"A CLINICAL AND ETIOLOGICAL STUDY OF PATIENTS WITH PANCYTOPENIA – A ONE YEAR CROSS SECTIONAL STUDY AT KLES DR. PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE, BELGAUM"

By

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Dissertation submitted to the
KLE University, Belgaum, Karnataka

In Partial Fulfillment
of the requirements for the degree of

M. D. MEDICINE

Under the Guidance of

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MAY - 2010

KLE UNIVERSITY, BELGAUM,

KARNATAKA

DECLARATION

I hereby declare that this dissertation entitled **"A CLINICAL AND ETIOLOGICAL STUDY OF PATIENTS WITH PANCYTOPENIA – A ONE YEAR CROSS SECTIONAL STUDY AT KLES DR. PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE, BELGAUM"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. VIJAYAKUMAR G. SOMANNAVAR** MD Professor, Department of Medicine, Jawaharlal Nehru Medical College, Nehru Nagar, Belgaum – 10.

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ACKNOWLEDGEMENT

The completion of the dissertation manuscript is possible only when many individuals have rendered their help and contribution towards it. Hence gratitude and thanks are owed to them all for their help in various ways in completion of this work.

I am extremely grateful to my respected teacher and guide **Dr. Vijayakumar G. Somannavar** MD, Professor, Department of Medicine, J. N. Medical College, Belgaum for his able guidance, supervision, invaluable suggestions and kind help rendered throughout the course of my study and in the preparation of this dissertation.

I am extremely grateful to **Dr. V. A. Kothiwale** MD, Ph.D Professor and Head, Department of Medicine, J. N. Medical College, Belgaum for his timely guidance, encouragement and help in preparing this dissertation.

I am grateful to **Dr. V. D. Patil** MD DCH, Principal, Jawaharlal Nehru Medical College, Belgaum for extending his help towards the completion of this dissertation.

I take this opportunity to thank **Dr. H. B. Rajashekhar** MD, FCCP, **Dr. S. B. Kalagate** MD, **Dr. Rekha S. Patil** MD, **Dr. B Srinivas** MD, **Dr. P. K. Phadnis** MD, **Dr. Arathi Darshan** MD, **Dr. Prakash B.** MD, **Dr. Raju Badiger** MD, **Dr. Rajeev Malipatil** MD, and **Dr. Naveen Angadi** MD for their kind support.

I sincerely thank **Dr. K. Ravishankar Naik** MD, DM (Neuro), **Dr. Saroja A.O**. MD, DM (Neuro), **Dr. Kutub Makandar** MD, DM (Neuro), **Dr. M. S. Khanpet** MD, DNB (Nephro) and all other teachers of J. N.

Medical College, Belgaum for their kind support and timely help during the study.

I take this opportunity to thank **Dr. M. V. Jali** $_{\text{MD}}$**,** Medical Director, KLES Hospital and Medical Research Centre, Belgaum for allowing me to conduct this study in the hospital.

I wish to offer my thanks to Department of Medical Education for their valuable information and support.

No amount of words can measure up to the deep sense of gratitude and thankfulness that I feel towards My **Parents** whose cherished blessings are behind whatever success I have achieved in my life. I whole heartedly thank my grand father **Dr. G. G. Sureja** whose countless sacrifices are behind whatever success I have achieved in my life.

I offer my sincere thanks to all my **Friends** and **Post Graduate Colleagues** for their companionship and support.

Last but not the least, this acknowledgement is incomplete if I fail in my duty to thank all the **Patients** who have whole heartedly participated in the study and have made this study complete.

I bow my head in respect before **God Almighty**.

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LIST OF ABBREVIATIONS USED

ALL	-	Acute lymphoblastic leukemia
AML	-	Acute myeloid leukemia
ATG	-	Anti thymocyte globulin
BFU	-	Blast forming unit
C/F	-	Clinical features
CFU	-	Colony forming unit
CSF	-	Colony stimulating factor
DNA	-	Deoxyribo nucleic acid
EBV	-	Ebstein barr virus
EPO	-	Erythropoietin
GEMM	-	Granulocyte erythrocyte monocyte megakaryocyte
GM	-	Granulocyte megakaryocyte
Hb	-	Haemoglobin
HIV	-	Human immunodeficiency virus
IF	-	Intrinsic factor
IL	-	Interleukin
ITP	-	Immune thrombocytopenic purpura
MCV	-	Mean corpuscular volume
MDS	-	Myelodysplastic syndrome
RBC	-	Red blood cell
RNA	-	Ribo nucleic acid
SLE	-	Systemic lupus erythematosus
TB	-	Tuberculosis
TLC	-	Total leukocyte count
TPO	-	Thrombopoietin
WBC	-	White blood cell

ABSTRACT

Background and Objectives

Pancytopenia means a disorder in which all three blood elements (RBCs, WBCs and platelets) are decreased than normal although it is a common clinical problem with an extensive differential diagnosis, there is a relatively little discussion of this abnormality. Detailed work up is generally required for total evaluation of such case. The objective of the present study was to determine various clinical presentations and etiological factors of pancytopenia.

Methods

The present cross sectional study was conducted in Department of Medicine, at KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during January 2008 to December 2008 on 50 patients (age ≥15 years) presenting with pancytopenia. Detailed history, clinical examination, blood investigations and bone marrow examination were performed. Other investigations like bone marrow trephine biopsy, serological tests, chest X-ray and electrophoresis were performed as necessary.

Results

In all cases, megaloblastic anemia constituted the largest group (36%), followed by aplastic anemia (16%), Malaria (10%) and acute leukemias (10%). Other causes as determined in the present study were myelodysplastic syndrome (MDS), hypersplenism, disseminated tuberculosis, multiple myeloma, human immunodeficiency virus infection, immune thrombocytopenic purpura (ITP), dengue fever and septicemia. The most common symptom was easy fatigability

(90%) followed by fever (70%) and decrease appetite (40%). Pallor (100%) and splenomegaly (52%) were the most common physical findings.

Conclusion

Megaloblastic anemia and aplastic anemia were the common causes of pancytopenia followed by malaria and acute leukemias. Bone marrow study or trephine biopsy was helpful in most of the cases to find out etiology.

Key words

Bone marrow; Pancytopenia; Megaloblastic anemia.

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INTRODUCTION

"Pancytopenia" is defined as the decrease in all the three formed elements in the blood that is leucocytes, erythrocytes and platelets which results in anemia, neutropenia and thrombocytopenia.[1] Pancytopenia therefore exists in the adult when haemoglobin level is less than 13.5 g/dL in males or 11.5 g/dL in females; leukocyte count is less than 4 x 10^9/L and platelet count is less than 150 x 10^9/L.[2]

The spectrum of disorders primarily or secondarily affecting the bone marrow may manifest with peripheral pancytopenia.[3] Patients usually present with complaints ascribed to anemia, thrombocytopenia and rarely leucopenia which in later stages is responsible for downhill course. Various factors encompassing geographical distribution and genetic disturbances may cause variation in the incidence of disorders causing pancytopenia.[4,5,6] Underlying pathology determines the management and prognosis of the patients.[7]

Over the last 30 years many conditions that cause pancytopenia were confused with aplastic anemia based on incomplete study of patient. The incidence of aplastic anemia is two to five cases per 1,000,000 populations per year. The incidence rate in Sweden (13 cases per 1,000,000 per year), Isarel (Eight cases per 1,000,000 per year) and the United States (five to 12 per 1,000,000 per year) suggest that the rate in industrialized countries is about five to 10 per 1,000,000 per year.[3] More recent surveys in Western Europe and the United States suggest that the incidence is in the order of 3.5 in one million population per year. The incidence in the far East, particularly China and Southeast Asia is higher than in West.[7]

Sometimes pancytopenia is detected as an incidental finding in a patient who has presented with symptoms of a disorder that is capable of depressing the levels of all cellular elements in the blood. Initially, mild impairment in marrow function is inapparent and pancytopenia may become apparent only during times of stress or increased demand (for example bleeding or infection). More severe degrees of cytopenias affect the peripheral blood count even in the steady state.[2]

However, major diagnostic problems occur when there are no specific features in the blood to suggest the diagnosis or when the clinical features are not sufficiently specific to point to the cause.[2]

No two studies of clinical profile of pancytopenia are the same. Apparent differences observed in profile may be due to number of factors including health care delivery system/availability of advanced laboratory back up facility/population risk factors (like occupations - rubber factory worker / radiation exposure/agricultural pesticide exposure/exposure to drugs and chemicals/infectious diseases), geographical and environmental factors and other comorbid conditions and study factors such as the type and number of sample collected; investigation performed and interpretation of results.

There are limited numbers of studies on the frequency of the various causes of pancytopenia and its clinical spectrum. Limited data has been reported from the Indian subcontinent. In view of above facts, present study was conducted to determine the various clinical manifestations and etiological factors of pancytopenia.

OBJECTIVES

Objectives of the present study were to determine the;

1. Various clinical presentations among patients with pancytopenia.

2. Etiological factors of pancytopenia.

REVIEW OF LITERATURE

HISTORICAL REVIEW

The study of bone marrow failure is traditionally dated to 1888, when Paul Ehrlich described an young woman who died after an explosive short illness marked by severe anemia, bleeding and high fever.[8] As a pathologist, Ehrlich was struck by the absence of nucleated RBCs and the fatty quality of the femoral marrow, opposite findings from the physiologic response to severe anemia and he inferred from the morphology a mechanism of failed blood cell regeneration.

Surgical trephine biopsy is an older procedure than needle aspiration of the marrow. Pianese in 1903 was first to obtain marrow from the epiphysis of the femur.

Vaquez and Aubertin in their 1904 case report of pernicious anemia with yellow marrow first named the disease and emphasized a pathophysiology of failed haematopoiesis.[9]

Pancytopenia is one of the well-known haematological manifestations of hypersplenism. Chalfford (1907) introduced the term hypersplenism to refer to this concept.[2]

In 1908, Ghedini trephined the upper third of the tibia.[10]

Tissue from patients with early cases of aplastic anemia could only be examined at autopsy and in practice as reflected in the medical literature of the

early 20th century, pancytopenia was often equated with aplastic anemia. Association of aplastic anemia with viral hepatitis first described in 1955.[11]

Adams E.B. (1951) reported pancytopenia associated with idiopathic aplastic anemia in twenty seven cases. He also reported pancytopenia with aleukaemic leukaemia in three patients.[12]

Under the title of 'familial infantile pernicious like anemia', Fanconi (1967) described a fatal autosomal recessive disorder that was characterized by clnical picture consisting of pancytopenia, skin abnormalities, neurologic and endocrine disorders, chrombosomal instability and increased rate of leukaemia and other tumour.[13]

Pancytopenia has been reported following infection with Human Immunodeficiency Virus (HIV). Marrow hypocellularity has been a common finding while aplastic anemia has been described rarely.[13]

In a study of fifty cases of aplastic anemia, reported forty three cases of idiopathic aplastic anemia. Remaining seven cases were attributed to benzol, phenylbutazone, chloramphenicol and arsenic fruit spray.[14]

Two other syndromes associated with aplastic anemia are; Dyskeratosis congenita characterized by aplastic anemia, reticulated hyperpigmentation, nail dystrophy and mucosal leukoplakia described in 1972[15] and Schwachman-Diamond syndrome associated with pancreatic insufficiency, pancytopenia and hypoplastic marrow described in 1964.[16]

In 1976 one study[17] reported haematological parameters in non-Hodgkin's lymphoma in about hundred and forty adults. Another study[21] in 1977 reported seven cases of hairy cell leukaemia, presenting as splenomegaly, pancytopenia and recurrent infection.

In 1976, Kirdly and Wheby found pancytopenia in thirteen patients with bone marrow necrosis. In a study[19] of paediatric patients with bone marrow necrosis, all the patients presented with pancytopenia.

Fatal pancytopenia in falciparum malaria was reported in the literature.[20]

Aplastic anemia associated with various drugs have been described which include OKT3[21], ibuprofen[22] and ciprofloxacin.[23]

In an Indian study[24] involving 50 patients of pancytopenia found megaloblastic anemia as the most common cause.

Aplastic anemia is strongly associated with a rare collagen vascular syndrome called eosinophilic fascitis. Pancytopenia with marrow hypolasia can also occur in Systemic Lupus Erythematosus (SLE).

One study[25] noticed global hypocellularity (47.6%), increased reticulin production (76.2%) and necrosis (19%) in 21 bone marrow specimens from patients with SLE. They concluded that bone marrow might be a target organ in SLE with cytopenias.

One Indian study[26] has described pancytopenia due to hemophagocytic syndrome as the presenting manifestation of the tuberculosis. Patients with

infection associated haemophagocytic syndrome have fever, severe constitutional symptoms and blood cytopenias. Another study[27] found that, panctyopenia associated with disseminated tuberculosis, reactive histiocytic haemophagocytic syndrome and tuberculous hyperplenism.

In 2007 one study[28] involving 148 cases of pancytopenia found that megaloblastic anemia was the most common cause.

NORMAL HAEMOPOIETIC SYSTEM AND HAEMOPOIESIS

Sites of blood formation[2]

Formation of blood cells occurs at different anatomical sites during the course of development form embryonic to adult life. Production of blood cells commences in the yolk sac of the embryo, but then shifts to the liver and to a lesser extent to the spleen, so that these organs become the dominant sites of production between the second and seventh month of gestation. The liver and spleen are then superseded by the bone marrow, which serves as the only important site of blood cell production after birth. An exception is lymphocyte production, which occurs substantially in other organs, in addition to the bone marrow, in adult life.

Haemopoietic tissue fills all of the cavities within the bones of the newborn, but with increasing age, becomes localized in the cavities of the upper shafts of the femur and the humerus, the pelvis, spine, skull and bones of the thorax. The total volume of haemopoietic tissue in adults is one to two litres.

Haematopoiesis[2,29]

In vitro methods for growing human haematopoietic stem cells in soft agar culture have provided further evidence for the hierarchical model of haematopoiesis. The pluripotential stem cell gives rise to multipotential stem cells, which then differentiate into committed stem cells. These committed stem cells then develop into mature, non-proliferating cells. The myeloid stem cell, called the colony forming unit - granulocyte, erythrocyte, monocyte, megakaryocyte (CFU-GEMM), and the lymphoid stem cell, called the colony-forming unit - lymphocyte (CFU-L), are derived from the pluripotential stem cell. These two progenitor cells have a limited capacity for self renewal but can still differentiate into several cell lines. Therefore, these progenitor cells are called multipotential stem cells. Eventually, under the control of the specific haematopoietic growth factors, the multipotential cells become committed to one cell line and then appropriately termed unilineage or committed progenitor cells. Each of these committed progenitor cells is named for the cell line to which it is committed. Each committed progenitor cell differentiates into morphologically identifiable blood cells platelets, erythrocytes, monocytes and granulocytes.

