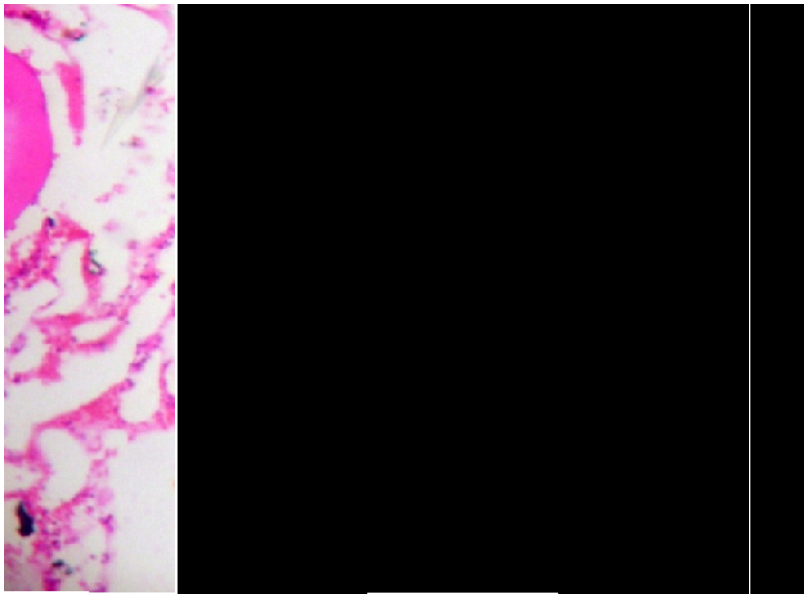

Figure 7:Bone marrow showing Hypocellularity
in 35yrs/M(Under 100x in Leishman's).

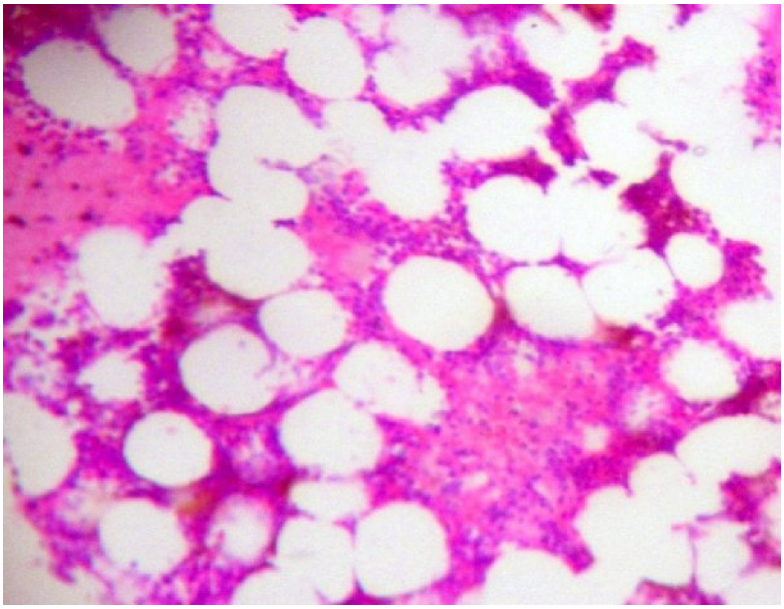

Figure 8:Bone marrow showing increased fat to
cell ratio (400x in Leishman's)

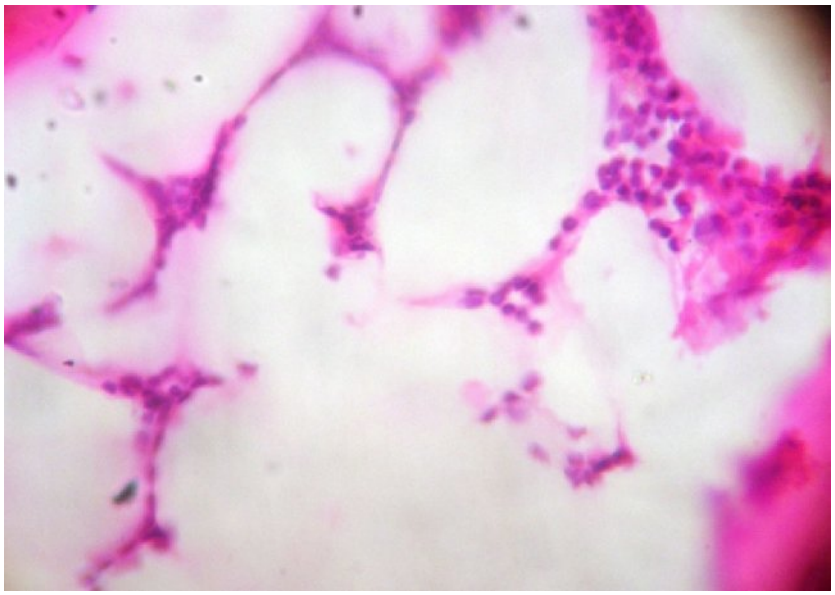

Figure 9:Bone marrow showing
Hypocellularity(1000x in Leishman's).

MYELOFIBROSIS

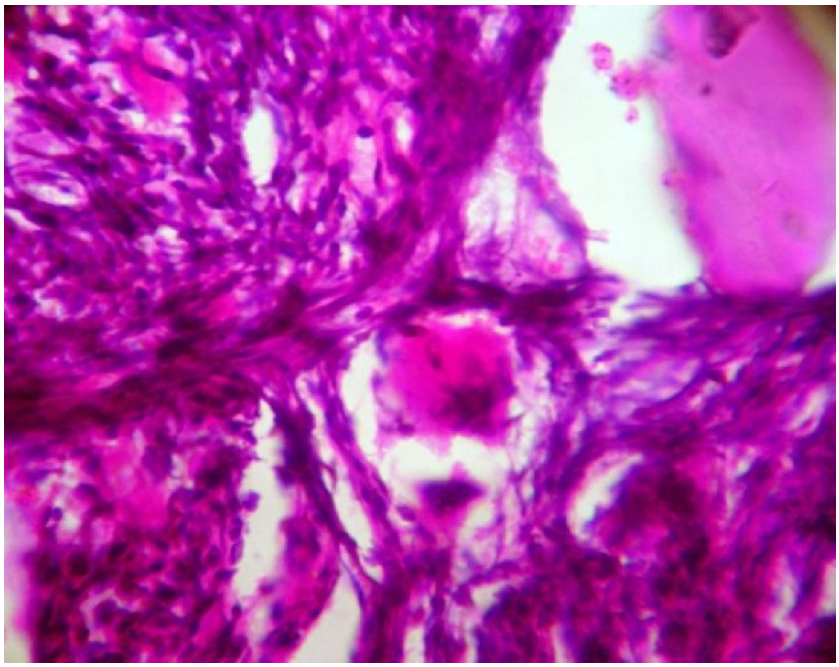

Figure 10:Trephine biopsy showing stromal fibrosis
streaming of cells and increased megakaryocytes.(400x in H&E).

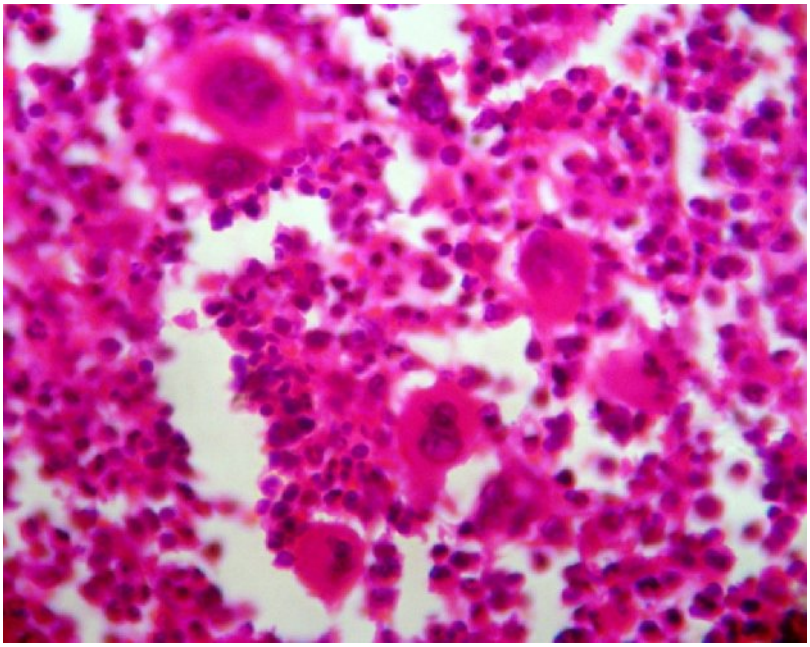

Figure 11 :Case of myelofibrosis showing increased megakaryocytes(400x in H&E).