Erythropoiesis[2]

In the erythroid culture system, progenitor cells give rise to two distinct types of erythroid colonies in the presence of the erythroid growth factor, erythropoietin (EPO). A primitive progenitor cell, blast forming unit – erythroid (BFU-E), derived from CFU-GEMM, is relatively insensitive to erythropoietin and forms large colonies after 14 days in the form of blasts. Another type of

colony grows to a maximal size in seven to eight days, matures and degenerates. The erythropoietin sensitive progenitor of this colony is termed colony forming unit – erythroid (CFU-E). The CFU-E gives rise to the first recognizable erythrocyte precursor, the pronormoblast. These progenitor cells are induced to prolieferate and differentiate by several growth factors acting synergistically with erythropoietin, including granulocyte-macrophase colony stimulating factor (GM-CSF), interleukin-3 (IL-3), interleukin-4 (IL-4).

CFU-GEMM → BFU-E → CFU-E → Pronormoblast

GRANULOPOIESIS AND MONOPOIESIS[2]

Granulocytes and monocytes are derived from a common bipotential stem cell, colony forming unit – granulocyte, monocyte (CFU-GM) that is derived from the CFU-GEMM. Specific growth factors for granulocytes and monocytes, acting synergistically with GM-CSF or IL-3, appear to determine which path of differentiations the CFU-GM tales. Monocyte colony stimulating factor (M-CSF) will induce monocyte differentiation, while granulocyte colony stimulating factor (G-CSF) induces neutrophil granulocyte differentiation.

Eosinophils are derived directly from the CFU-GEMM under the influence of growth factors GM-CSF, IL-3 and IL-5.

Basophils are also derived directly from the CFU-GEMM under the influence of IL-3.

Thrombopoiesis

Platelets (thrombocytes) are derived from CFU-GEMM. CFU-Megakaryocyte is induced to proliferate and differentiate into megakaryocytes by the growth factors IL-3 and GM-CSF, while megakarycytes are stimulated to increase in size and to produce platelets by a substance termed thrombopoietin (TPO).

Lymphoid stem cell

The lymphoid stem cell is derived from the pluripotential stem cell and gives rise to T and B lymphocytes. Lymphocytes mature at multiple sites, including the bone marrow, thymus, lymphnodes and spleen.

Table No. 1: Haematopoietic growth factors and some of their characteristics[26]

Factor	Cells stimulated	Production sources
M-CSF (CSF-I)	Monocytes	Endothelial cells, monocytes fibroblasts
GM-CSF	All granulocytes, megakaryocytes, erthrocytes, stem cells, leukaemic blasts	T-cells, endothelial cells, fibroblasts
G-CSF	Granulocytes, macrophages, endothelial cells, fibroblasts, leukemic blasts	Endothelial cells, placenta, monocytes
IL-1	Stimulates expression of growth factors by other cells, mediates inflammation	Monocytes, macrophages, endothelial cells
IL-2	T and B lymphocytes	T lymphocyte
IL-3	Granulocytes, erythroid cells, multipotential progenitor, leukemic blasts	T cells
IL-4	B and T cells	T cells
IL-5	B cells, CFU-E	T cells
IL-6	B, T cells, CFU-GEMM, CFU-GM, BFU-E, macrophages, neural cells, hepatocytes	Fibroblasts, leukoctyes, epithelial cells
IL-7	B cells	Leukocytes
IL-8	T cells and Neutrophils	Leukocytes
IL-9	BFU-E, CFU-GEMM	Lymphocytes
IL-11	B and T cells, CFU-GEMM, CFU-GM, Macrophages, BFU-E, BFU-B, Myokaryocytes and Leukoerythroblasts	Macrophages
EPO	CFU-E, BFU-E	Kidney and liver

Stromal cells in the bone marrow microenvironment serve to support stem cell renewal proliferation and differentiation by secreting by specific haematopoeitic growth factors as well as providing physical support and points of adhesion.

Erythroid series[2]

1. Proerythroblast – the least mature of the morphologically identifiable members of the erythorid series. It has diameter of 14 to 20μm. There are several nucleoli in the nucleus which is round and occupies most of the cell. The chromatin in the nucleus consists of network of fine red-purple strands characteristic feature is that the peripheral cytoplasm is more basophilic than myeloblast.

2. Basophilic erythroblast – It is a round cell of 12-16μm diameter. The nucleus of the pro-erythroblast by having coarser and more basophilic chromatin strands.

3. Polychromatic erythroblast (intermediate erythroblast) – It is a round cell between 12 and 14μm in diameter. The characteristic polychromatic appearance of the cytoplasm is derived from the mixture of the basophilic ribonucleic acid (RNA) and acidophilic hemoglobin. Nuclear chromatin in coarse, deeply basophilic and proliferate activity ceases after this stage.

4. Orthochromatic erythroblasts (late erythroblast) – It has a diameter between 8 and 12μm. The nucleus is relatively small and pyknotic with a

homogeneous blue-black appearance. The cytoplasm is predominantly acidophilic due to the presence of large amounts of haemoglobin.

5. Reticulocytes – The nucleus is extruded from the orthochromatic erythroblast to form the reticulocytes. Reticulocytes have the same biconcave discoid shape as mature red cells, although they have a slightly greater volume and diameter than the later. Reticuloctye cytoplasm is similar in staining characteristics to that of orthrochromatic erythroblasts, which are distinguished form mature red cells by a diffuse basophilic hue. Reticuloyctes lose their mitochondria and ribosomes over the course of a few days and in doing so, lose the basophilic tint and evolve into the mature erythrocytes.

GRANULOPOIESIS[2]

The colour of the numerous granules in the cytoplasm after staining with Romanovsky stains is the basis of the classification of granulocytes into neutrophil, eosinophil and basophil series. Mature granulocytes are produced by the proliferation and maturation of precursors from the earliest recognizable stage, the myeloblast, through the promyelocyte, myelocyte, metamyelocyte and the stab-form stage, until the mature segmented stage is reached. Development of the neutrophil, eosinophil and basophil series follows a similar pattern, except that the characteristic distinction between the colour of the granules becomes obvious at the myelocyte stage.

1. Myeloblast: The myeloblast is a relatively large cell 15-20μm in diameter, with a round to oval nucleus which occupies a large proportion of the cell. There are no typical granules in the moderately basophilic cytoplasm. Nuclear chromatin is arranged in a fine network of red-purple strands with occasional small aggregates. Nucleoli are typically prominent.

2. Promyelocyte: The features of this cell are similar to those of the myeloblast, except for the development of some cytoplasmic granules and a slightly more coarse appearance of the chromatin. Nucleoli are still present.

3. Myelocyte: It has prominent cytoplasmic granules, and the area of cytoplasm relative to the nucleus is greater than in the promyelocyte. The cytoplasm is also less basophilic, nucleoli are no longer present and the chromatin appears more aggregated than in the promyelocyte. Granulocyte precursors undergo active proliferation until after the myelocyte stage. Subsequent steps in the maturation process consequently occur in non dividing cells and in particular involve progressive changes in the conformation of the nucleus from round in the myelocyte to segmented in the mature form.

4. Metamyelocyte: The nucleus becomes indented and assumes a kidney-shaped appearance in the metamyelocyte granules are prominent in the cytoplasm.

5. Band or stab form: When the degree of indentation of the nucleus is greater than 50 percent of the nuclear diameter, the precursor has reached this stage cytoplasmic granules are identical to those in the mature segmented form.

6. Segmented or polymorphonuclear granulocytes: The cell 12-14μm in diameters, characterized by a lobulated nucleus with two to five lobes of clumped chromatin. An abnormally high number of nuclear lobes is indicative of disordered granulopoiesis.

7. Polymorphonuclear eosinophils: They are slightly larger than segmented neutrophils and have a diameter of upto 16μm. The number of nuclear lobes is usually two. The cytoplasm has a pale hue similar to that of the segmented neutrophil and contains many granules which are larger than those in the segmented neutrophil. These granules stain bright orange with Romanovsky stains.

8. Polymorphonucelar Basophils: They are similar to the mature eosinophil, with the characteristic distinction that the granules are intensely basophils and tend to overlie and obscure the nucleus.

THE MONOCYTE – MACROPHAGE SERIES[2]

1. Monoblasts are the least mature of the morphologically recognizable members of the monocyte macrophage series and are very similar in appearance to myeloblasts.

2. Promonocyte: It is similar in size to the promyelocyte, but has a more regularly shaped and often deeply cleft, nucleus containing nucleoli. The cytoplasm contains granules often arranged in a localized region and the granules are larger and more basophilic than in the mature monocyte.

3. Monocyte: It is slightly larger than the segmented granulocyte. Is has a regularly shaped nucleus with a relatively fine chromatin pattern. Cytoplasm, abundant and of a pale grey-blue tint. It contains some small neturophilic or basophilic granules, which are less common than in granulocytes.

4. Macrophages: Range form 15 to 80µm in diameter. They have one or more oval nuclei and an irregular or oval cytoplasmic outline. Cytoplasm in larger macrophages is particularly abundant and contains granules and in some instances, vacuoles which may contain phagocytosed material.

THE LYMPHOID SERIES[2]

1. Lmphoblasts are slightly smaller than the myeloblasts which they resemble, except that the ratio of the diameter of the nucleus to that of the cell tends to the greater and the number of nucleoli per nucleus tends to be fewer than the myeloblast.

2. Large lymphocyte – It is between 12 and 16µm in diameter and round in outline. The nucleus is round or slightly indented and its chromatin is more clumped than in the lymphoblast. The cytoplasm is more abundant than in the lymphoblast, usually pale blue in colour.

3. Small lymphocyte – They are between 9 and 12µm in diameter and are thus smaller than segmented granulocytes. The cytoplasm usually forms only a thin, medium to deeply basophilic rim encircling a round or

marginally indented nucleus which contains, deeply staining, heavily clumped chromatin.

4. Plasma cells – At the most immature stage of development resemble lymphoblasts, except for possessing more basophilic cytoplasm. In the next stage of development, the nucleus is smaller and the chromatin is more clumped. The nucleus at this intermediate stage has assumed the eccentric location at the periphery of the cell which is characteristic of the mature plasma cell.

THE MEGAKARYOCYTIC SERIES[2]

1. Megakaryoblast – It is the most immature stage of platelet development which resembles the myeloblast in its basic features.

2. Promegakaryoctye – It is larger than its precursor, has deeply basophilic cytoplasm containing some basophilic granules. The nucleus may be lobulated and the chromatin is more deeply basophilic than in the megakaryoctye.

3. Megakaryoctye – Range from 30 to 90μm in diameter and contain 4 to 16 nuclear lobes with coarsely clumped chromatin. The larger expanse of cytoplasm stains light blue and contains many small red-purple granules.

4. Platelets – These are the small, anucleate, terminal stage of development of the megakaryocytic series. They are discoid and have a diameter of 1-4μm. The cytoplasm stains light blue and contains small red-purple granules which are centrally located in platelets in blood films.

Normal bone marrow structure[2]

The red marrow interspersed between the trabeculae of bone within the bony cavity contains specialized connective tissue cells, reticulin fibrils, blood vessels, fat cells, nerves and macrophages in addition to cells of the lymphoid and haemopoietic series.

A supportive framework for the components of the bone marrow is provided by a network of fine reticulin fibrils. These fibrils stretch from the endosteum of the bony trabeculae to the vascular sinusoids and appear to be produced by the adventitial reticular cell.

Arteriolar blood passes into the relatively large lumen of sinusoids lined by a single layer of endothelial cells. Entry of newly formed blood cells into the circulation occurs at this site.

Fat cells make up approximately half the extra vascular volume of red marrow and nearly all of the extravascualr volume of yellow marrow in the more peripheral parts of the long bones. Distribution of fat cells is irregular in red marrow and an adequate sample size is necessary in order to obtain a reliable indication of the cellularity of haemopoietic tissue.

Bone marrow biopsy[2,29]

The advantages of aspiration are that films prepared from aspirated material can be examined almost immediately and the morphological detail is superior to that in histological sections of core biopsies obtained by the trephine procedure. The bone marrow trephine on the other hand, provides a more reliable

index of the cellularity of haemopoietic elements and reveals certain abnormalities such as neoplastic cells or fibrotic material which may not be dislodged from the marrow cavity by suction. The information obtained by each procedure is therefore additive, so that combined data is of greater diagnostic value than that provided by either procedure alone.

Needle Aspiration biopsy of the bone marrow

Satisfactory samples of bone marrow can usually be aspirated form the sternum, iliac crest or anterior or posterior iliac spines. However, the sternum is no longer favoured because unless the needle is correctly inserted there is a danger of perforating the inner cortical layer and damaging the underlying large blood vessels and right atrium with serious consequences.

Overlying skin at the site, is cleaned with 70% alcohol (for example ethanol) or 0.5% chlorhexidine after giving atropine and lignocaine test dose. The skin, subcutaneous tissue and periosteum overlying the site selected for the puncture are carefully infiltrated with two percent lignocaine.

With a boring movement, pass the needle perpendicularly into the cavity of the ileum at a point just posterior to the anterior superior iliac spine two cms posterior and two cms inferior to the anterior superior iliac spine. When the bone has been penetrated remove the stillete and with a well-fitting two or five ml syringe suck up not more than 0.3 ml of marrow contents – bone marrow diluted with a variable amount of blood.

The posterior iliac spine overlies a large marrow containing area and relatively large volumes of marrow can be aspirated from this site. Posterior iliac puncture can be carried out with the patient lying prone or on his side. An advantage of puncturing the ileum rather than the sternum is that the patient can lie on his side and cannot see what is happening.

Marrow puncture needles

The most commonly used needles are the salah and klima needles.

Bone marrow films

Deliver single drops of aspirate on to slides about 1cm from one end, place the slides on a slope to allow the blood to drain away. The irregularly slipped marrow fragments tend to adhere to the slide and most of them will be left behind. Then make films 3-5cms in length of the marrow fragments and the reminding blood using a smooth-edged glass spreader of not more than two cms in width.

Fix the films of bone marrow and stain them with Romanovsky dyes as peripheral blood films. Some workers add the aspirated marrow routinely to an anti-coagulant.

Cellularity of marrow

The degree of cellularity can be altered within broad limits as increased, normal or reduced by inspection of a stained film containing marrow particles and for practical purposes, this is all that is usually necessary. As a rough guide,

if less than 25% of the particle is occupied by haemopoietic cells it is probably hypocellular and if more than 75-80% it is hypercellular.

In one study, by means of point counting of sections from the iliac crest, the range of cellularity in children under 10years was reported as 59-95% with a mean of 79%, at 30 years the mean was 50% and at 70 years it was 30% with a range of 11 – 47%.[30]

Ratio

The M:E ratio is based on a count of 200 to 500 marrow cells. In the normal adult the ratio is about three or 4:1.

Bone marrow trephine biopsy[31]

A trephine biopsy is usually most easily carried out on the posterior superior iliac spine, with the patients in the left or right lateral position and with the knees drawn up. An alternative site is the ilium, just below the anterior superior iliac spine. Various needle designs are satisfactory including Jamshidi and Islam needles.

A trephine biopsy and aspiration biopsy can be carried out through the same skin incision but with the bone being entered at two different points, about 1cm apart. Aspiration is performed first. It is easier to perform the least painful procedure first. Local anaesthesia must be adequate with particular attention being paid to infiltrating an adequate area of the periosteum. In anxious patients sedation is useful. The trephine should be inserted by to-and-fro rotation through approximately 90°. The biopsy needle should be firmly fixed in the cortex of the

bone before the trocar is removed. Ideally, the biopsy should measure at least 20 mm in length after processing.

Trephine biopsies can be carried out safely on patients with severe thrombocytopenia but prolonged pressure is indicated to achieve primary haemostasis and reduce bleeding to a minimum.

Bleeding problems are more likely in patients with coagulation defects and if patients with severe liver disease or disseminated intravascular coagulation require a biopsy, the coagulation defect should be corrected, as far as possible, before the procedure is undertaken.

Fix the specimen in 10% formalin solution buttered to pH 7. Sections of marrow should be stained as a routine by haematoxylin and eosin and by a reticulin impregnation method. H and E staining is excellent for demonstrating the cellularity and pattern of the marrow and for revealing pathological changes such as fibrosis or the presence of granuloma or carcinoma.

PANCYTOPENIA

Pancytopenia referes to a reduction in all three formed elements of the blood erythrocytes, leukocytes and platelets. It is not a disease entity, but the triad of findings that may result from a number of disease processes.[13]

Pancytopenia therefore exists in the adult when the haemoglobin level is less than 13.5g/dl in males, or 11.5g/dl in females, the leukocyte count is less than 4 X 10^9/L and the platelet count is less than 150 X 10^9/L.[2]

PATHOPHYSIOLOGY

The mechanisms by which pancytopenia develops appear to be varied.

1. Some conditions are associated with a decrease in haematopoietic cell production in bone marrow as a result of
    a. Destruction of marrow tissue by toxins (cellular or hypoplastic marrow).
    b. Replacement by abnormal or malignant tissue.
    c. Suppression of normal marrow growth and differentiation.
2. In other conditions the marrow may be normally cellular or even hyper-cellular and no abnormal cells may be present. The mechanisms are
    a. Ineffective haematopoiesis with cell death in the marrow.
    b. Formation of defective cells that are rapidly removed from the circulation.
    c. Trapping of normal cells in hypertrophied and overactive reticuloendothelial system.
    d. Sequestration and/or destruction of cells by action of antibodies.

CAUSES[3]

The diverse causes of pancytopenia are listed below.

I. Pancytopenia with hypocellular bone marrow.
    i. Acquired aplastic anemia
    ii. Inherited aplastic anemia (Fanconi's anemia)
    iii. Some myelodysplastic syndrome
    iv. Rare aleukaemic leukaemia
    v. Some acute lymphoid leukaemia

vi. Some lymphomas of bone marrow

II. Pancytopenia with cellular bone marrow

i. Primary bone marrow disease

1. Myelodysplastic syndrome (MDS)
2. Paroxysmal nocturnal haemoglobinuria
3. Myelofibrosis
4. Some aleukaemic leukaemias
5. Bone marrow lymphoma
6. Hairy cell leukemia

ii. Secondary to systemic diseases.

1. Systemic lupus Erythematosus
2. Hypersplenism
3. Vitamin B_{12}, folate deficiency
4. Overwhelming infections
5. Alcohol
6. Brucellosis
7. Sarcoidosis
8. Tuberculosis
9. Leishmaniasis
10. Malaria

Severe overwhelming infections, rather than being a direct cause of pancytopenia, usually reflects the exhaustion of marrow reserves already depleted for example as a result of vitamin B_{12} deficiency or folate, alcoholism, the effect

of drugs or chemicals to which the patients may be sensitive or previous treatment with cytotoxic agents.

Pancytopenia has been reported occasionally in asosication with tuberculosis and other mycobacterial infections, such as mycobacterial kanasii, clinical features included fever, sweating, weight loss, lymphadenopathy and splenomegaly. Macrocytosis and severely hypoplastic or aplastic bone marrow containing caseating tubercles are present in some patients, in others, the marrow is cellular.

Rarely, pancytopenia has been reported in association with brucellosis, sarcoidosis, pregnancy, idiopathic siderobiastic anemia and refractory anemia, some of which conditions after a variable period, may evolve into acute myelomonocytic leukemia, erythroleukaemia. Myelofibrosis or aplastic anemia.

Clinical Featrues[2,24]

The initial clinical picture in patients with pancytopenia varies widely. The onset often is insidious. Manifestations depend on the severity of the anemia, thrombocytopenia or leucopenia.

Sometimes pancytopenia is detected as an incidental feature in a patient who has presented with symptoms of a disorder that is capable of depressing the levels of all cellular elements in the blood.

The clinical features and simple laboratory findings reflect the underlying disease process and usually serve to reduce the number of possible diagnosis quickly. Thus, the presence of splenomegaly calls attention to the possibility of

leukemia, Myelofibrosis, congestive splenomegaly. the presence of enlarged lymph nodes further supports the possibilities of leukemia, one of the lymphomas, or lupus erythematosus. On the other hand, lack of these signs and absence of evidence of vitamin B_{12} or folate deficiency should suggest multiple myeloma or aplastic anemia. The presence of rouleaux on the blood smear or Bence Jones protein in the urine suggests myeloma. Immature erythrocytes and leukocytes in the blood smear (leukoerythroblastic blood picture) should lead the clinician to consider infiltrative disease in the bone marrow (for example, metastatic carcinoma, leukemia or Myelofibrosis), except in the event of greatly accelerated blood formation and destruction, such as occurs in cases with frank haemolytic anemia.

The anemia usually is normochromic and normocytic, but occasionally it is mildly macrocytic. The leucopenia usually results from a reduction in the absolute number of cells of the myeloid series and thus relative lymphocytosis is noted. If, however, the reduction is sufficiently great, lymphocytopenia is found as well.

Difficulties in diagnosis

Difficulty arises when atypical features are encountered, for example when a patient thought to have aplastic anemia has normally cellular or even hypercellular marrow. One explanation for such a contradictory finding is that the biopsy needle entered an area in which the bone marrow is regenerating after severe damage, such as after benzene intoxication or irradiation. Another dilemma involves finding that several marrow aspirations are acellular in a

patient thought to have leukemia. In most situations, a larger marrow sample obtained by biopsy will solve the problem. With a few conditions, such as congestive splenomegaly or hypersplenism the diagnosis is made largely by excluding the other possibilities. Finally, in a few patients, no clearly defined syndrome can be recognized.

Treatment

The treatment of pancytopenia is dictated by the nature of the underlying disease.

Aplastic anemia [2,32,33,34]

Definition

Aplastic anemia is pancytopenia with bone marrow hypocellularity. Classification of aplastic anemia.

Acquired aplastic anemia

a. Idiopathic aplastic anemia
b. Secondary aplastic anemia
    i. Irradiation
    ii. Drugs and chemicals
        1. Regular effects
            a. Cytotoxic agents
            b. Benzene
        2. Idiosyncratic reactions

a. Chloramphenicol
b. Nonsteroidal anti-inflammatory drugs
c. Antiepileptics
d. Gold
e. Other drugs and chemicals

iii. Virus

1. Ebstein-barr virus (infectious mononucleosis)
2. Hepatitis virus (non A, non B, non C, non G hepatitis)
3. Parvo virus (transient aplatic crises, some pure red cell aplasia)
4. Human immunodeficiency virus infection

iv. Immune diseases

1. Eosinophilic fascities
2. Hypoimmunoglobulinemia
3. Thymoma and thymic carcinoma
4. Graft-versus-host disease in immunodeficiency

v. Paroxysmal nocturnal haemoglobinuria

vi. Pregnancy

c. Inherited aplastic anemia

i. Fanconi anemia

ii. Dyskeratosis congenita
iii. Shwachman-Diamond syndrome
iv. Reticular dysgenesis
v. Amegakaryocytic thrombocytopenia
vi. Familial aplastic anemia
vii. Preleukemia
viii. Nonhaematologic syndromes

Pathophysiology

Bone marrow failure results form severe damage to the haematopoietic cell compartment. The magnetic resonance imaging of the spine, cells bearing the CD34 antigen, a marker of early haematopoietic cell, are greatly diminished; and in functional studies, committed and primitive progenitor cells are virtually absent. Aplastic anemia does not appear to result from defective stroma or growth factor production.

Drug injury

Extrinsic damage to the marrow follows massive physical or chemical insults such as high doses of radiation and toxic chemicals. For the more common idiosyncratic reaction to modest doses of medical drugs, altered drug metabolism has been involved as a likely mechanism. The metabolic pathways of many drugs and chemicals, especially if they are polar and have limited water solubility, involve enzymatic degradation to highly reactive electrophilic compounds, these intermediates are toxic because of their propensity to bind cellular macromolecules.

Immune mediated injury

Blood and bone marrow cells of patients can suppress normal haematopoietic progenitor cell growth and removal of T cells from aplastic anemia bone marrow improves colony formation in vitro. Increased numbers of activated cytotoxic T cells are observed in aplastic anemia patients and usually decline with successful immuno-suppressive therapy. Interferon and tumour necrosis factor induce Fas expression on CD34 cells, leading to apoptotic cell death.

Early immune system events in aplastic anemia are not well understood. Many different exogenous antigens appear capable of initiating a pathologic immune response, but at least some of the active T cells recognize true self-antigens. The rarity of occurrence of aplastic anemia despite common exposures (medical drugs, hepatitis viruses) suggests that genetically determined features of the immune response can convert a normal physiologic response into a sustained abnormal autoimmune process.

Clinical features[2,24,32]

History

Aplastic anemia can appear with seeming abruptness or have a more insidious onset. Bleeding is the most common early symptom; a complaint of days to weeks of easy bruising, oozing form the gums, nose bleeds, heavy menstrual flow and sometimes petechiae will have been noticed. Symptoms of anemia are also frequent including lassitude, weakness, shortness of breath and a

pounding senstation in the ears. Infection is an unusual first symptom in aplastic anemia. A striking feature of aplastic anemia is the restriction of symptoms to the haematologic system. History of drug use, chemical exposure and preceding viral illness must often be elicited with repeated questioning.

Physical examination

Pallor of the skin and mucous membranes is common. Petechiae and ecchymoses are often present and retinal hemorrhages may be present. Infection on presentation is unusual but may be present if the patient has been symptomatic for a few weeks. Lymphadenopathy and splenomegaly are highly atypical of aplastic anemia. Cafe au lait spots and short stature suggest Fanconi's anemia.

Laboratory studies

Blood

The smear shows erythrocytes and a paucity of platelets and granulocytes. Reticulocytes are absent or few and lymphocyte numbers may be normal or reduced.

Bone marrow

The bone marrow is usually readily aspirated but appears dilute on smear and the fatty biopsy specimen may be grossly pale on withdrawal. The biopsy is superior for determination of cellularity and shows mainly fat under the microscope, with haematopoietic cells occupying, by definition, <25% of the

marrow space. The correlation between marrow cellularity and disease severity is imperfect.

Ancillary studies

Chromosome breakage studies of periperhal blood using diepoxybutane or mitomycin C should be performed on children to exclude Fanconi's anemia. Chromosome studies of bone marrow cells are often revealing in MDS and should be negative in typical aplastic anemia. Flow cytometric studies have replaced the Ham test for the diagnosis of PNH. Serologic studies may show evidence of viral infection, especially EB virus and HIV. Post hepatitis aplastic anemia is typically seronegative.

Prognosis

The major prognostic determinant is the blood count, severe disease is defined by the presence of two of three parameters, absolute neutrophil count less than 500 /µl, platelet count less than 20,000 /µl and corrected reticulocytes count less than one percent. Survival of patients who fulfill these criteria is about 20% at one year after diagnosis; patients with very severe disease, defined by an absolute neutrophil count less than 200 /µl, do even more poorly.

Treatment

Treatment includes therapies that reverse the underlying marrow failure and supportive care of the pancytopenic patient. Severe acquired aplastic anemia can be cured by replacement of the absent haematopoietic cells by stem cell transplant, or ameliorated by suppression of the immune system to allow

recovery of the patients residual bone marrow function. Haematopoietic growth factors have limited usefulness and glucocorticoids are of no value. Suspect exposures to drugs or chemicals should be discontinued.

Bone marrow transplantation

This is the best therapy for the young patient with a fully histo-comptible sibling donor. For allogenic transplant from fully matched siblings, long-term survival rates for children are about 80%. Transplant morbidity and mortality are increased among adults, due mainly to the increased rate of chronic graft-versus-host disease and serious infections. Survival using alternative donors is about half that of conventional sibling transplants.

Immunosuppression

Used alone, antithymocyte globulin (ATG) induces haematologic recovery in about 50% of patients. The addition of cyclosporine to ATG has further increased response rates to about 70% to 80%. Combined treatment is now standard for patients with severe disease.

Horse ATG is given at 40mg/kg per day for four days. Most patients are given methylprednisolone, one mg/kg per day for two weeks to ameliorate the immune consequences of heterologous protein infusion. Cyclosporine is administered orally at an initial dose of 12 mg/kg per day in adults, with subsequent adjustment according to blood levels obtained every two weeks.

Other therapies

The effectiveness of androgen therapy has not been verified in controlled trials, but occasional patients will respond. For patients with moderate disease or those with severe pancytopnia who have failed imunosuppression, a three to four months trail is appropriate. Haematopoietic growth factors, G-CSF, GM-CSF and interleukin-3, are not recommend as initial therapy for severe aplastic anemia.