SUBLEUKEMIC LEUKEMIA-ACUTE MYELOID LEUKEMIA

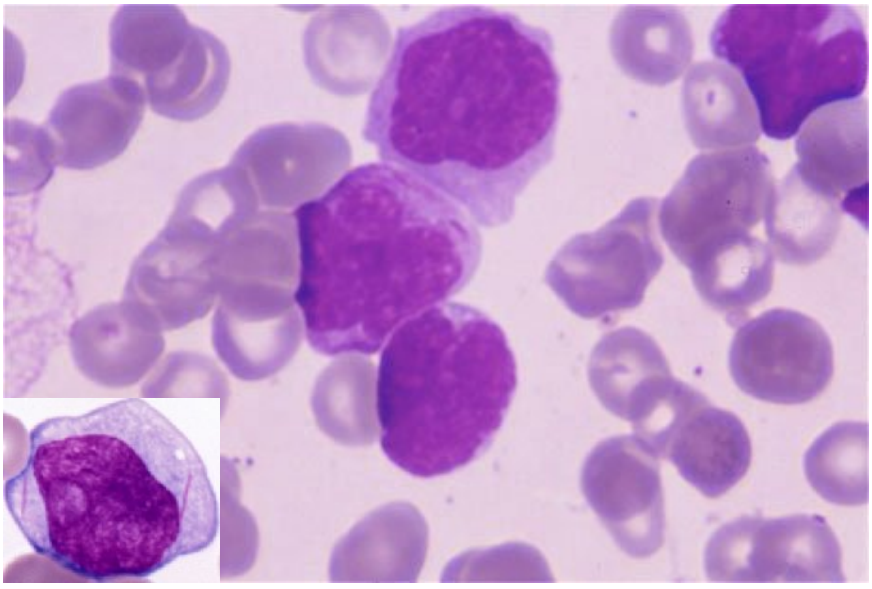

Figure 12:Peripheral smear showing Myeloblasts (400x in Leishman's).Inset showing myeloblast.

SUBLEUKEMIC LEUKEMIA-ACUTE LYMPHOBLASTIC LEUKEMIA

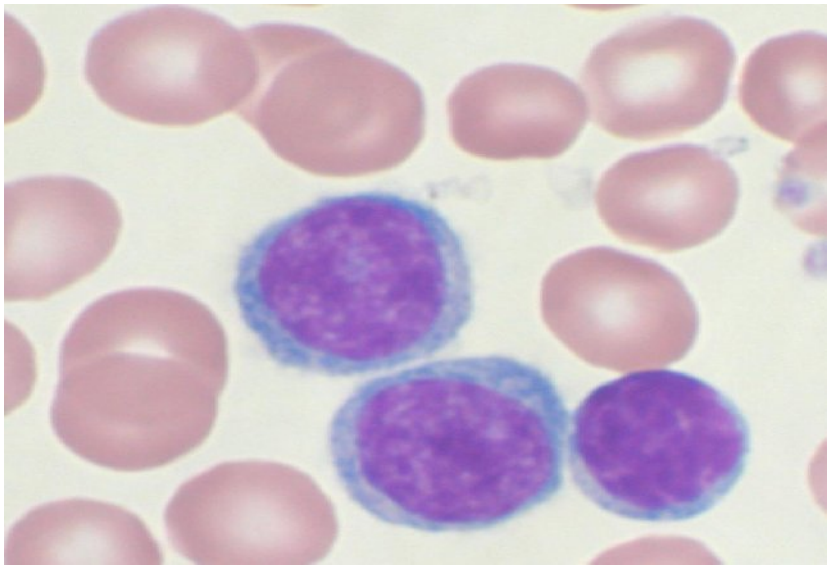

Figure 13: Peripheral Smear showing small lymphoblasts(400x in Leishman's).

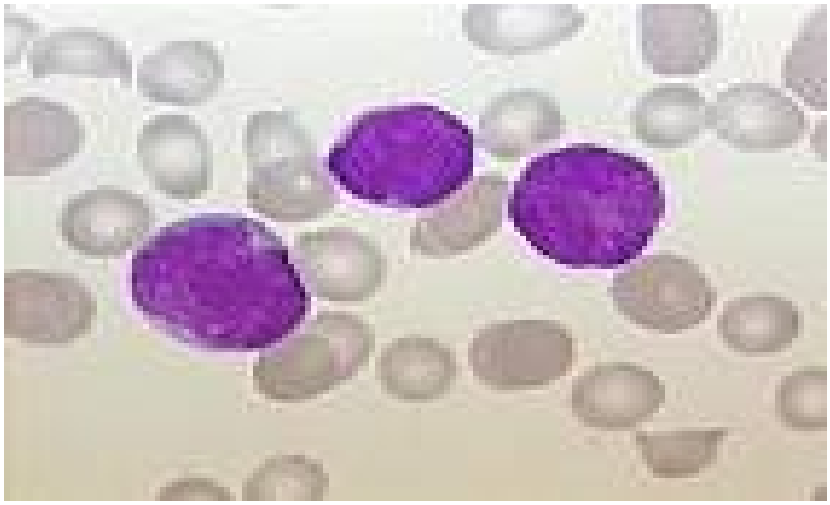

Figure 14:Bone Marrow showing Lymphoblasts (1000x in Leishmans).

MYELODYSPLASTIC SYNDROME

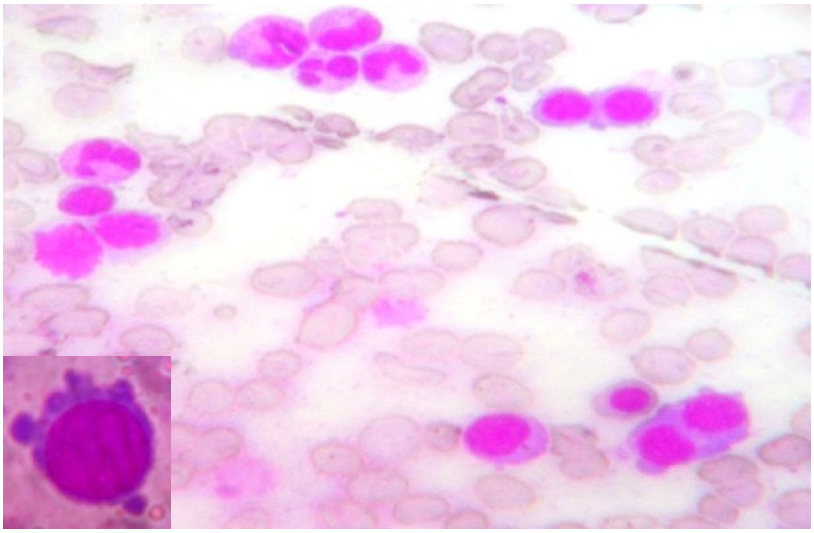

Figure15:Bonemarrow showing dysplastic erythroblast with nuclear budding and binucleation.(400x in Leishman's). Inset showing erythroblast with cytoplasmic blebs.

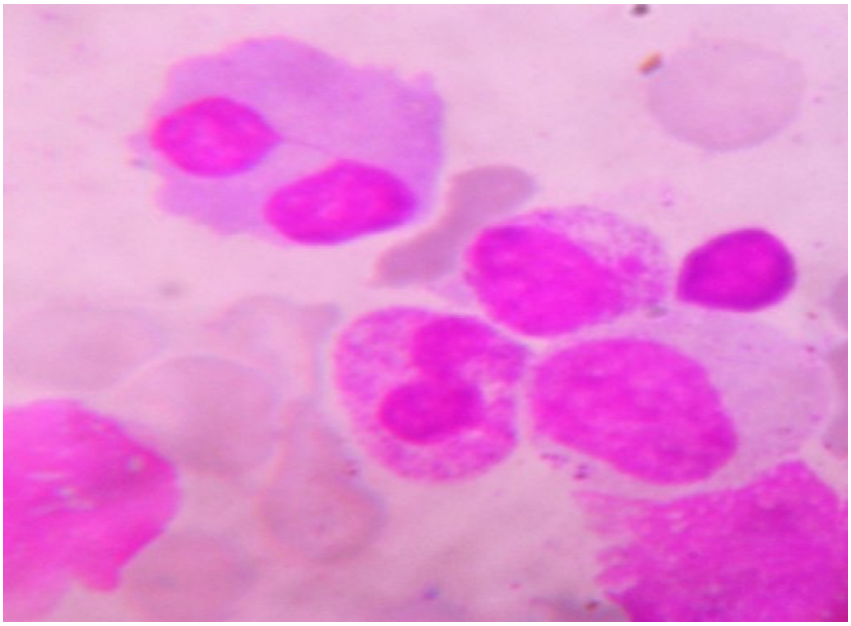

Figure 16:Bonemarrow showing dysplastic erythroblast with chromatin bridging.(1000x in Leishman's).

RETICULIN STAIN PATTERN

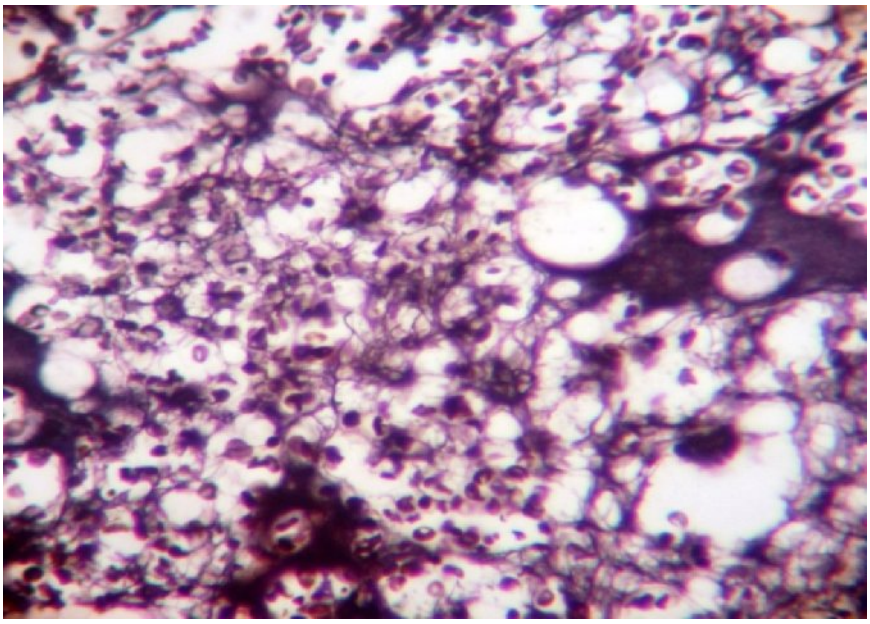

Figure 17: Bonemarrow showing Grade 1 myelofibrosis with thin, short reticulin fibres, but these do not intersect to form a network (400x).

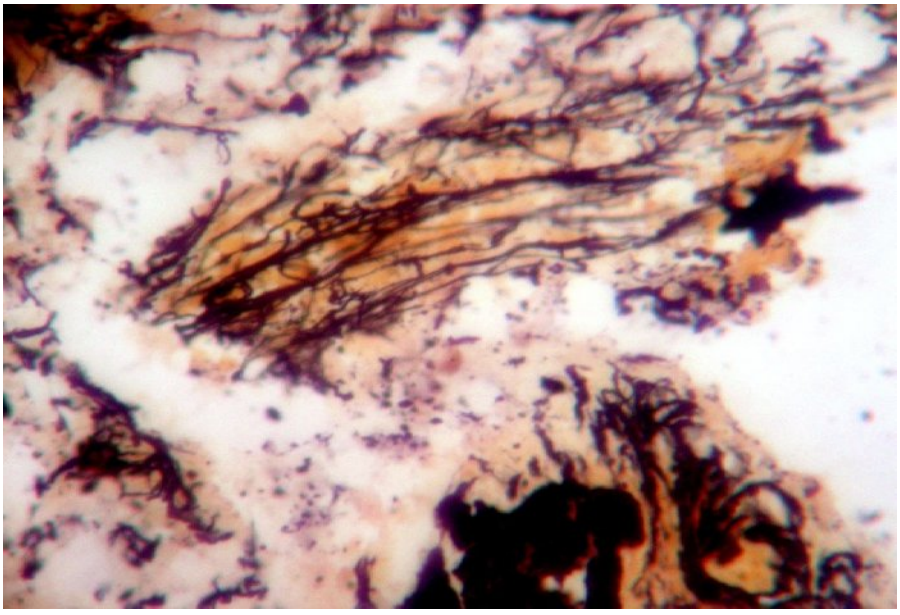

Figure 18:Bone marrow showing Grade 2 myelofibrosis with thin reticulin fibres which intersect to form a network (400x).

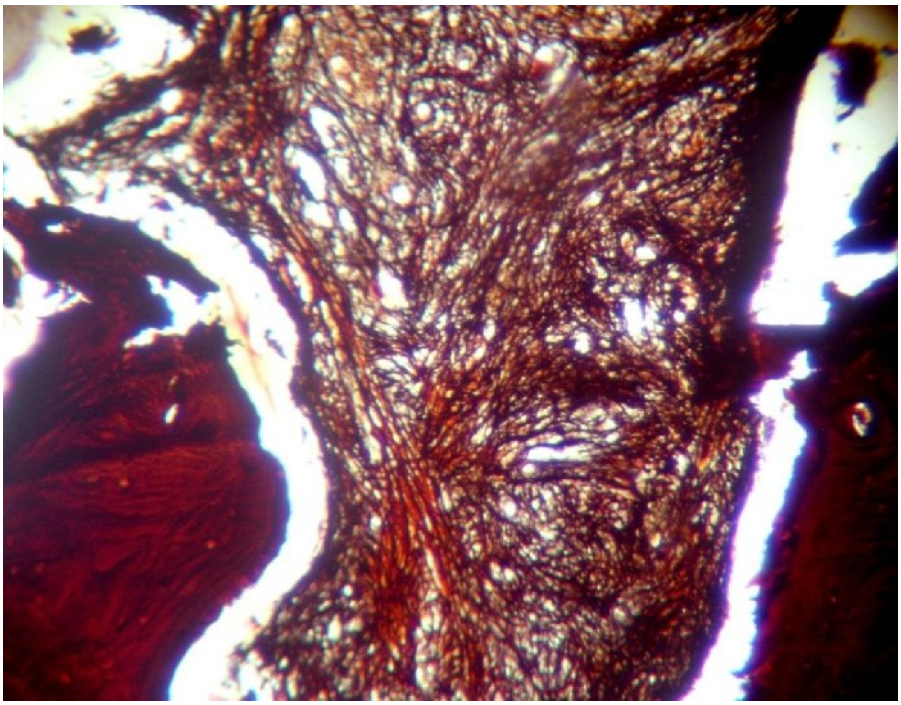

Figure 19:Bonemarrow showing Grade 3 myelofibrosis thick and reduplicated reticulin fibres in the absence of collagen.(400x).

DISCUSSION

50 cases of pancytopenia during the period of March 2010 to August 2011 were studied.