Supportive care

Both platelet and erythrocyte numbers can be maintained by transfusion. Any rational regimen of prophylaxis requires transfusions once or twice weekly in order to maintain the platelet count more than 10,000/cmm. Menstruation should be suppressed by oral estrogen. Red blood cells should be transfused to maintain a normal level of activity, usually at a haemoglobin value of 70g/L (90 g/L if there is underlying cardiac or pulmonary disease), a regimen of two units every two weeks will replace normal losses in a patient without a functioning bone marrow.

HYPERSPLENISM[2,5]

Definition

It has been known for many years that certain patients with splenomegaly secondary to a number of disorders develop neutropenia, anemia or thrombocytopenia, either singly or in combination and that splenectomy results in varying degrees of improvement in the peripheral blood picture, even to normal.

The fact that the peripheral blood picture is corrected by splenectomy led to the concept of hypersplenism.

Etiology

Secondary

- Portal hypertension with congestive splenomegaly
- Lymphomas
- Sarcoidosis
- Felty's syndrome
- Lipid storage disease – Gaucher's disease
- Kala azar, Chronic Malaria, tropical splenomegaly
- Bacterial infections : tuberculous, brucellosis, bacterial endocarditis, chronic bacteraemia
- Thallassemia
- Chronic lymphocytic leukemia
- Myelofibrosis
- Hairy cell leukaemia

Primary (idiopathic)

Mechanism of hypersplenism

The processes involved in depression of the red cell count is pooling of red cells within the enlarged spleen. Studies with radio-isotope labelled red cells indicate that passive pooling of red cells in the spleen has a greater impact on

lowering the red cell count in the blood than accelerated destruction of entrapped red cells, although the latter can occur to some extent in some instances.

Diagnostic criteria of hypersplenism[2]

1. Anemia, leucopenia or thrombocytopenia, either singly or in combination.
2. Cellular or hyprplastic bone marrow
3. Splenomegaly
4. Significant improvement in the peripheral blood picture following splenectomy.

In many cases of hyperplenism, the cause of the splenomegaly is suggested by the presence of manifestations of the underlying disease for example portal hypertension or lymphoma and is confirmed by appropriate investigation.

Blood picture

There is nothing specifically diagnostic in the peripheral blood picture. Anaemia is usually normocytic and normochromic. Leukopenia is due primarily to neutropenia, but in severe cases all while cells are reduced in number. The white cell count is usually not reduced sufficiently to predispose to infection – total counts from 3 to 4 X 10^9/L, with neutrophil counts of 1 to 2 X 10^9/L being usual. Moderate thrombocytopenia occurs with a platelet count of about 100 X 10^9/L being usual, but occasionally values are 50 to 100 X10^9/L.

Bone marrow

The bone marrow is either of normal or increased cellularity and may be infiltrated by a disease process that has been responsible for the enlargement of the spleen.

Treatment

Splenectomy produces partial or complete recovery of the abnormal blood picture in otherwise uncomplicated cases. When the effect of hypersplenism is not sufficient to cause symptoms, splenectomy offers no benefit to the patient. Splenectomy is indicated when significant problems are caused by the sole or the additional effect of hypersplenism in reducing the count of blood cells, usually anemia of sufficient severity to cause symptoms, neutropenia predisposing to infectious or thrombocytopenia causing spontaneous bleeding.

MEGALOBLASTIC ANAEMIA[2,3,5]

The megaloblastic anemia's are disorders caused by impaired DNA synthesis. Cells primarily affected are those having relatively rapid turnover, especially haematopoietic precursors and gastrointestinal epithelial cells. Cell division is sluggish, but cytoplasmic development progresses normally, so megaloblastic cells tend to be large, with an increased ratio of RNA to DNA. Megaloblastic erythroid progenitors tend to be destroyed in the marrow (ineffective erythropoiesis).

Classification of the megaloblastic anemia's

Cobalamine deficiency

1. Inadequate intake – Vegetarians
2. Malabsorption
    a. Defective release of Cobalamine from food
        i. Gastric achlorhydria
        ii. Partial gastrectomy
        iii. Drugs and block acid secretion
    b. Inadequate production of intrinsic factor (IF)
        i. Pernicious anemia
        ii. Total gastrectomy
        iii. Congenital absence or functional abnormality of IF (rare)
    c. Disorders of terminal ileum
        i. Tropical sprue
        ii. Non-tropical sprue
        iii. Regional enteritis
        iv. Intestinal resection
        v. Neoplasms and granulomatous disorder (rare)
    d. Competition for Cobalamine
        i. Fish tapeworm (Diphyiloborthrium latum)
        ii. Bacteria : "blind loop" syndrome
    e. Drugs : p-amino salicylic acid, colchicines, neomycin
3. Others
    a. Nitrous oxide

b. Congenital enzyme defect

Folic acid deficiency

1. Inadequate intake : Unbalanced diet (common in alcoholics, teenagers)
2. Increased requirements
    a. Pregnancy
    b. Infancy
    c. Malignancy
    d. Increased haematopoiesis
    e. Chronic exfoliative skin disorders
    f. Haemodialysis
3. Malabsorption
    a. Tropical sprue
    b. Non-tropical sprue
    c. Drugs : Phenytoin, barbiturate, (?) ethanol
4. Impaired metabolism
    a. Inhibitors of dihydrofolate reductase : methotrexate, pyrimethamine, triamterene, pentamidine.
    b. Alcohol

Other cases

1. Drugs that impair DNA metabolism
    a. Purine antagonists : 6-mercaptopurine, azathioprine.
    b. Pyrimdine antagonists : 5-flurouracil, cytosine arabinoside.
    c. Others : Procarbazine, hydroxyurea, acyclovir, zidovudine.

2. Metabolic disorders
    a. Hereditary orotic aciduria
    b. Lesch-Nyhan syndrome
3. Megaloblastic anemia of unknown etiology
    a. Refractory megaloblastic anemia
    b. Di Guglielmos syndrome
    c. Congenital dyserythropoietic anemia

Clinical features

1. Cobalamine deficiency

The clinical features of Cobalamine deficiency involve the blood, the gastrointesintal tract and the nervous system.

The haematologic manifestations are almost entirely the result of anemia, although very rarely prupura may appear, due to thrombocytopenia. Symptoms of anemia may include weakness, light-headedness, vertigo and tinnitus as well as palpitation, angina and the symptoms of congestive failure. On examination, the patient is pale with slight icterus of skin and eyes. The gastrointestinal manifestation are sore tongue, which on inspection will be smooth and beefy red. Anorexia with moderate weight loss, possibly accompanied by diarrhea. The neurologic manifestations include numbness, paraesthetia in the extremities, weakness and ataxia, due to demyelination, followed by axonal degeneration of peripheral nerves, the spinal cord and the cerebrum itself.

2. Folate deficiency

Patients with folic acid deficiency are more often malnourished than those with Cobalamine deficiency. The haematologic and gastrointestinal manifestations are the same as those of Cobalamine deficiency. However, in contrast to Cobalamine deficiency, neurologic abnormalities do not occur.

Diagnosis

The finding of significant macrocytosis (MCV>100fl) suggests the presence of a megaloblastic anemia. The reticulocyte count may also be decreased, particularly in severely anaemic patient. The blood smear demonstrates marked anisocytosis and poikilocytosis, together with macroovalocytes. In the white blod cell series, the neutrophils show hyper-segmentation of the nucleus. Bizarre, misshapen platelets are also observed.

The bone marrow is hypercellular with a decreased myeloid/erythroid ratio. RBC precursors are abnormally large and have nuclei that appear much less mature than would be expected from the development of the cytoplasm. The nuclear chromatin is more dispersed than expected, and it condenses in a peculiar fenestrated pattern. Granulocyte precursors are also affected, many being larger than normal including meta-myelocytes. Megakarycyte are decreased and show abnormal morphology. Enhanced intramedullary destruction of erythroblasts results in an increase in unconjugated bilirubin and lactic acid dehydrogenase in plasma. Once Cobalamine deficiency has been established, its pathogenesis can be delineated by means of a Schilling test.

Treatment

Cobalamine deficiency

Apart from specific therapy related to the underlying disorder, the mainstay of treatment for Cobalamine deficiency is replacement therapy. Because the defect is nearly always malabsorption, patients are generally given parenteral treatment, specifically in the form of intramuscular cyano-cobalamine. Parental treatment begins with 1000µg cyanocobalamine intramuscularly every month for the rest of the patient's life.

Folate deficiency

Folate deficiency is treated by replacement therapy. The usual dose of folate is 1mg/d by mouth, but higher doses (up to 5mg/d) may be required for folate deficiency due to malabsorption.

MYELODYSPLASIA[2,5]

Definition

The myelodysplastic syndromes are a heterogeneous group of haematologic disorders broadly characterized by cytopenias associated with dysmorphic (or abnormal appearing) and usually cellular bone marrow and consequent ineffective blood cell production.

Classification of myelodysplasia (MDS)

1. Refractory anemia (RA)
2. Refractory anemia with ringed sideroblasts (RARS)

3. Refractory cytopenia with multilineage dysplasia (RCMD)
4. Refractory cytopenia with multilineage dysplasia with ringed sideroblasts (RCMD-RS)
5. Refractory anemia with excess blasts (RAEB-1)
6. Refractory anemia with excess blasts in transformation (RAEB-2)
7. Myledysplastic Syndrome with unclassified (MDS-U)
8. Myledysplastic Syndrome with isolated deletion (5q)

Epidemiology

Idiopathic MDS is a disease of the elderly, the mean age at onset is 68 years. There is a slight male preponderance.

Etiology and Pathophysiology

The myelodysplastic syndromes have been convincingly linked to environment exposures such as radiation and benzene, secondary MDS occurs as a stereotypical late toxicity of cancer treatment. Myelodysplastic syndrome is a clonal haematopoietic stem cell disorder leading to impaired cell proliferation and differentiation. Cytogenetic abnormalities are found in about half of patients. Both presenting and evolving haematologic manifestations result from the accumulation of multiple genetic lesions, loss of tumour suppressor genes, activating oncongene, mutations or other harmful alteration. Sideroblastic anemia may be related to mutations in mitochondrial genes. Ineffective erythropoiesis and disordered iron metabolism are the functional consequence of the genetic alterations.

Clinical features

Anaemia dominates the early course. Most symptomatic patients complain of the gradual onset of fatigue, weakness, dyspnoea and pallor, but at least half the patients are asymptomatic and MDS is discovered only incidentally on routine blood counts. Previous chemotherapy or radiation exposure is an important historic fact. Children with Down's syndrome are susceptible to MDS. The physical examination is remarkable for signs of anemia, about 20% of patients have splenomegaly. Some unusual skin lesions, including Sweets syndrome (febrile neutrophilic dematosis) have been associated with MDS.

Laboratory studies

Blood

Anaemia is present in the majority of cases, either alone or a part of pancytopenias. Macrocytosis is common and the smear may be dimorphic with a distinctive population of large red blood cells. Platelets are also large neutrophils are hypogranulated have hyposegmented ringed or abnormally segmented nuclei. Circulating myeloblasts usually correlate with marrow blast numbers and this is important for classification and prognosis.

Bone marrow

The bone marrow is usually normal or hypercellular but in 20% of cases is sufficiently hypocellular to be confused with aplasia. No single characteristic feature of marrow morphology distinguishes MDS but the following are commonly obsdrved, dyserythropoietic changes (especially nuclear

abnormalities) and ringed sideroblasts in the erythroid lineage; hypogranulation and hyposegmentation in granulocytic precursors, with an increase in myeloblasts; and megakaryocytes showing reduced numbers of disorganized nuclei.

Treatment

The therapy of MDS is generally unsatisfactory. Only stem cell transplantation offers cure. Survival rates of 40% have been reported. Myelodysplastic syndrome has been regarded as particularly refractory to cytotoxic chemotherapy. Immunosuppressive therapies, including ATG and cyclosporine, that are effective in aplastic anemia may induce sustained remissions in a high proportion of patients with refractory anemia, especially in those with hypocellular marrow.

Haematopoietic growth factors can improve blood counts but, as in most other marrow failure state, have been most beneficial in patients with the least severe pancytopenia. The combination of G-CSF and erythropoiesis increased blood counts in one third to one half of patients, but survival advantage is not yet proven.

Prognosis

The median survival varies greatly with FAB type and ranges from years for patients with 5q or sideroblastic anemia to a few months in refractory anemia with excess blasts or severe pancytopenia cases with monosomy.

METHODOLOGY

The present study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on patients with pancytopenia during the period of January 2008 to December 2008.

Study design

One year cross-sectional study.

Study period

The present study was conducted during January 2008 to December 2008.

Method of collection of data

Source of Data

Patients with pancytopenia admitted in the wards and ICUs of Department of Medicine at KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

Sample size

Fifty (50) patients with pancytopenia.

Sampling procedure

As the data was not available, all the cases presented with pancytopenia during the study period were included in the study. Fifty (50) patients with

pancytopenia admitted at KLES Dr. Prabhakar Kore Hospital and Medical Research were studied.

Selection criteria

Inclusion Criteria

- Patients with age more than or equal to 15 years.
- Patients with pancytopenia
    - Haemoglobin: Less than 13.5 gm/dL in males; 11.5 gm/dL in females.
    - Total leucocyte count less than 4000 /cmm.
    - Platelet count less than 150,000 /cmm.

Exclusion Criteria

- Patients with age less than 15 years.
- Patients on cancer chemotherapy.

Procedure

The study was approved by the Ethical and Research Committee of Jawaharlal Nehru Medical College, Belgaum. During the study period, all patients presenting with and fulfilling the inclusion criterion were included in this study after obtaining informed written consent (Annexure–I).

Detailed relevant history and clinical examination was done according to predesigned and pretested proforma (Annexure-II). All patients underwent bone

marrow examination. These patients were subjected to routine haematological investigations like;

- Haemoglobin.
- Total count, differential count and ESR.
- Platelet count.
- Peripheral smear study.
- Bone marrow smear study.

  Other investigations as necessary such as;

- Bone marrow trephine biopsy.
- Peripheral smear for malarial pigment
- Serological study.
  - For HIV infection.
  - For leptospirosis.
  - For Dengue.
- Blood culture study.
- Chest x-ray.
- Electrophoresis.