Statistical data of age, sex, presenting complaints, various causes of cytopenias, peripheral smear and bone marrow aspiration smears were studied, and compared with those published in the literature.

TABLE 25

AGE, SEX DISTRIBUTION COMPARED TO OTHER STUDIES OF PANCYTOPENIA

Sl.No.	Authors	No.of.cases	Age range	M : F
1	Khunger JM et al[7] (2002)	200	2-70	1.2:1
2	Kumar R et al[4] (2001)	166	12-73	2.1:1
3	Khodke K et al[28] (2001)	50	3-69	1.3:1
4	Tilak V et al[5] (1999)	77	5-70	1.14:1
5	Gayathri et al(2005)	104	2-80	1.2:1
6	Present study	50	2-60	1:1.2

The age of the patients ranged from 2 years to 60 years with a mean age of 39.5 years. Cytopenias were observed more in females (54 %) than males (46 %) with a M:F ratio of 1:1.2 .

In comparison to all other studies female predominance was noted in this study.

TABLE 26

PHYSICAL FINDINGS COMPARED TO OTHER STUDIES

Diseases	Physical Findings								
	Spleenomegaly			Hepatomegaly			Lymphadenopathy		
	A	B	C	A	B	C	A	B	C
Megaloblastic anemia	40	22	11	42	23	13	1	3	-
Aplastic anemia	-	4	-	1	3	-	-	1	-
Myelofibrosis	-	2	5	-	1	5	-	-	-
Sub leukemic leukemia	8	1	1	10	-	1	6	-	1
MDS	4	-	1	4	-	1	-	-	-

A – Khunger JM et al [7] study(n=200), B – Tilak V et al[5] study(n=77), C – Present Study(n=50).

The most common presenting complaint in our study was generalized weakness (100%) and dyspnoea (46%). The most common physical finding was pallor (86%) ,followed by hepatomegaly (40%) and splenomegaly (36%).

The presenting symptoms were usually attributed to anaemia, 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.

TABLE 27

A comparison of the most common causes of pancytopenia in different studies:

Study group	Country	Year	No. of cases	Commonest cause
IAASG	Israel & europe	1987	319	Hypoplastic anemia
Keisu&Ost	Israel	1990	100	Post radiation
Hossain et al	Bangladesh	1992	50	Hypoplastic anemia
Verma &Dash	India	1992	202	Hypoplastic anemia
Tilak & Jain	India	1999	77	Megaloblastic anemia
Kumar et al	India	1999	166	Hypoplastic anemia
Khodke et al	India	2000	50	Megaloblastic anemia
Bajracharya et al	Nepal	2005	23	Hypoplastic anemia
Present study	India	2010	50	Megaloblastic anemia

IAASG-International Agranulocytosis and Aplastic Anemia Group

The commonest cause of pancytopenia, reported from various studies throughout the world has been aplastic anaemia[5]. This is in sharp contrast with the results of various Indian studies where the commonest cause of pancytopenia is megaloblastic anaemia[2,4.7,28]. Results observed in present study were also similar to those Indian studies. This seems to reflect the higher prevalence of nutritional anemia's in Indian subjects.

TABLE 28
COMPARISON OF HAEMATOLOGICAL PARAMETERS IN MAJOR SUBGROUPS OF CYTOPENIAS

	Aplastic anemia		Megaloblastic anemia	
Parameters	A	B	A	B
Hb g/dl	1.3-8	1.8-5	2.4-7	1.8-8
TLC$\times 10^3$/cumm	0.2-3.0	0.1-2.5	0.7-3.6	1-3.9
Platelets /cumm	8000-86,000	10,000-80,000	10,000-1,30,000	10,000-80,000

A – Kumar R et al[4] study (2001), B – Present study.

Haemoglobin, total leucocyte count and platelet count were comparable with other study.

Table 29 COMPARISON OF PERIPHERAL BLOOD FINDINGS WITH OTHER STUDIES.

Diseases	Total no of cases			anisopoiklocytosis			Nucleated RBC			Hypersegmented neutrophils			Immature WBC			reticulocytosis		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
MA	53	144	34	51	140	34	13	18	7	45	-	30	-	18	-	5	-	2
AA	6	28	7	2	2	1	-	-	-	5	-	-	-	-	-	3	-	-
MF	1	2	6	1	2	2	-	1	2	-	-	-	-	-	-	-	-	-
Sub LL	1	10	2	1	1	1	1	4	1	-	-	-	1	10	2	-	-	-
MDS	-	4	1	-	-	1	-	2	-	-	1	1	-	3	-	-	-	-

1-Tilak V et al(n=77), 2- Khunger JM et al(n=200)[7], 3 – present study(n=50)

Hypersegmented neutrophils was noted in 88 % compared to 84.9% in Tilak V et. al. study and Khunger JM et al demonstrated no hypersegmented neutrophils in megaloblastic anaemia. In the present study of 50 cases, 23 cases had dimorphic anaemia, macrocytic anaemia was found in 12 cases, normocytic normochromic and normocytic hypochromic anaemia constituted rest of the cases.

There are limited number of studies on the frequency of various causes of pancytopenia[78].To the best of our knowledge ,limited data has been reported from Indian subcontinent . The variation in the frequency of various diagnostics entities causing pancytopenia has been attributed to differences in methodology and stringency of diagnostics criteria , geographic area ,period of observation , genetic differences and varying exposure to myelotoxic agents etc[79].

The incidence of megaloblastic anaemia varied from 0.8 to 32.26% of all pancytopenic patients[5]. Our incidence of megloblastic anaemia was 68 %. Incidence of 72% was reported by Khunger JM et al and 68% by Tilak V et al. All the above studies

done in India, stress the importance of megaloblastic anaemia being the major cause of pancytopenia. It is a rapidly correctable disorder and should be promptly notified[7].

Bone marrow aspiration showed megaloblastic erythroid hyperplasia (fig1&2). Although bone marrow aspiration study is uncommon in a suspected megaloblastic anaemia, if the diagnosis does not appear straight forward or if the patient requires urgent treatment and haematological assays are not available, bone marrow aspiration is indicated (fig 3). 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[4]. Figures 5 & 6 depicts the bone marrow picture of megaloblastic dyserythropoesis.

Incidence of aplastic anaemia varies from 10-52 % among pancytopenic Patients[7]. Our incidence of hypoplastic anaemia was 14 %, which correlated with the studies done by Khodke K et al and Khunger JM et al whose incidence for the same was 14%[7, 28]. A higher incidence of 29.5% was reported by Kumar R et al[4]. Most of the cases of aplastic anaemia were idiopathic (fig9).

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. Hypoplastic MDS is a differential diagnosis of aplastic anaemia. But the presence of blasts indicates the diagnosis of hypoplastic MDS[24](fig15&16).

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[28].

We encountered 4 % of subleukemic leukemia compared to Khunger JM et al who has reported 5% of subleukemic leukemia, Kumar R et al reported 12% of aleukemic leukemia. Pancytopenia was the common feature of subleukemic leukemia in our study, this correlated with Kumar R et al, Gayathri et al and Khunger JM et al studies[4,7]. The diagnosis of acute leukemias was based on bone marrow aspiration study, and we reported 1 case of AML-M2 (fig12) and 1 case of ALL –L2 (fig13&14). Khodke K et al reported a single case of AML-M2 of 50 cases of pancytopenia. Kumar R et al reported 5 cases of ALL, 13 cases of AML, 2 cases of hairy cell leukemia out of 166 cases of pancytopenia, over a 6 year study period[4,28].

Pancytopenia with few abnormal cells as seen in MDS was seen 2% of cases. Hypercellularity of the marrow with the abnormal cells confirmed the diagnosis. Myelodysplastic syndrome is a disease characterized by ineffective erythropoesis. It differs from AML by its early apoptosis in early and mature hematopoetic cells.MDS, may thus be suspected in various cases of pancytopenia as also revealed in various other Indian studies[80]. Same 2%wasalso noted by Khunger et al.

Myelofibrosis was seen in 2% of our cases of pancytopenia (fig10&11). Literature also reveals pancytopenia in cases of myelofibrosis by keiss et al[81] and khunger et al.