Statistical methods

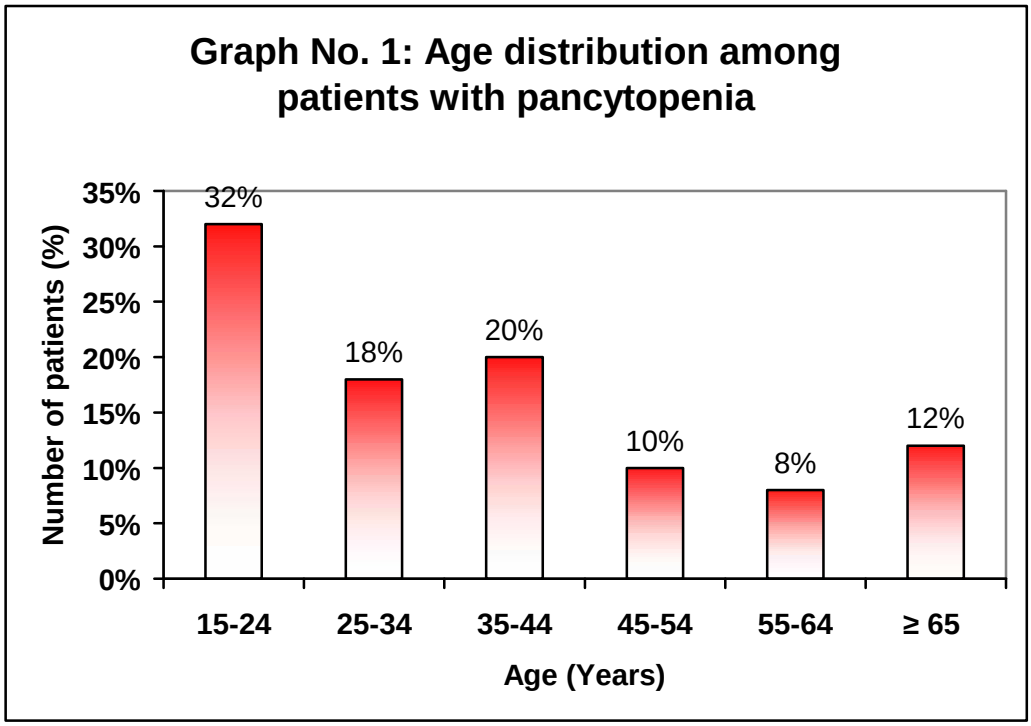
The data obtained was tabulated and was analysed using proportion and percentages.

RESULTS

The present study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the study period from January 2008 to December 2008. Fifty (50) cases were studied and the findings obtained are tabulated as below.

Table No. 2: Age distribution among patients with pancytopenia

Age at presentation (years)	Patients	
	Number	Percentage
15 - 24	16	32%
25 – 34	09	18%
35 – 44	10	20%
45 – 54	05	10%
55 – 64	04	08%
≥ 65	06	12%

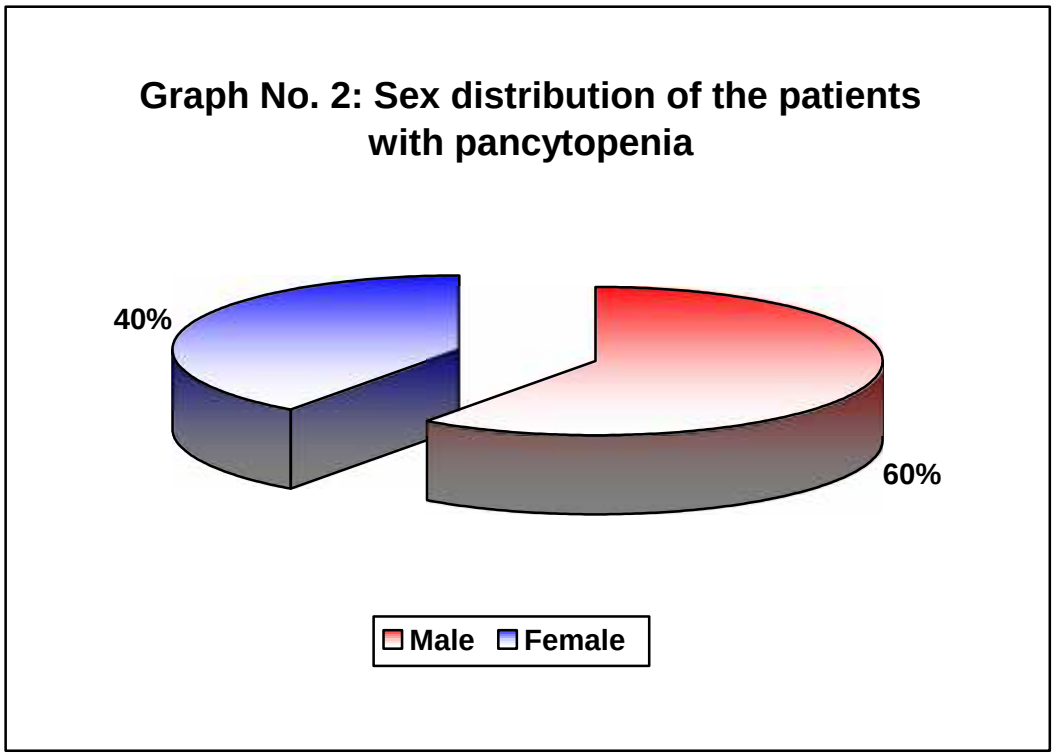


Patients age ranged from 15 to 75 years. Maximum number of cases were in the age group of 15 to 24 years (32%) and four cases were in the age group of 55 to 64 years (8%). Average age at presentation was 36.74 ± 17.71 years.

Inference: Majority of patients were young

Table No. 3: Sex distribution of the patients with pancytopenia

Sex	Patients	
	Number	**Percentage**
Male	30	60%
Female	20	40%

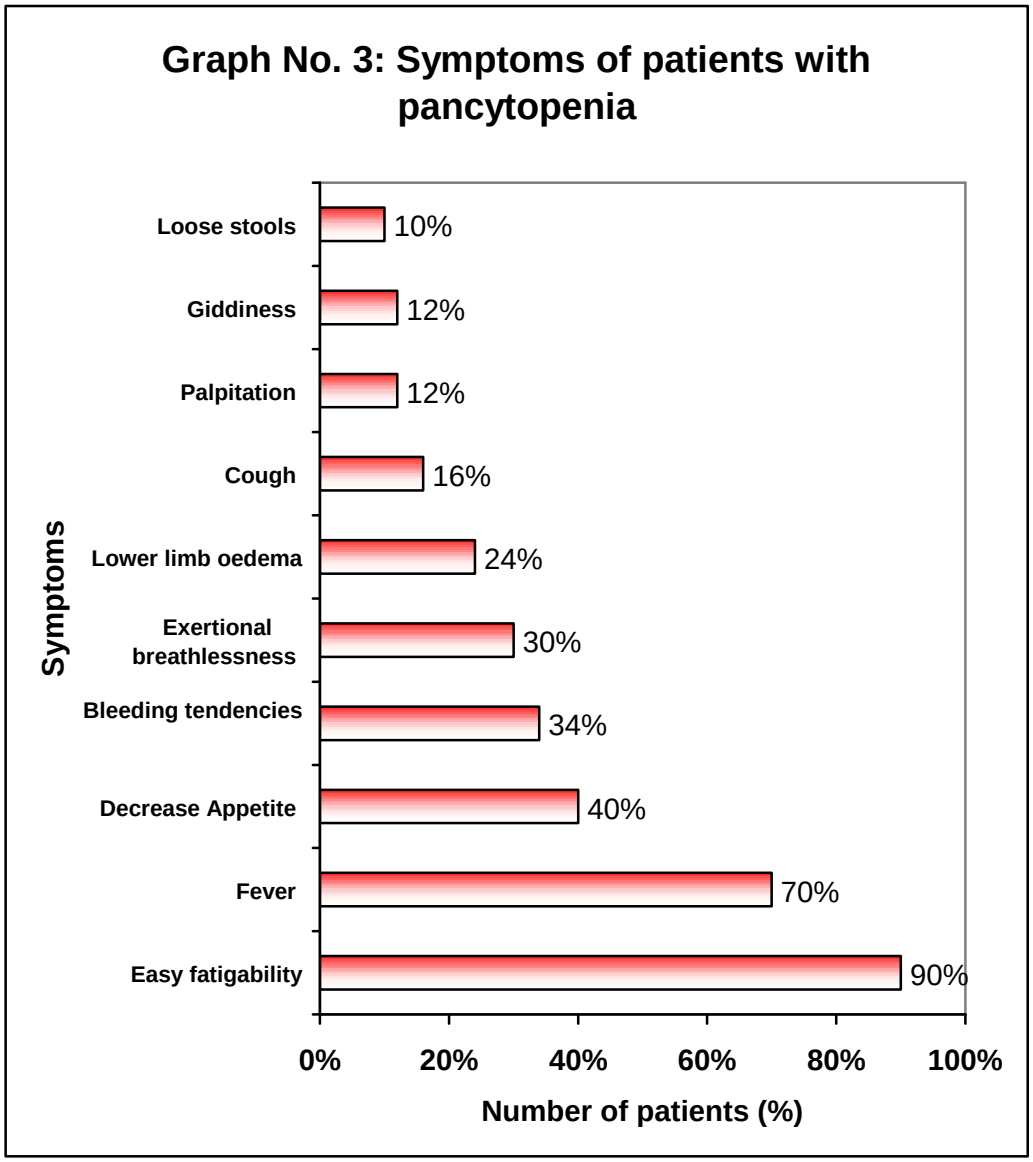


Out of 50 patients, 30 patients (60%) were males and 20 patients (40%) were females. Accounting a ratio of male to female was 3:2.

Inference: Male preponderance was noticed.

Table No. 4: Symptoms of patients with pancytopenia

Symptoms	Patients	
	Number	**Percentage**
Easy fatigability	45	90%
Fever	35	70%
Decrease appetite	20	40%
Bleeding tendencies	17	34%
Exertional breathlessness	15	30%
Lower limb oedema	12	24%
Cough	08	16%
Palpitation	06	12%
Giddiness	06	12%
Loose stools	05	10%

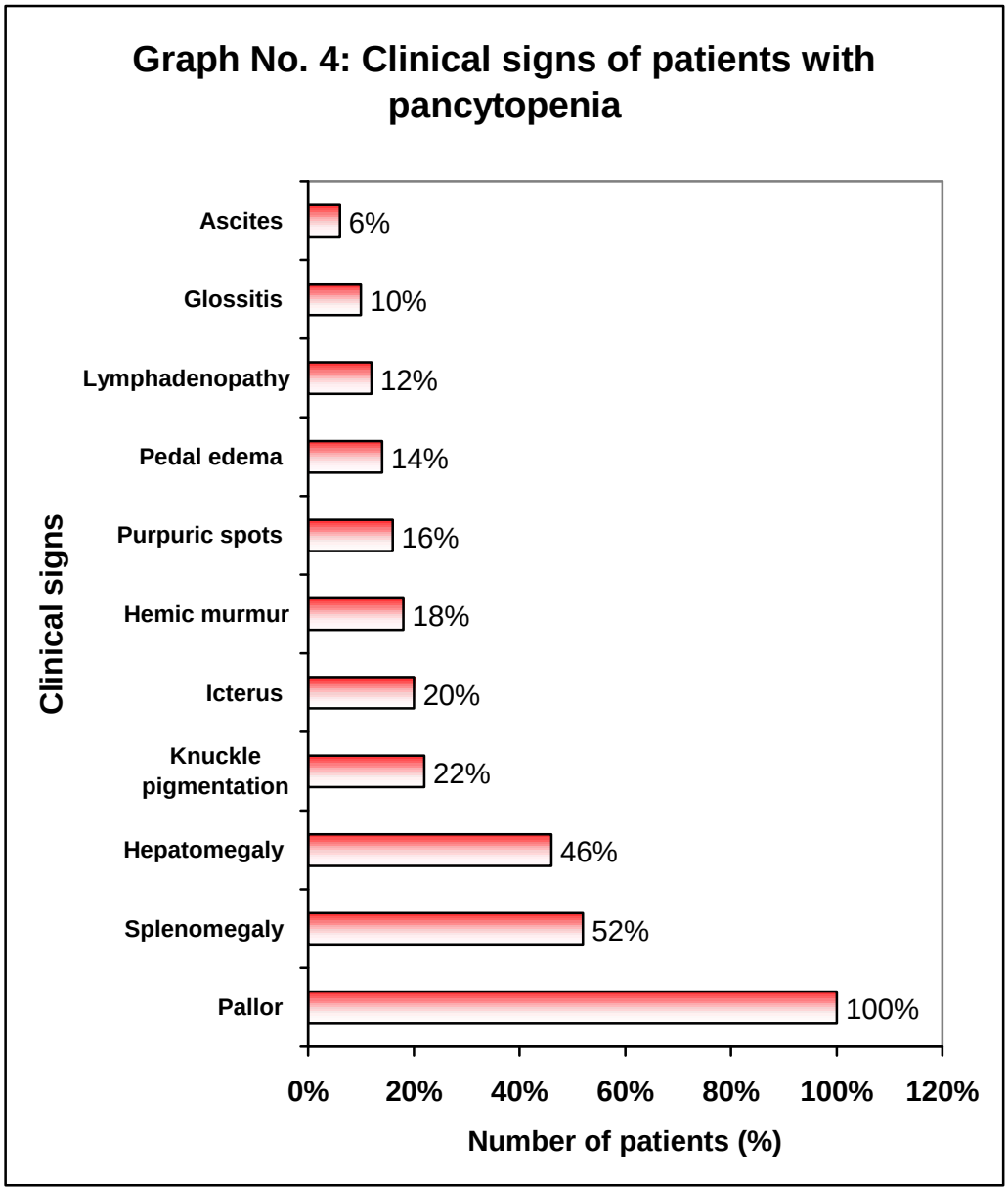


The commonest symptom was easy fatigability (90%) followed by fever (70%), decrease appetite (40%), bleeding tendencies (34%), exertional breathlessness (30%) and lower limb oedema (24%). Other symptoms like cough, palpitation, giddiness and loose stools were accounting for 16%, 12%, 12% and 10% respectively.

Inference: Majority of patients were with the symptoms suggestive of anemia. Followed by fever and decreased appetite.