Megaloblastic Anaemia was observed to be the commonest cause of Pancytopenia in this study. This fact indicates the acute Vitamin B12 and folate deficiency. If this nutritional deficiency, which is widely prevalent now in this region, is set right, the number of Pancytopenia cases reported in the Hospitals will no doubt get drastically reduced.

SUMMARY

- This is a prospective Clinico-Hematological study on Pancytopenia over a period of 18months from March 2010 to August 2011 in the Haematology Unit, Department of Pathology, Coimbatore Medical College, Coimbatore.
- 50 patients in age group between 2-60 yrs presenting with cytopenias were evaluated.
- A combined evaluation of physical findings, primary haematological investigations and bone marrow aspiration were done in cytopenic patients.
- The age of the patients ranged from 2 years to 60 years with a mean age of 39.5 years. Males accounted for 23 cases (46 %) and female 27 cases (54 %) with a M:F ratio of 1:1.2.
- Commonest presenting complaint was generalised weakness and fever.
- Commonest physical finding was pallor followed by hepatomegaly and splenomegaly.
- Megaloblastic anemia (68 %) was the commonest cause of cytopenia followed by hypoplastic /aplastic anemia(14 %), myelofibrosis (12 %), subleukemic leukemia (4 %) and MDS(2 %).
- Lowest haemoglobin percentage was 1.8 gm/dl and noted in a case of megaloblastic anaemia.
- Lowest total leucocyte count was 500 cells/mm3 and noted in a case of megaloblastic anaemia.
- Lowest platelet count of 20,000 cells/mm3 was noted in a case of megaloblastic anaemia.
- Dimorphic anaemia was predominant blood picture in cytopenic patients

- Hypercellular marrow was noted in 38 patients and the common cause was megaloblastic anaemia, followed by leukemia,myelofibrosis, MDS.
- Hypocellular marrow was noted in 11 patients and commonest cause was hypoplastic/aplastic anaemia.
- Grading of associated marrow fibrosis by reticulin stain showed the predominance of grade 2 observed in 22 cases.

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CONCLUSION

Pancytopenia is not an uncommon haematological problem encountered in clinical practice and should be suspected on clinical grounds when a patient presents with unexplained anaemia, 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 anaemia, 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 haematology which helps to evaluate various cases of cytopenia. Bone marrow examination is an 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 anaemia in our region.

The other common causes were hypoplastic/aplastic marrow. However, uncommon and rare causes such as multiple myeloma, storage disease should be kept in mind while planning investigation for complete work up of cytopenic patients.

In patients presenting with cytopenia and hepatosplenomegaly, smear revealed dimorphic picture , but marrow showed megaloblastic change indicating acute vitamin B12 and folate deficiency in patients.

Tuberculosis being highly prevalent and endemic in India, it is essential to be aware of its manifestation as pancytopenia.

Present study concludes that detailed primary haematological investigations along with bone marrow examination 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.

ANNEXURE 1

PROFORMA

COIMBATORE MEDICAL COLLEGE

DEPARTMENT OF PATHOLOGY

COIMBATORE

Particulars of the patient :

Name : Hospital:

Case No: Date :

Age/sex : I.P No. :

Address : Ward No. :

Occupation Religion :

Presenting complaints and duration :

Weakness / Dyspnoea / Palpitation / Guiddiness / Angina + / -

Fever / Sweats / Infection + / -

Purpura / Ecchymosis / Bleeding diathesis / Skin infections / parasthesia + / -

Past history :

Previous history of anemia

Transfusions + / -, Drugs + / -, Liver disease + / -, Chronic diseases + / -,

Exposure to radiation + / - chemicals + / -.

Family history :

Anemia + / -, Bleeding diathesis + / -, Malignancy + / -, Recurrent jaundice + / -.

Personal history :

Diet : Appetite : Bowel / Bladder habits :

Sleep : Alcohol intake : Smoking :

Menstrual history:

General physical examination :

Built : Nourishment : Conscious : Weight

Pulse : RR : BP: Febrile / Afebrile :

Pallor : Jaundice : Cyanosis : Clubbing :

Lymphadenopathy : Edema : Mouth : Skin :

Systemic examination :

P/A : Hepatomegaly : Splenomegaly : Ascites :

CVS : RS : CNS : Musculo-skeletal-bone pain:s + / -

Clinical diagnosis :

Investigations :

1.Complete haemogram(autoanalyser)

Sl. No.	**Tests**	**Observed Value**
i.	Hb%	
ii.	RBC count.	
iii.	WBC count.	
iv.	Platelet count.	
v.	PCV.	
vi.	MCV.	
vii.	MCH.	
viii.	MCHC.	
2.	Retic count.	

2.Peripheral smear :

RBC :

WBC :

Platelets :

Parasites :

Impression :

3.Bone marrow study (Bone marrow aspiration No.)

Aspirate :

Cellularity :

Myeloid : erythroid ratio :

Erythropoiesis

Leucopoiesis :

Megakaryopoiesis :

4. Bone marrow trephine (Hpe no.)

Cellularity :

Architecture:

Presence of fibrosis:

Focal lesions:

5.RETICULIN STAIN:

Grading of fibrosis.

ANNEXURE 2
MASTER CHART

sl.no	Biopsy. No.	Data			Mode of presentation				Physical examination				Complete hemogram				BMA	BMT	Reticulin (Grades)	Final diagnosis
		Ip.no	age	sex	G.W	fever	dyspnoea	bleeding	pallor	hepatomegaly	spleenomegaly	lymphadenopathy	Hb(g%)	TLC(cells/mm^3)	Platelets (cells/mm^3)	Peripheral smear				
1	480/10	11029	54	M	+	+	-	-	+	-	-	-	2	1800	42,000	MA	Hypo cellular	Hypo plastic	0	AA
2	686/10	18953	55	M	+	-	-	-	+	-	-	-	8	3800	95,000	NN	MB	EH	2	MA
3	730/10	21231	24	M	+	+	+	-	+	+	+	-	1.8	500	40,000	MA	MB	EH	2	MA
4	909/10	25670	43	M	+	+	+	-	+	+	+	-	6.8	2100	70,000	DA	MB	EH	0	MA
5	1047/10	26372	38	F	+	+	-	-	+	-	-	-	2.4	1600	25,000	NH	Hypo cellular	Hypo plastic	1	AA
6	1082/10	30590	42	F	+	+	+	-	+	-	-	-	8	3800	90,000	NN	MB	EH	1	MA
7	1086/10	30032	45	F	+	+	+	-	+	+	-	-	7	1600	20,000	DA	MB	EH	1	MA
8	117410	33274	15	F	+	+	-	-	+	+	+	-	3.4	1600	60,000	DA	MB	EH	1	MA
9	1288/10	35009	58	F	+	-	-	-	-	+	+	-	6.2	3300	77,000	LEB	DRY	Hypo cellular	3	MF
10	1289/10	34945	28	F	+	+	-	-	+	-	-	-	2.5	1000	34,000	MA	MB	EH	2	MA
11	1439/10	37013	60	F	+	-	-	-	-	-	-	-	5.6	2300	43,000	DA	Hypo cellular	Hypo plastic	0	AA
12	1470/10	39528	10	F	+	-	-	-	-	-	-	-	4.2	2600	49,000	MH	Hypo cellular	Hypo plastic	1	AA
13	1563/10	41420	40	M	+	-	+	-	+	-	-	-	8.5	3600	64,000	NN	MB	EH	1	MA
14	1574/10	39628	15	F	+	-	-	-	+	-	-	-	7.6	3600	90,000	MA	MB	EH	1	MA
15	1645/10	42922	48	F	+	-	-	-	+	-	+	-	2.4	2600	46,000	MA	MB	EH	2	MA
16	1648/10	45224	47	M	+	+	+	-	+	-	-	-	4.2	2900	57,000	DA	MB	EH	2	MA
17	1668/10	17107	60	M	+	+	-	-	+	-	-	-	6.6	3500	62,000	NH	MB	EH	1	MA
18	1972/10	53257	58	M	+	-	+	-	+	-	-	-	4.2	2900	47,000	LEB	DRY	Hypo cellular	3	MF
19	2049/10	55156	50	F	+	+	-	-	+	-	-	-	3.6	3800	54,000	DA	MB	EH	2	MA
20	2112/10	58445	50	M	+	+	-	-	+	+	+	-	8.2	2900	44,000	DA	MB	EH	1	MA