Table No. 5: Clinical signs of patients with pancytopenia

Signs	Patients	
	Number	**Percentage**
Pallor	50	100%
Splenomegaly	26	52%
Hepatomegaly	23	46%
Knuckle pigmentation	11	22%
Icterus	10	20%
Hemic murmur	09	18%
Purpuric spots	08	16%
Pedal oedema	07	14%
Lymphadenopathy	06	12%
Glossitis	05	10%
Ascites	03	06%



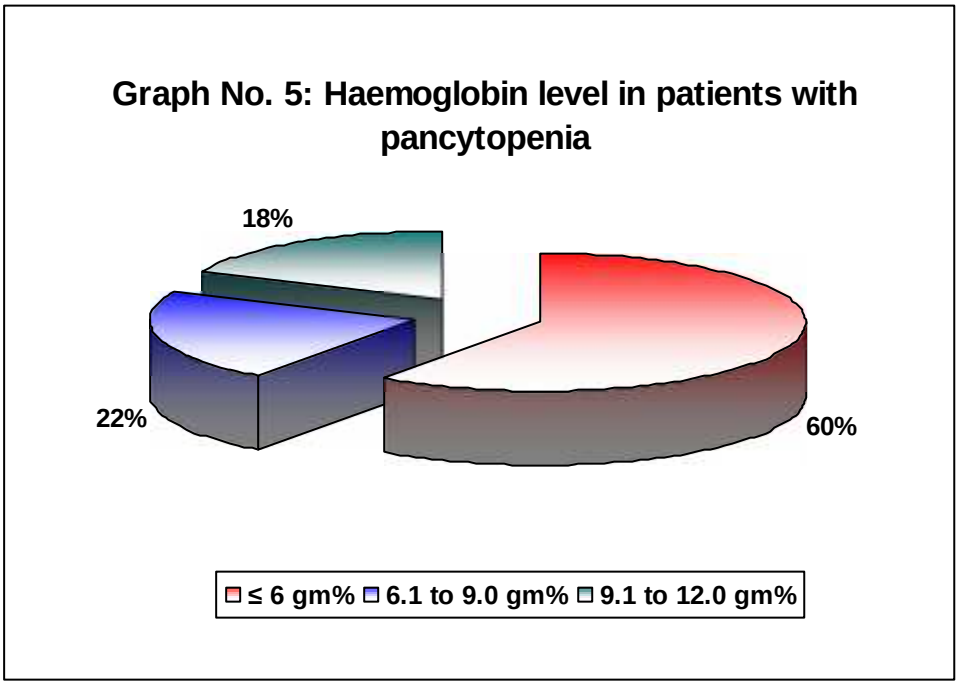
Pallor was universally present in all the patients followed by splenomegaly (52%), hepatomegaly (46%), knuckle pigmentation (22%), icterus (20%), hemic murmur (18%), purpuric spots (16%), pedal edema (14%), lymphadenopathy (12%), glossitis (10%) and ascites (6%).

Inference: Commonest physical sign was anemia followed by splenomegaly and hepatomegaly.

HAEMATOLOGICAL PARAMETERS

Table No. 6: Haemoglobin level in patients with pancytopenia

Haemoglobin (gm%)	Severity of anemia	Patients	
		Number	Percentage
≤ 6	Severe Anemia	30	60%
61. to 9.0	Moderate Anemia	11	22%
9.1 to 12.0	Mild Anemia	9	18%



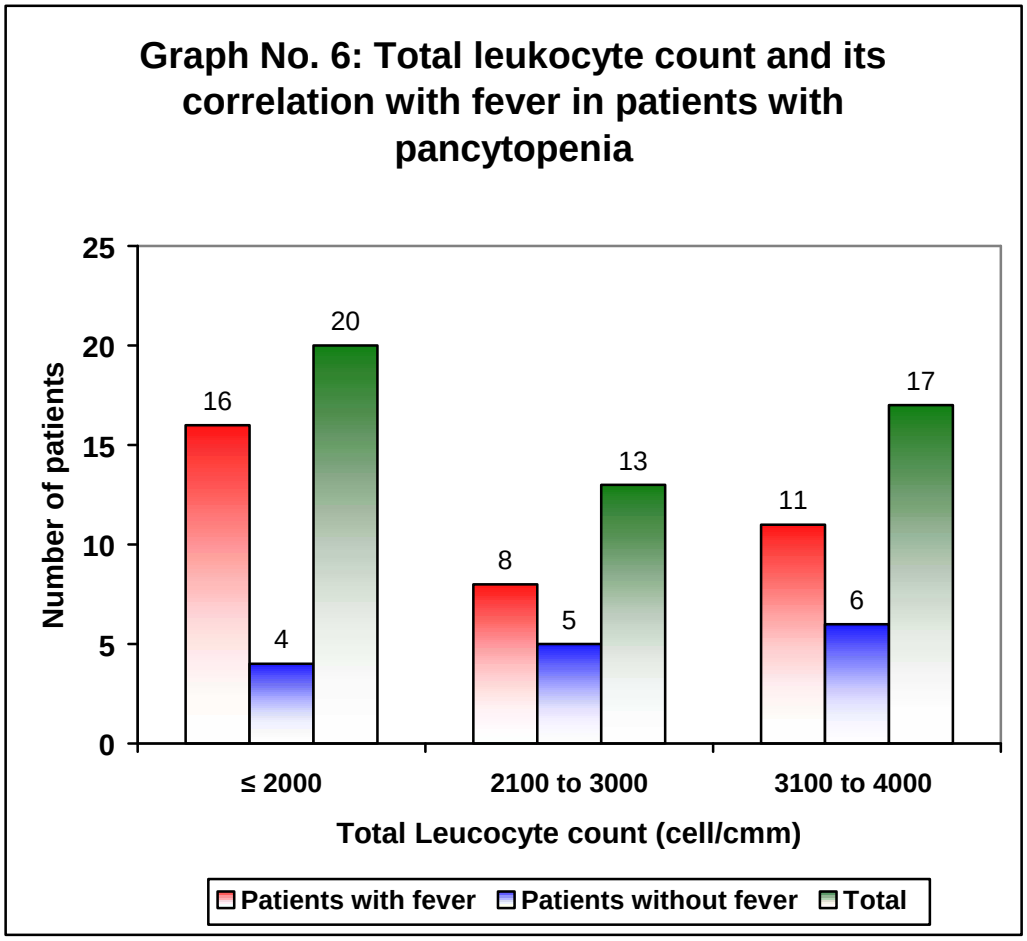
Haemoglobin level ranged from 1.8 gm% to 10.4 gm%. The mean hemoglobin level was 5.8 ± 2.5 gm%. Sixty percent (60%) of patients had haemoglobin value less than or equal to 6 gm% followed by 6.1 to 9 gm% in 22% and 9.1 to 12.0 gm% in 18% of patients.

Inference: Majority of patients had severe anemia.

Table No. 7: Total leukocyte count and its correlation with fever in patients with pancytopenia

Leucocyte count (cells/cmm)	No. of patients with fever	No. of patients without fever	Total	
			Number	Percentage
≤ 2000	16	04	20	40%
2100 – 3000	08	05	13	26%
3100 – 4000	11	06	17	34%

x^2=1.622; p=0.4443



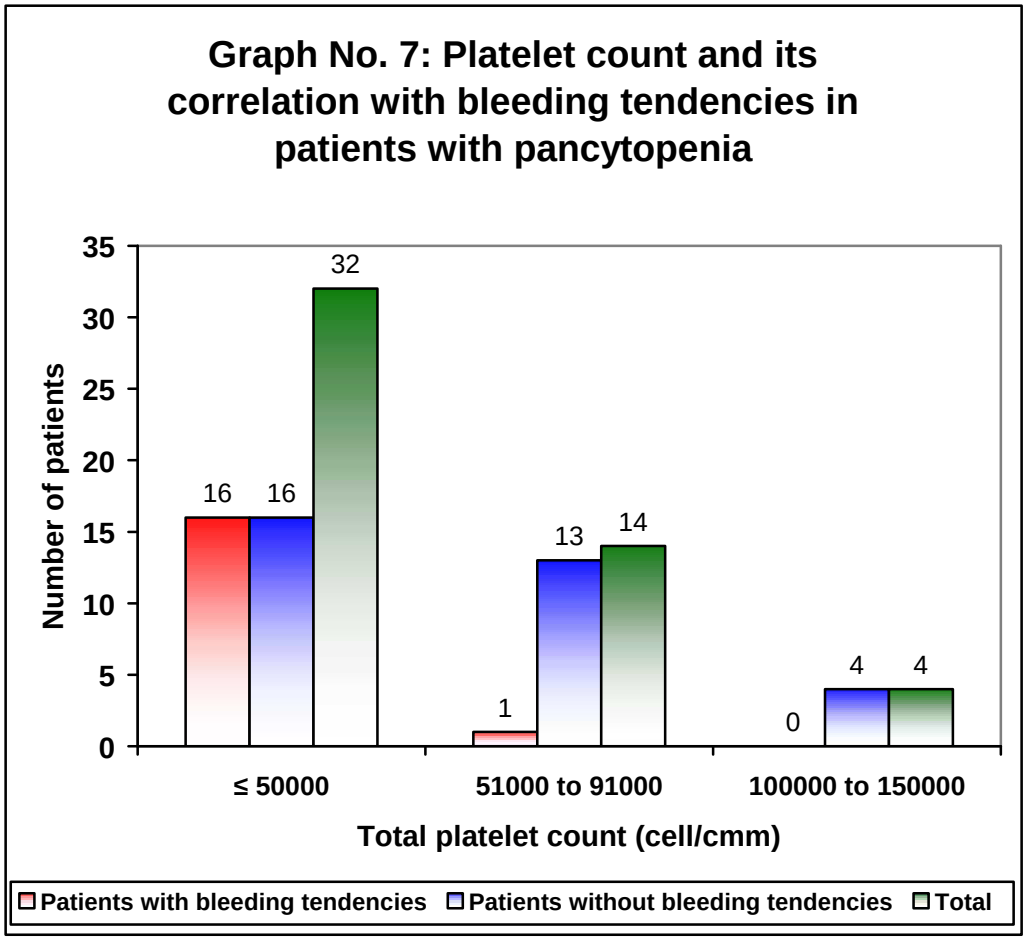
Total leukocyte count ranged from 200 cells to 3900 cells/cmm. The mean total leukocyte count was 2360 ± 984 cells/cmm. Majority (40%) had total leukocyte count less than or equal to 2000 cells/cmm followed by 26% had between 2100 to 3000 cells/cmm and 34% had between 3100 to 4000 cells/cmm.

Inference: Correlation between total leukocyte count and incidence of fever was statistically non significant (p=0.4443).

Table No. 8: Platelet count and its correlation with bleeding tendencies in patients with pancytopenia

Platelet count (cells/cmm)	No. of patients with bleeding tendencies	No. of patients without bleeding tendencies	Total	
			Number	Percentage
≤ 50000	16	16	32	64%
51000 – 99000	01	13	14	28%
100000 – 150000	00	04	04	8%

x^2=10.211; p=0.006



Platelet count ranged form 2000 to 120000 cells/cmm. Mean platelet count was 41160 ± 34492 cells/cmm. Majority (64%) patients had platelet count less than or equal to 50000 cells/cmm followed by 28% patients had between 51000 to 99000 cells/cmm and eight percent patients had between 100000 to 150000 cells/cmm.

Inference: Patients (64%) whose platelet count was less than or equal to 50000 cells/cmm had more bleeding tendencies as compared to patients who had platelet count of more than 50000 cells/cmm. This correlation was statistically significant (p=0.006).

Table No 9: Red blood cells morphology on peripheral smear in patients with pancytopenia

Morphology on peripheral smear	Patients	
	Number	**Percentage**
Normocytic hypochronic	32	64%
Dimorphic picture	10	20%
Microcytic hypochronic	03	6%
Macrocytic/Megaloblastic	03	6%
Normocytic normochronic	02	4%

Majority (64%) of the patients had normocytic hypochronic blood picture on peripheral smear followed by dimorphic picture (20%).

Inference: All 50 patients had one or the other type of anemia at the time of presentation but commonest was normocytic hypochromic blood picture.

Table No. 10: Bone marrow cellularity in patients with pancytopenia

Bone marrow cellularity	Patients	
	Number	**Percentage**
Hypercellular	31	62%
Normocellular	11	22%
Hypocelluar/acellular	8	16%

Majority (62%) of the patients had hypercellular bone marrow followed by normocellular (22%) and hypocellular / acellular (16%).

Inference: Majority of patients had hypercellular bone marrow.

Table No. 11: Etiology of pancytopenia in study population

Etiology	Patients	
	Number	**Percentage**
Megaloblastic anemia	18	36%
Aplastic anemia	8	16%
Malaria	5	10%
Acute leukemias	5	10%
Myelodysplastic syndrome	2	4%
Hypersplenism	2	4%
Disseminated tuberculosis	2	4%
Multiple myeloma	2	4%
HIV Infection	2	4%
Immune thrombocytopenic purpura	2	4%
Dengue fever	1	2%
Septicemia	1	2%

The most common cause of pancytopenia was megaloblastic anemia (36%) followed by aplastic anemia (16%), malaria (10%) and acute leukemias (10%). Other etiologies of pancytopenia is shown in the table.

Inference: Megaloblatic anemia was the commonest cause of pancytopenia followed by aplastic anemia, malaria and acute leukemias.

Table No. 12: Bone marrow cellularity in different etiologies

Etiology	Hypercellular		Normocellular		Hypocellular /Acellular	
	No.	**%**	**No.**	**%**	**No.**	**%**
Megaloblastic anemia (n=18)	16	88.89%	2	11.11%	0	0%
Aplastic anemia (n=8)	0	0%	0	0%	8	100%
Malaria (n=5)	2	40%	3	60%	0	0%
Acute leukemia (n=5)	5	100%	0	0%	0	0%
MDS (n=2)	1	50%	1	50%	0	0%
Hypersplenism (n=2)	1	50%	1	50%	0	0%
Disseminated tuberculosis (n=2)	1	50%	1	50%	0	0%
Multiple myeloma (n=2)	2	100%	0	0%	0	0%
HIV infection (n=2)	1	50%	1	50%	0	0%
ITP (n=2)	1	50%	1	50%	0	0%
Dengue fever (n=1)	0	0%	1	100%	0	0%
Septicemia (n=1)	1	100%	0	0%	0	0%

In megaloblastic anemia 88.89% cases had hypercellular marrow, remaining had normocellular. All cases of aplastic anemia had hypocellular/acellular marrow. In malaria 60% of cases had normocellular marrow, remaining had hypercellular. All cases of acute leukemias had hypercellular marrow.

Inference: The commonest etiology was megaloblastic anemia in which majority had hypercellular bone marrow. All patients of aplastic anemia had hypocellular / acellular marrow.

DISCUSSION

In the present study of 50 patients it was observed that megaloblastic anemia was the most common cause of pancytopenia. The incidence of megaloblastic anemia varies from eight percent to 68% as a cause of pancytopenia in various studies.[35,36,37] In the present study incidence of megaloblastic anemia was 36%.

The incidence of aplastic anemia varies from 10% to 52.7% as a cause of pancytopenia in various studies.[35,36,37] In this study incidence of aplastic anemia was 16% which was second common cause of pancytopenia. The incidence of malaria in the present study was 10% which is third common cause of pancytopenia.

In a study[38] involving 75 cases found malaria was the most common cause of pancytopenia in adult population with an incidence of 17.3%.

One Indian study[20] reported fatal pancytopenia in falciparum malaria.

The incidence of hypersplenism varies from 4.9% to 19% as a cause of pancytopenia in various studies.[38,39] In the present study incidence was 4% compared to one Indian study[36] where the incidence was 11.4% as a cause for pancytopenia.

In the present study 10% of cases of acute leukemias were the cause of pancytopenia. One study[38] reported 13.3% of cases of acute leukemias.

In the present study other etiologies for pancytopenia were MDS (4%), disseminated tuberculosis (4%), HIV infection (4%), multiple myeloma (4%) and ITP (4%). One case (2%) of each dengue fever and septicemia detected. Septicemia was secondary to left lower lobe pneumonia. In blood culture pseudeomonas was isolated as causating organism. Pancytopenia in malaria, dengue and septicemia was transient and resolved with treatment.

The commonest cause of pancytopenia, reported from various studies throughout the world has been aplastic anemia. This is in sharp contrast with the results of our study, where the commonest cause of pancytopenia was megaloblastic anemia. This seems to reflect the higher prevalence of nutritional deficiency of vitamin B_{12} and folic acid in Indian subjects. Similar results have been reported in other Indian studies.[24,35]

One Indian study[37] involving 65 pancytopenic patients found megaloblastic anemia in 25.4% of cases.

Another Indian study[40] found megaloblastic anemia to be the commonest cause (39%) in a study of 191 patients. All cases of megaloblastic anemia in our study responded very well to treatment with supplementation of adequate dosages of vitamin B_{12} and folic acid.

In a study[41] in France involving 213 cases, found malignant myeloid disorder (42%) was commonest cause followed by aplastic anemia (10%). In our study incidence of acute leukemias was 10%.

Another study[30] conducted on 50 cases of pancytopenia found aplastic anemia as the commonest cause followed by hypersplenism.

An Indian study[35] involving 77 cases of pancytopenia found megaloblastic anemia (68%) followed by aplastic anemia (7.7%).

Majority of patients (32%) in the present study were in the 15 to 24 years age group. Average age at presentation was 36.74 ± 17.71 years. In our study there was a male preponderance (60%) with male to female ratio 3:2. In the present study majority cases of megaloblastic anemia and acute leukemias were in the 15 to 24 years age group.

An Indian study[36] involving 166 patients with pancytopenia found mean age at presentation 30.6 years (range 12 – 73 years) with male preponderance (67.47%).

Common presenting symptoms in our study were easy fatigability (90%) followed by fever (70%), decrease appetite (40%), bleeding tendencies (34%), exerctional breathlessness (30%) and lower limb oedema (24%).

Common presenting symptoms in a study[24] carried out in 50 cases were fever (40%) followed by weakness (30%) and bleeding manifestations (20%).

Another study[38] reported fever (86.7%) as a most common presenting symptom followed by easy fatigability (76%) and dizziness (64%).

In the present study most of the patients had symptoms suggestive of anemia like easy fatigability, weakness and exerctional breathlessness followed

by fever and decrease appetite. Some patients presented with bleeding manifestations.

In the present study pallor was universally present in all the patients. Fifty two percent (52%) of the patients had splenomegaly and 46% had hepatomegaly. Knuckle pigmentation due to megaloblastic anemia was observed in 22% of cases. All the cases of malaria had icterus either because of hemolysis or hepatocellular injury. Angular stomatitis and glossitis (10%) were observed due to nutritional deficiency. Pedal oedema (14%) was seen in most of severe anemia cases due to hypoproteinemia. Five patients of megaloblastic anemia had signs of peripheral neuropathy. Both cases of disseminated tuberculosis and HIV infection had lymphadenopahty (12%).

One Indian study[24] found pallor in all the patients followed by splenomegaly (40%), hepatomegaly (38%), purpuric spots (28%) and lymphadenopahty (12%).

Another study[38] conducted in Aden, Yemen involving 75 patients with pancytopenia found pallor (100%) and splenomegaly (44%) were the most common physical findings. Followed by purpuric spots (38.67%), hepatomegaly (21.33%) and lymphadenopahty (14.67%).

The mean haemoglobin in the present study was 5.8 ± 2.5 gm%, the mean total leukocyte count was 2360 ± 984 cells/cmm and mean platelet count was 41160 ± 34492 cells/cmm.

In the present study majority (60%) of patients had severe anemia (Hb ≤ 6 gm%). This finding was consistent with Indian study.[36]

In this study 40% of cases had total leukocyte count less than or equal to 2000 cells/cmm and 26% of cases had between 2100 to 3000 cells/cmm, 34% of cases had between 3100 to 4000 cells/cmm. We studied correlation between total leukocyte count and incidence of fever which was statistically non significant (p=0.4443)

In the present study platelet count ranged from 2000 to 1,20,000 cells/cmm. Majority (64%) patients had platelet count less than or equal to 50000 cells/cmm, in which 50% of cases had bleeding manifestations. Thirty six percent (36%) of cases had platelet count between 51000 to 150000 cells/cmm. In which bleeding manifestations were negligible. Correlation between platelet count and bleeding tendencies was statistically significant (p=0.006).

In this study, on peripheral smear majority (64%) of cases had normocytic hypochromic RBCs morphology followed by dimorphic picture (20%), macrocytic/megaloblastic (6%), microcytic hypochronic (6%) and normocytic normochromic (4%).

Anisopoikilocytosis was the predominant finding in megaloblastic anemia. Other findings like macroovalocytes and hyper segmented neutrophils were seen in megaloblastic anemia. Blast cells with atypical cells were seen in two cases of acute leukemia which later on diagnosed to be acute myeloid leukemia.

An Indian study[24] involving 50 cases found hyper segmented neutrophils in 40% of cases, dimorphic picture in 20% of cases, microcytic hypochronic picture in 20% of cases, circulating erythroblasts in 8% of cases and reticulocytosis was seen in 6% of cases.

Bone marrow examination was of great diagnostic value in patients of pancytopenia. in the present study bone marrow was hypercellular majority (62%) of patients, hypocellular in 16% of patients and normocellular in 22% of patients.

One study[41] in France involving 213 cases found hypercellular marrow (66%) in patients with pancytopenia, where malignant myeloid disorder (42%) was the commonest cause for pancytopenia.

Most patients with megaloblastic anemia had hypercellular marrow. Typical megaloblasts with sieved chromatin and asynchronic nuclear cytoplasmic ratio were found in megalobastic anemia patients. All cases of aplastic anemia (16%) had hypoplatic/aplastic marrow. All these cases underwent trephine biopsy and aplastic anemia was confirmed. The etiology of aplastic anemia was idiopathic.

All five cases of acute leukemia (10%) had hypercellular marrow. Blast cells were seen in all the cases. After detailed marrow examination three cases turned out to be acute lymphoblastic leukemia stage L_2 and two cases turned out to be acute myeloid leukemia stage M_4.

Five cases of malaria (10%) observed in our study as a cause of pancytopenia in which four cases had plasmodium falciparum infection and one case had mixed malarial infection (plasmodium falciparum + plasmodium vivex). In two patients gametocytes of plasmodium falciparum seen on bone marrow smear examination.

Two patients of refractory anemia (4%), on doing bone marrow study /trephine biopsy turned out to be MDS. Bone marrow of these patients shown normo to hypercellularity with increase number of immature myeloid precursor cells and presence of ring sideroblasts. Further one case of MDS confirmed by chromosomal sequence and analysis.

In a study[28] involving 148 patients found on bone marrow examination that hypoplastic bone marrow (29.05%), megaloblastic marrow (23.64%), hematological malignancies (21.62%), erythroid hyperplasia (19.6%) and normal bone marrow (93.38%).

Disseminated tuberculosis (4%) had multiple granuloma on trephine biopsy. In patients of multiple myeloma, marrow smear study shown plasmacytosis (plasma cells > 25%). On bone marrow smear study, ITP (4%) had shown megakaryocytosis, majority were immature with granular cytoplasm.

CONCLUSION

Amongst the patients presenting with Pancytopenia, 36% had megaloblastic anemia and 16% had aplastic anemia.

Pancytopenia was observed in younger age group more commonly than in elderly. In younger age group causes of pancytopenia were megaloblastic anemia, acute leukemias and infection like malaria. In older age group causes were MDS and multiple myeloma.

In majority of megaloblastic anemia cases, cause was nutritional deficiency of vitamin B_{12} and folic acid.

Features suggestive of anemia were commonly seen followed by leucopenia and thrombocytopenia.

Peripheral smear was inconclusive to arrive at diagnosis of pancytopenia in most of the cases. Bone marrow study/trephine biopsy was helpful in most of the cases to find out etiology.

In megaloblastic anemia, infection like malaria, pancytopenia was transient and responded well to treatment.

SUMMARY

The present study was conducted to know the various clinical manifestations and etiological factors of pancytopenia. This study was conducted on 50 patients aged ranged from 15 to 75 years, admitted in medicine wards of KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the period of January 2008 to December 2008.

Results of the present study showed that the commonest cause of pancytopenia was megaloblastic anemia. Various other causes of pancytopenia were aplastic anemia, malaria, acute leukemias, MDS, hypersplenism, disseminated tuberculosis, multiple myeloma, HIV infection, ITP, septicemia and dengue fever.

Amongst various cases of pancytopenia, detailed examination and investigations including bone marrow study/trephine biopsy helped us to arrive at a proper diagnosis and treatment of the same. Peripheral smear study at times was inconclusive, only bone marrow smear study and trephine biopsy helped in proper evaluation of pancytopenia. Majority of cases had megaloblatic anemia as a cause of pancytopenia followed by aplastic anemia, malaria and acute leukemias. In cases of megaloblastic anemia and malarial infection, pancytopenia was transient and responded promptly to treatment.

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ANNEXURE I

CONSENT FORM

Title of the topic

"A CLINICAL AND ETIOLOGICAL STUDY OF PATIENTS WITH PANCYTOPENIA-A ONE YEAR CROSS SECTIONAL STUDY AT KLES DR. PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE, BELGAUM"

Principle Investigators

Name**: Dr Bhautik V. Tilva**

Guide: **Dr Vijayakumar G. Somannavar**

You have been requested to participate in research because your profile matches with the study group. During the study you will be asked some questions and you are supposed to answer to the best of your knowledge. Your participation in the research is absolutely voluntary. Your decision to participate in the study or otherwise will not affect your relationship with KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. If you decide not to participate, you are free to withdraw at any time.

Purpose of study

To study the various clinical presentation and etiological factors of patients with pancytopenia.

Procedure Involved

1. Venepuncture for estimation of routine and specific blood tests.
2. Bone marrow aspiration for smear study.
3. Other investigations as necessary.

Risks and benefits

Venipuncture and bone marrow procedure may cause pain, hematoma, inflammation and rarely infection.Benifits of this study are many. The study helps to identify various clinical features of the patients with pancytopenia and identify different causative factors which will be useful for further management. The results deduced at the end of study will help all similar patients admitted in the hospital.

Alternatives

Even if you decline to participate, there will be no any change in the line of your management or the relationship with your doctor. You will be told about all the new information that may affect your decision to participate in the study.

Privacy and confidentiality

The only people to know that you are a research subject are the members of research team. No information about you or provided by you during the research will be disclosed to others without your written permission except:

1. In emergency to protect your rights and welfare.
2. If required by law.

Financial incentives for participation

You will not be paid any monetary benefits or free gifts for participation in the research. You will not be reimbursed for expenses.

Authorization to publish results

When the results of the research are published or discussed in a conference, no information will be displayed that would disclose your identity. Any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Consent statement

I, the undersigned, have been explained in my own vernacular language about the study and my participation in the study is voluntary. If I want I can withdraw at any time. Also I have been given enough time to clear my doubts about the study and my rights as a study participant.

In case you have any questions related to the study you can contact Dr. BHAUTIK V. TILVA (Ph.9341478383) Or Dept. of medicine (0831-2473777) Extension no: 1520

In case you have any questions about your rights as a study participant you can contact Dr V. D. Patil (0831-2471350).

Signature or the left thumb impression of the participant or legally authorized representative.

Participant's name_____ Signature_____

Witness name_____ Signature_____

Experimenter's name_____ Signature_____

Place _____ Date _____

ANNEXURE II

PROFORMA

A. IDENTIFICATION **IP No:**

- Name:
- Age:
- Sex:
- Religion:
- Address:
- Occupation:
- DOA/DOD:
- Final Diagnosis:

B. HISTORY

1) PRESENTING COMPLAITS WITH DURATION

- Weakness
- Fatigability
- Fever
- Shortness of breath
- Bleeding from any site
- Joint pain
- Rashes
- Bruises
- Lethargy

- Loss of Appetite
- Others

2) DRUG HISTORY

- Chloramphenicol
- NSAIDS
- Chemotherapy
- Others

3) PAST HISTORY

- Radiation Exposure
- Pesticide Exposure
- Jaundice
- Blood Transfusion
- Diabetes
- Hypertension

4) PERSONAL HISTORY

- Diatary Habit
- Alcohol Consumption
- Smoking Habit

5) FAMILY HISTORY

- Malignancy
- Others

C. EXAMINATION

1) General Examination

- General Condition

- Temperature
- Blood pressure
- Pulse rate
- Respiratory Rate
- Pallor
- Icterus
- Edema
- Lymphadenopathy
- Rashes
    - Easy Bruisiability
    - Petechia
    - Ecchymosis
    - Bruises
- Mouth
    - Gum Hypertrophy
    - Gum Bleeding
    - Oral Ulceration
    - Glositis
- Other

2) Systemic Examination

- Abdomen
    - Liver
    - Spleen
    - Aortic/Paraaortic lymphnodes

- Mass
- Ascites
- Other

- Cardio Vascular system
  - Systolic Flow Murmur
  - Organic Murmur
  - Other
- Respiratory System
  - Breath Sound
  - Added Sound
  - Percussion Note
  - Other
- CNS
  - Higher mental function
  - Motor
  - Sensory
  - Reflexes
  - Sign of meningeal irritation
  - Fundoscopy
  - Other
- Musculo skeletal system
  - Joint Swelling
  - Bony Tenderness