21	2151/10	58996	16	F	+	+	+	-	+	-	-	+	5.3	3300	29,000	SLL	ALL-L2	Infil -trative	0	ALL-L2
22	2157/10	59821	20	M	+	+	+	-	+	-	-	-	5.8	2600	49,000	MA	MB	EH	2	MA
23	G400/10	11818	27	F	+	-	-	-	+	-	-	-	6.2	3200	68,000	MA	MB	EH	2	MA
24	G394/10	11014	23	F	+	+	+	-	+	+	+	-	6.8	3400	66,000	DA	MB	EH	2	MA
25	2361/10	65496	16	F	+	-	+	-	+	+	+	-	4.5	3500	72000	DA	DRY	Hypo cellular	3	MF
26	2368/10	63825	5	F	+	-	+	-	+	-	-	-	8.8	1900	56,000	NN	MB	EH	0	MA
27	2369/10	63597	46	M	+	-	-	-	+	+	+	-	7.6	2800	72,000	DA	MB	EH	2	MA
28	2419/10	66875	52	F	+	-	+	-	+	+	-	-	6.8	2100	46,000	NN	MB	EH	0	MA
29	2491/10	69509	28	F	+	-	+	-	+	-	-	-	9	3900	67,000	NN	MB	EH	2	MA
30	2604/10	69461	29	M	+	-	-	-	+	-	-	-	6.4	3200	18,000	MA	MB	EH	2	MA
31	2605/10	71211	50	F	+	-	-	-	+	+	+	-	2.6	650	20,000	MA	MB	EH	2	MA
32	2653/10	73500	41	F	+	+	+	-	+	-	-	-	6	2200	55,000	DA	Hypo cellular	Hypo plastic	1	AA
33	2654/10	71577	40	F	+	-	+	+	+	+	+	-	2.4	3300	33,000	MA	MB	Normal	1	MA
34	2660/10	73345	23	M	+	+	+	-	+	-	-	-	8.6	1250	73,000	NH	MB	EH	0	MA
35	2707/10	73337	57	F	+	+	+	-	+	-	+	-	2.8	2700	69000	DA	MB	EH	2	MA
36	109/11	1727	46	M	+	-	-	-	-	+	-	-	4.3	2300	43000	NH	Hypo cellular	Hypo plastic	1	AA
37	404/11	9961	58	M	+	+	+	-	+	+	+	-	7.6	3400	46,000	DA	MB	EH	2	MA
38	642/11	14972	23	M	+	+	-	-	+	-	-	-	3.6	3300	39,000	MA	MB	EH	2	MA
39	676/11	16125	50	M	+	-	-	-	-	+	+	-	4.2	3800	64,000	DA	Normal	Hyper cellular	2	MF
40	854/11	22713	55	F	+	-	+	-	+	-	-	-	4.6	2800	52,000	MA	MB	EH	0	MA
41	875/11	21836	55	F	+	-	+	-	+	+	+	-	5.2	3600	60,000	SLL	AML	Infil- trative	1	AML-M2
42	886/11	20489	5	F	+	+	-	-	+	-	-	-	5.6	3500	49,000	DA	MB	EH	2	MA
43	942/11	24896	33	M	+	-	+	-	+	+	+	-	6.4	2200	70,000	NN	MB	EH	0	MDS
44	1008/11	26251	41	M	+	-	-	-	+	-	-	-	4.2	2600	57,000	DA	MB	EH	2	MA
45	1346/11	36024	58	M	+	-	-	-	+	-	-	-	6.2	2100	65,000	DA	MB	Hyper cellular	2	MA
46	1347/11	34528	28	M	+	+	-	-	+	-	-	-	2.3	1100	40,000	DA	Hypo cellular	Hypo plastic	0	AA
47	1397/11	36043	45	F	+	+	+	-	+	+	-	-	8.6	2700	54,000	NN	MB	EH	2	MA
48	1401/11	35433	55	F	+	-	-	-	-	+	+	-	7.2	3200	34,000	NN	Normal	Hyper cellular	2	MF
49	1475/11	38453	41	M	+	-	-	-	-	+	+	-	6.3	3500	62,000	LEB	DRY	Hypo cellular	3	MF
50	1663/11	40111	60	M	+	-	-	-	+	-	-	-	7.2	3400	75,000	DA	MB	EH	1	MA

KEY TO MASTER CHART

MA-Macrocytic anaemia

AA-Aplastic anemia

MB-Megaloblastic anemia

DA-Dimorphic anemia

NN-Normocytic Normochromic

LEB-Leukoerythroblastic picture

NH-Normocytic Hypochromic

MF-Myelofibrosis

MDS- Myelo Dysplastic Syndrome

AML-M2-Acute Myeloid Leukemia (M2 type)

ALL-L2-Acute Lymphoblastic Leukemia (L2 type)

Hb(g%)-Hemoglobin

TLC- Total leukocyte count.

SLL – Subleukemic leukemia

ANNEXURE 3

NORMAL REFERENCE VALUES

TABLE. 1 : HAEMATOLOGY REFERENCE VALUES IN NORMAL ADULTS[29]

TEST	MEN	WOMEN
Hemoglobin	14-17g/dl	12.3-15.3g/dl
Hematocrit	41.5-50.4%	36-45%
Red cell count	4.5-5.9×10^6/µl	4.5-5.1×10^6/µl
White cell count	4.4 -11.3×10^3/µl	4.4 - 11.3×10^3/µl
MCV	80 - 96 fl	80 - 96 fl
MCH	27.5 - 33.2pg	27.5 – 33.2pg
MCHC	33.4 - 35.5g/dl	33.4 - 35.5g/dl
Platelet count	150-450×10^3/µl	150-450×10^3/µl
Reticulocyte count	0.5-2.5%	0.5-2.5%
ESR	0-15mm/hr	0-20mm/hr

TABLE. 2 : DIFFERENTIAL COUNTS OF BONE MARROW ASPIRATE[29]

	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
Myelocyte	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
ERYTHROID SERIES(TOTAL)	18.4-33.8	25.6
Pronormoblast	0.2-1.3	0.6
Basophilic	0.5-2.4	1.4
Polychromatophilic	17.9-29.2	21.6
Orthochromatic	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 : Erythroid	1.5-3.3	2.3

TABLE 3 :AUTOANALYSER REFERENCE RANGES[39]:

PARAMETERS	RANGE FOR FEMALES	RANGE FOR MALES
WBC(per μL)	3.1-10.3	2.6-8.8
RBC(per μL)	3.2-4.6	3.6-5.3
Hg(g/dl)	9.9-13.6	11.3-15.7
HCT(%)	30.2-42.3	32.6-47.5
MCV(fl)	78.6-102.2	80.3-103.4
MCH(pg)	25.2-34.7	26-34.4
MCHC(g/dl)	31.3-35.4	31.8-36.3
Plt(per μL)	128-434	134-377
Lym%	15-45.8	17.5-47.9
Mxd%	1.3-25.9	1.9-24.6
Neut%	43.7-77.1	38.3-69
Lym#(per μL)	0.9-2.8	0.8-2.7
Mxd#(per μL)	0.1-1.6	0.1-1.5
Neut#(per μL)	1.6-6.9	1.2-5.3
RDW-CV(%)	10.6-15.7	10.8-14.9
RDW-SD(fl)	35.3-48.9	33.4-49.2
PDW(fl)	9.4-18.1	9.8-18.0
MPV(fl)	8.5-12.4	8.1-12.4
P-LCR(%)	14.3-44	10.7-45.0

BIBLIOGRAPHY

1) Guinan EC, Shimamura A. Acquired and inherited aplastic anemia syndromes In : Greer JP, Foerster J, Lukens JN, Rodgers GM, Paraskevas F, Glader B edts, Wintrobe's Clinical Hematology, 11th edn, Philadelphia : Lippincott Williams and Wilkins 2004:p.13971419.

2) Ryan DH, Cohen HJ. Bone marrow aspiration and morphology. In : Hoffman R,Benz EJ, Shathil SJ, Furie B, Cohoen HJ, Silberstein LE et al, edts. Haematology basic principles and practice, 3rd edn. Philadelphia : Churchill Livingstone 2002;p.2460-248.

3) Guinan EC, Shimamura A. Acquired and inherited aplastic anemia syndromes In : Greer JP, Foerster J, Lukens JN, Rodgers GM, Paraskevas F, Glader B edts, Wintrobe's Clinical Hematology, 12th edn, Philadelphia : Lippincott Williams and Wilkins 2009:p.1173-1195.

4) Tilak V, Jain R, Pancytopenia-A Clinco-hematologic analysis of 77 cases. Indian J Pathol Microbiol 1992;42(4):399-404.

5) Kumar R, Kalra SP, Kumar H, Anand AC, Madan M. Pancytopenia-A six year study. JAPI 2001;49:1079-81.

6) Knodke K, Marwah S, Buxi G, Vadav RB, Chaturvedi NK. Bone marrow examination in cases of pancytopenia. J Academy Clin Med 2001;2(1-2):55-59.

7) . Khunger JM, Arulselvi S, Sharma U, Ranga S, Talib VH. Pancytopenia--a clinico haematological study of 200 cases. Indian J Pathol Microbiol 2002; 45:375-9.

8)Issaragil S, Chansung K,Kaufman et al.Aplastic Anaemia Study Group.Am J Public Health 1997;84;923-927.

9) Mackey MC. Unified hypothesis for the origin of Aplastic Anaemia and Periodic Haematopoiesis blood 1978;51(5):1456-60.

10)Mathe G,Amiel JL,Schwarzenberg L,et al.Bone marrow graft in man after conditioning by antilymphocyte serum.BMJ 1970;2;131-136.

11)Shimamura A,Guinan EA.Acquired aplastic anaemia .In:Nathan DG,Orkin,eds.Hematology of infancy and childhood.Philadelphia:WB Saunders,2003:256.

12)Scott JL,Finegold SM,Belkin GA et al 1965.A controlled double –blind study of the hematological toxicity of chloramphenicol.New England Journal of Medicine272:1137-1142.

13) Singh H, Kishore K, Gupta M, Singh S, Marwah N, Khetarpal S Aplastic anemia associated with antituberculous chemotherapy – A Case Report. Ind J HematolBlood Transf 2001;19(1):18-19.

14) Hanada T, Koike K, Takeya T, Nagasawa T, Matsanaga Y, Takita H. Human Parvovirus B19-induced transient pancytopenia in a child with hereditary spherocytosis. Br J Hematol 1988 ;70 :113-115.

15)Hagler L,Pastore RA,Bergin JJ,et al.Aplastic anemia following Viral hepatitis: report of two cases and literature review.Medicine 1975;54:139-164.

16) Matloub YH, Brunning RD, Arthur DC, Ramsay NKC. Severe Aplastic Anemia preceding Acute Lymphoblastic Leukemia. Cancer 1992;71:264-268.

17)Catrwright RA,Alexander FE,Mckinney PA.Leukemia and Lymphoma .An atlas of distribution within areas of England and Wales 1984-1988.LRF.

18)Williamson PJ,Kruger A,Reynolds PJ et al 1994.establishing the incidence of myelodysplastic syndromes.British journal of Haematology 87:743-745.

19) Tuncer MA, Pagliuca A, Hicsonmez G, Yetgin S, Ozsoyler S, Mufti GJ. Primary myelodysplastic syndrome in children : the clinical experience in 33 cases. Br J Hematol 1992;82:347-53.

20) Kini J, Khadilkar UN, Dayal JP. A study of the haematologic spectrum of Myelodysplastic Syndrome. Indian J Pathol Microbiol 2001;44(1):9-12.

21) Williams DM. Pancytopenia, Aplastic anemia, and Pure Red Cell Aplasia. In : Lee GR, Foerster J, Leukens J, Paraskenas F, Greev JP, Rodgers GM, edts, Wintrobe's Clinical Hematology, 10th edn, Maryland : Williams and Wilkins, 1999:1449-1476.

22) Marshall A Lichtman,Ernest Beutler,Thomas J Kipps.Aplastic anaemia.Williams hematology seventh edition.McGraw-Hill 33:419-437.

23) Sunitha N,Wickramasinghe.Normal bone marrow :histology and histochemistry.Blood and Bonemarrow Pathology,1st edn2003.32(2):54-70

24)Aster JC. Red blood cell and bleeding disorders. In : Kumar V, Abbas AK, Faustro N edts. Robbins pathological basis of disease, 7th edn. New Delhi: Saunders; 2004:p.620-622.

25) Marris MW, Davey FR. Basic Examination of blood. In : Henry JB edt, Clinical diagnosis and management by laboratory methods, 21st edn. New Delhi: WB Saunders; 2001:457-483.

26)Miller J,Alley K,McGlave 1994.Differentiation of progenitor cells in a stroma based culture .Blood83:2594-2603

27) Wilkins BS, Clark D. Recent advances in bone marrow pathology. In : Lowe DG, Underwood JCE edts. Recent advances in histopathology number 20. London, Royal Society Med press Ltd. 2003:145-161.

28)Jacobsen KM1941.Untersuchungen das knockmarkpunktat normalen verschidner Altersklassen.Acta medica scandinavica 106:417-446.

29) Perkins SL. Normal Blood and Bone Marrow values in humans. In : Lee GR,Foerster J, Lukens J, Paraskenas F, Greev Jp, Rodgers GM, edts. Wintrobe's Clinical Hematology, 10th edn, Maryland : Williams and Wilkins 1999;2:p.2738-2748.

30) Burkhardt R,Frisch B,Bartl R1982.Bone biopsy in hematological disorders. Journal of clinical pathology 35:257-284.

31)Firtin Frank,Chestermann colin,Penington David:Pancytopenia:Aplastic anaemia:Degruchy's clinical hematology in medical practice,Oxford university press.Fifth edition.Delhi 1989:119-136.

32) Iqbal W, Hassan K, Ikram N, Nur S. Aetiological breakup of 208 cases pancytopenia. J Rawal Med Coll 2001;5(1):7-9

33) Gordon-Smith EC, Marsh JCW. Acquired aplastic anaemia, other acquired bone marrow failure disorders and dyserythropiesis. In : Hoffbrand AV, Catovsky D, Tuddenham ECD edts, Post graduate hematology, 5th edn. Malden Black well Publishing 2005:p.90-204.

34) Judith CW Marsh and Neal S Young.Acquired aplastic anaemia.Hoffbrands post graduate Hematology 6th edn. Blackwell 2011;13:206-225.

35) Adams JA, Barrett AJ. Haematopoetic stimulators in the serum of patients with severe aplastic anaemia. Br J Hematol 1982;52:327-335.

36) Burns WA, Yook CR. Plastic sections and Ultrastructural techniques in the evaluation of bone marrow pathology. Hematology / Oncology Clin North Am 1988;2(4):525-535.

37)Neal S.Young,Jarolaw P.Maciejewski.Aplastic anaemia.Hematology basic principles and practice by Hoffman 5th edition.Churchill livingstone 2009:29:359-383.

38)Mole RH.The LD50 for uniform low LET irradiation of man.Br J Radiology 1984;57:355-369.

39) Operator's manual of automated hematology analyser. KX – 21- Sysmex corporation Kobe, Japan – April 2004.

40) Choudhry VP, Bhattacharyya M. Inherited Bone Marrow Failure Syndrome. I:Choudhry VP, Saxena R, Pati Hp edts. Recent Advances in Haematology. New

Delhi: Jaypee Brothers Medical Publishers; p.147-161.

41) Young NS, Alter BP. The Bone Marrow Failure Syndrome. In : Nathan DG, Orkin SH edts, Nathan and Oski's Hematology of Infancy and Childhood, 5th edn. Philadelphia W.B. Saunders 1998;1:259-275.

42) Cone TE,Abelson SM.Aplastic anemia.Blood –Textbook on hematology by James H.Jandl 1996;4:201-248.

43)Van Tongeren JHM et al:Folic –acid deficiency in chronic arsenic poisoning.Lancet 1:784,1965.

44) Lazarus KH,Baehner RL.Aplastic anaemia complicating infectious mononucleosis:a case report and review of literature .Pediatrics 1981;907-910.

45) Brown KE, Tisdale J, Barrett J, Dunbar CE, Young NS. Hepatitis-Associated Aplastic Anemia. N Engl J Med 1997;336:1059-64.

46) Young N:Hematologic and hematopoietic consequences of viral infection.Semin hematol 25:159,1988.

47) Karcher DS, Frost AR. The bone marrow in human immunodeficiency virus (HIV) related disease. Morphology and Clinical Correlation. Am J Clin Pathol 1991;95:63-71.

48) Nakakuma H, Nagakura S, Iwamoto N, Kawaguchi T, Hidaka M, Horikawa K et al. Paroxysmal Nocturnal Hemoglobinuria clone in bone marrow of patients with pancytopenia. Blood 1995;85(5):1371-76.

49) Babu SY. Clinico-Haematological study of pancytopenia. Dissertation submitted to the Faculty of medicine, Kuvempu University, M.D (Path) 1998.

50) Tater ML, Gupta BD, Singh RN, Gupta R. Fanconi's Anemia. Indian Paediatrics 1991;28:301-303.

51) Bhatnagar S, Chandra J, Narayan S, Jain V. Fanconi's constitutional aplastic anemia. Indian Paediatrics 1999;36:722-724.

52)Sasaki MS,Tonomura A.Ahigh susceptibility of Fanconis anemia to chromosome breakage by DNA cross linking agents.Cancer Res 1973;33:1828-1836.

53) Patel A, Renge R, Tule V. Fanconi's anemia. Ind J Hematol Blood Tranasf 2001;19(1):26-27.

54) Segel GB, Lichtman MA. Aplastic Anemia. In : Lichtman MA, Kipps TJ, Kaushansky K, Beutler E, Seligsohn U, Prchal JT edts. Williams Haematology 7th edn. Newyork McGraw – Hill Publication 2006:p.419-430.

55)Lawler SD, Robert PD, Hoff brand AV: chromosome studies in megaloblastic anemia before and after treatment .Scand J Hematol 8: 309,1971.

56)Gordon-Smith EC, Marsh JCW. Acquired aplastic anaemia, other acquired bone marrow failure disorders and dyserythropiesis. In : Hoffbrand AV, Catovsky D, Tuddenham ECD edts, Post graduate hematology, 6th edn. Malden Black well Publishing 2011:p.186-226.

57) Carmel R. Megaloblastic anemias : Disorders of Impaired DNA synthesis. In Greer JP, Foerster J, Lukens JN, Rodgers GM, Paraskenas F, Glader B. Wintrobes Clinical Hematology12th edn. Philadelphia, Lippincott Williams and Wilkins 2004: p 1143-1165.

58)Neal S.Young,Jarolaw P.Maciejewski.Megaloblastic anemia.Hematology basic principles and practice by Hoffman 5th edition.Churchill livingstone 2009:39:491-523.

59) Pancytopenia, Aplastic Anaemia, In : Firkin F,Chesterman C, Penington D, Rush B edts. De Gruchy's Clinical Haematology in medical practice 5th edn, London: Black well Science; 1989:p.119-134.

60) Brunning RD, Bennett JM, Flandrin G, Matutes E, Head D, Vardiman J et al.Myelodysplastic syndromes In : Jaffe ES, Harris NL, Stein H, Vardiman JW edts.Pathology and Genetics of Tumors of Haematopoietic and Lymphoid tissues. Lyon, IARC Press; 2001:61-66.

61)Peter J Campbell,Anthony R Green.Myeloprolifrative neoplasms. Hematology basic principles and practice by Hoffman 5th edition.Churchill livingstone 2009;36:697-700.

62) Thiele J, Pierre R, Imbert M, Vardiman JW, Brunnning RD, Flandrin G. Chronic idiopathic myelofibrosis. In : Jaffe ES, Harcis NL, Stein H, Vardiman JW edts.Pathology and genetics of tumors of haematopoietic and lymphoid tissues. Lyon IARC Press 2001:85-88.

63) Grogan TM, Camp BV, Kyle RA, Hermelink HKM, Harris NL. Plasma cell neoplasma. In : Jaffe ES, Harris NL, Stein H, Vardiman JW edts, Pathology and genetics of tumors of haematopoietic and lymphoid tissues. Lyon IARC Press 2001:142-146.

64)Moscinski LC. Laboratory and bone marrow evaluation in patients with cancer. http://www.moffitt.org/moffittapps/ccj//v5ns/article3.html-6/24/2007.

65)Lewis SM. The spleen. In : Hoffbrand AV, Catonsky D. Tuddenlam EGD edts. Post graduate haematology, 5th edn, Malden Black well publication. 2005:363-365.

66) Hebert KJ, Hubner SA, Willis K, Monier PL. A young woman with fever and pancytopenia. J La State Med Soc 2003;155:192-195.

67) Fritsche TR, Smith JW. Medical parasitology. In : Henry JB, edt. Clinical diagnosis and management of laboratory methods, 20th edn, New Delhi : WB Saunders 2001: p 1196-1270.

68) Aouba A, Noguera ME, Claunel JP, Quint L. Haemophagocytic syndrome associated with plasmodium vivax infection. Br J Hematol 2000;108:832-833.

69) Shrivastava MP, Madhu SV, Grover AK. Pancytopenia – A Rare Presentation of Miliary Tuberculosis. JAPI 1993;41(5):311-312.

70) Sign KJ, Ahlucvalia G, Sharma SK, Saxena R, Chaudhary VP, Anant MSignificance of hematological manifestations in patients with tuberculosis. JAPI2001;49:788-794

71) Yadav TP, Mishra S, Sachdeva KJS, Gupta VK, Siddhu K. Pancytopenia indisseminated tuberculosis. Indian paediatrics 1969;33:597-599.

72) Infective and reactive changes. In : Bain BJ, Clark DM, Lampert IA edts, Bone marrow pathology, 2nd edn, Australia. Black Well Science; 1992: p 51-87.

73) Miscellaneous disorders. In Bain BJ, Clark DM, Lampert IA edts, Bone marrow pathology, 2nd edn Australia. Black Well Science Ltd; 1992 :p 261-286.

74) Young NS. Aplastic anemia, myelodyplasia, and related bone marrow failuresyndromes. In : Kasper DL, Fauci AS, Longo DL, Braunwald E, Hauser SL, Jameson JL edts. Harrison's Principles of Internal Medicine, 16th edn. Vol. 1, New York McGraw-Hill 2005: p 617-625.

75) Ishtiaq O, Baqai HZ, Anwer F, Hussai N. Patterns of pancytopenia patients in a general medical ward and a proposed diagnostic approach. www.ayubmed.edu.pk/JAMC/PAST/16-1/osama.htm-206K-6/24/2007.

78)Varma N and Dash S:Reappraisel of underlying pathology in adult patients presenting with pancytopenia.Trop Geogr. Med,44:322-327,1992.

79)International agranulocytosis and aplastic anemia study.Incidence of aplastic anemia :the relevance of diagnostic criteria.Blood 70 ;1718-1721,1987.

80)Albitar M.Manshuri t ,Shen Yet al.Myelodysplastic syndrome is not merely a preleukemia.Blood100:791-8,2002.

81)Kiss e,Gai I,Sinkovis E ,et al.Myelofibrosis in SLE.Leuk lymphoma 39;661-5.2002.