D. INVESTIGATIONS

- Hb
- TC
- DC
- ESR
- Platelet count
- Peripheral smear study

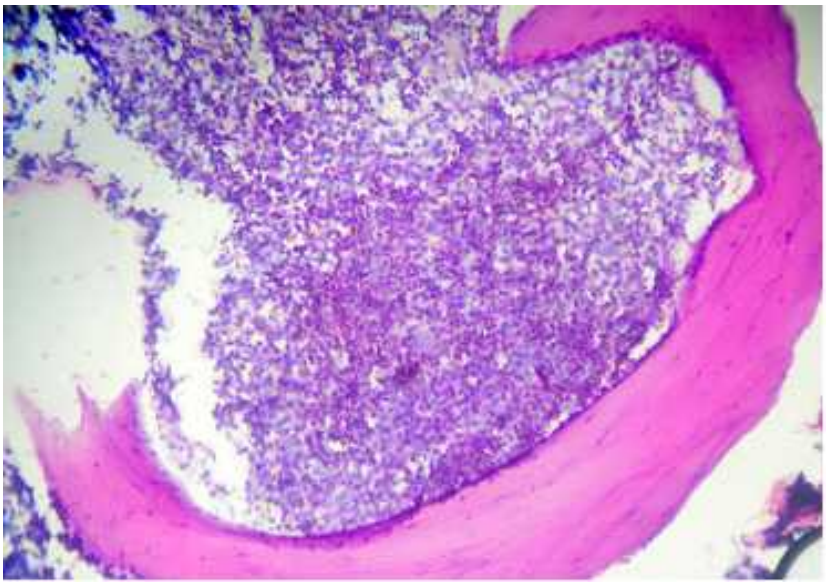
Other investigations as necessary such as:-

- Bone marrow smear study
- Bone marrow trephine biopsy
- Peripheral smear for Malarial pigment
- Serological study
    - For HIV infection
    - For Leptospirosis
    - For Dengue
- Blood culture study
- Chest X-ray

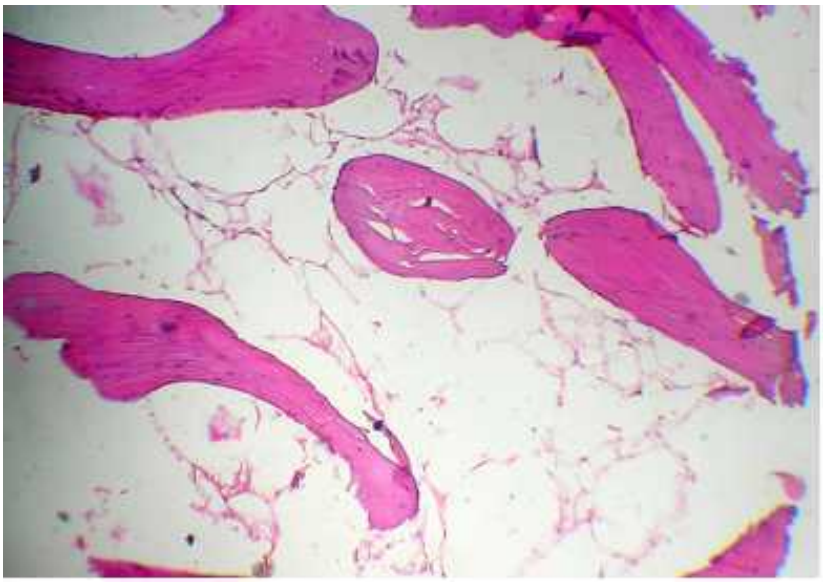
Final diagnosis:

ANNEXURE III

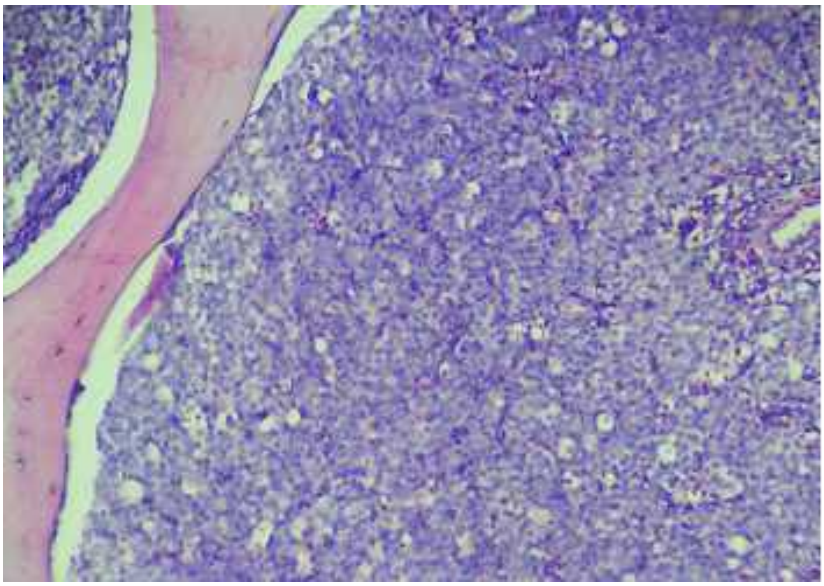
PHOTOGRAPHS



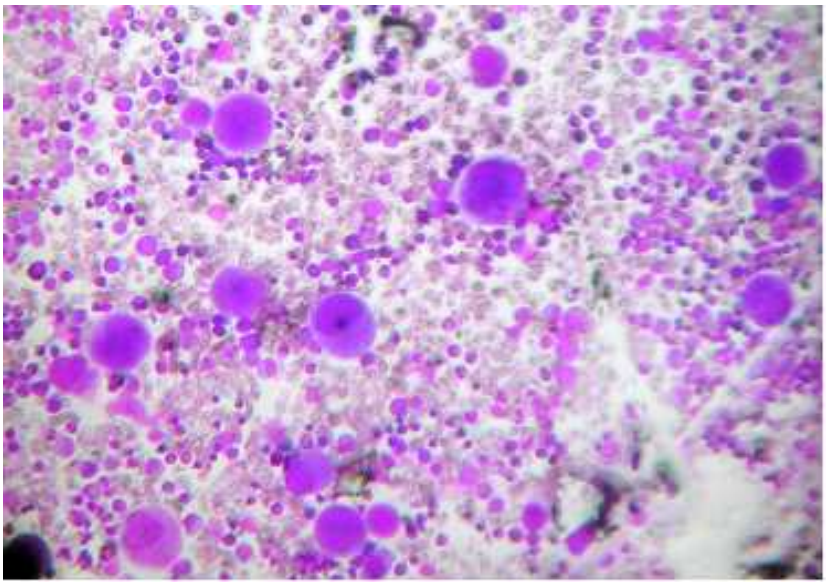
Photograph 1: Biopsy section from a case of megaloblastic anemia showing hypercellular marrow



Photograph 2: Biopsy section from a case of aplastic anemia



Photograph 3: Biopsy section from a case of AML showing a sheets of blasts



Photograph 4: Bone marrow aspirate smear from a case of ITP showing mature and immature megakaryocytes.

ANNEXURE IV - MASTER CHART

ANNEXURE IV

KEY TO MASTER CHART

-	-	Negative
+	-	Positive
AA	-	Aplastic anemia
ALL	-	Acute lymphoblastic leukemia
AM	-	Aplastic / Acellular marrow
AML	-	Acute myeloid leukemia
DP	-	Dimorphic picture
DTB	-	Disseminated tuberculosis
F	-	Female
HIV	-	Human immunodeficiency virus
HM	-	Hypercellular marrow
HS	-	Hypersplenism
IP No.	-	In patient number
ITP	-	Immune thrombocytopenic purpura
M	-	Male
MA	-	Megaloblastic anemia
MDS	-	Myelodysplastic syndrome
MH	-	Microcytic hypochromic
MM	-	Macrocytic megaloblastic
mm	-	Millimeter
MML	-	Multiple myeloma

NH	-	Normocytic hypochromic
NM	-	Normocellular marrow
NN	-	Normocytic normochromic
PS	-	Peripheral smear
RBC	-	Red blood cell
RVD	-	Retro viral disease
Sr. No.	-	Serial Number

Sr. No.	IP No.	Demography		Presenting symptoms											Clinical signs											Investigations							Diagnosis
		Age (Years)	Sex	Easy fatiguability	Fever	Bleeding tendencies	Palpitation	Breathlessness	Cough	Decrease appetite	Lower limb oedema	Giddiness	Loose stool	Bony pain	Pallor	Icterus	Knuckle pigmentation	Pedal oedema	Lymphadenopathy	Purpuric spots	Glossitis	Splenomegaly	Hepatomegaly	Ascites	Hemic murmur	Haemoglobin (gm%)	Total leukocyte count (/cmm)	Platelet count (/cmm)	RBC morphology on PS	Malarial parasite	HIV	Bone marrow cellularity	
1	264877	28	M	+	+	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	6.0	1400	25000	NN	-	-	HM	MA
2	262919	18	F	+	+	-	+	+	-	+	+	-	-	+	+	-	-	+	-	-	-	+	-	-	+	1.8	3800	90000	NH	-	-	HM	ALL
3	263307	20	F	+	+	-	-	-	-	+	+	+	-	-	+	-	-	+	+	-	-	-	-	-	+	5.3	1000	16000	NH	-	-	HM	ALL
4	250330	26	M	+	+	-	-	-	-	+	+	-	+	-	+	+	+	+	-	-	-	-	-	-	-	3.2	1100	35000	DP	-	-	HM	MA
5	251005	24	F	+	+	+	-	+	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	5.1	1500	2000	NH	-	-	NM	ITP
6	248379	20	F	+	-	-	-	-	-	-	+	-	+	-	+	-	+	-	-	-	-	-	+	-	-	3.4	2600	120000	MH	-	-	HM	MA
7	248448	21	F	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	9.4	2300	110000	MM	-	-	HM	MA
8	243512	19	M	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	-	-	3.8	3000	10000	DP	-	-	HM	MA
9	253220	36	M	+	+	+	-	+	+	-	+	-	-	-	+	-	-	+	-	+	-	+	+	-	-	3.1	1800	4000	NH	-	-	HM	MA
10	251083	28	M	+	-	-	-	-	-	-	-	+	-	-	+	+	-	+	+	-	+	-	-	-	-	5.6	3400	100000	NH	-	-	HM	ALL
11	261316	30	F	+	+	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	+	+	+	-	5.9	2300	65000	DP	-	-	NM	HS
12	253879	20	M	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+	-	-	10.4	3200	77000	NH	-	-	NM	MA
13	259194	40	M	+	+	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	7.0	2800	70000	NH	-	-	AM	AA
14	260333	35	M	+	-	-	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	3.0	2100	10000	MH	-	-	AM	AA
15	257295	34	M	+	+	-	-	-	-	+	+	-	-	-	+	-	-	-	+	-	-	+	+	-	-	9.9	2900	81000	NH	-	+	NM	RVD+TBM
16	244712	75	M	-	+	+	-	+	+	+	+	-	-	-	+	+	-	-	-	+	-	+	-	-	-	8.0	200	6000	NH	-	-	HM	SEPTICEMIA
17	247611	65	F	+	-	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	5.2	3500	13000	DP	-	-	HM	MA
18	260179	20	F	+	-	-	+	-	-	-	-	-	+	-	+	-	+	-	-	-	-	+	+	-	-	4.2	1700	60000	DP	-	-	NM	MA
19	254111	42	M	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	3.8	2200	91000	MH	-	-	HM	MA
20	274219	74	M	+	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	10.3	800	55000	MM	-	-	HM	MA
21	295683	28	F	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	9.4	1800	33000	NH	+	-	NM	MALARIA
22	295089	20	M	+	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	2.0	3800	3000	NN	-	-	AM	AA

Sr. No.	IP No.	Demography		Presenting symptoms											Clinical signs											Investigations							Diagnosis
		Age (Years)	Sex	Easy fatiguability	Fever	Bleeding tendencies	Palpitation	Breathlessness	Cough	Decrease appetite	Lower limb oedema	Giddiness	Loose stool	Bony pain	Pallor	Icterus	Knuckle pigmentation	Pedal oedema	Lymphadenopathy	Purpuric spots	Glossitis	Splenomegaly	Hepatomegaly	Ascites	Hemic murmur	Haemoglobin (gm%)	Total leukocyte count (/cmm)	Platelet count (/cmm)	RBC morphology on PS	Malarial parasite	HIV	Bone marrow cellularity	
23	296618	28	F	+	-	+	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	6.9	3200	15000	NH	-	-	HM	MA
24	293933	36	M	+	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	3.0	3500	14000	NH	-	-	AM	AA
25	298619	42	M	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	+	-	-	5.2	1100	20000	MM	-	-	HM	MA
26	309114	65	F	+	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	2.4	2500	86000	NH	-	-	AM	AA
27	299390	52	F	+	-	-	-	+	+	-	+	-	-	-	+	-	-	+	-	-	+	-	-	-	-	3.9	1300	63000	DP	-	-	HM	MML
28	302451	38	M	-	+	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	8.0	900	4000	NH	-	-	AM	AA
29	296435	15	F	+	-	-	+	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	3.1	3100	5000	NH	-	-	AM	AA
30	324989	70	F	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	+	+	-	-	7.4	3900	18000	NH	-	-	HM	MML
31	277080	72	M	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	9.3	3700	20000	NH	-	-	NM	MDS
32	276898	45	F	+	+	-	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-	+	+	-	-	9.4	2300	16000	NH	+	-	NM	MALARIA
33	267786	22	M	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	4.0	800	36000	NH	+	-	HM	MALARIA
34	269095	22	M	+	+	+	-	+	-	-	-	-	+	-	+	-	+	-	-	-	-	+	-	-	+	4.8	3600	91000	DP	-	-	HM	MA
35	269246	60	M	+	+	-	+	+	-	+	-	-	-	-	+	-	-	-	-	-	+	-	+	-	-	2.0	1800	80000	DP	-	-	HM	AML
36	288662	45	M	+	+	-	-	+	+	+	+	-	-	-	+	-	-	-	+	-	-	+	+	-	+	3.0	3100	104000	NH	-	-	HM	DTB
37	283222	50	F	+	+	+	-	+	+	+	-	-	-	-	+	-	-	-	+	-	-	+	+	+	-	9.3	3500	27000	NH	-	-	NM	DTB
38	278666	64	M	+	+	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	6.4	1600	19000	NH	-	-	HM	AML
39	289750	24	M	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	5.9	2400	47000	DP	-	-	HM	MA
40	285113	18	F	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	5.1	1500	59000	NH	-	-	HM	MA
41	288644	55	M	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	9.0	3700	50000	NH	-	-	HM	MDS
42	276413	35	M	+	+	+	+	-	-	+	+	-	-	-	+	-	-	-	-	-	-	+	+	-	+	2.4	2000	12000	NH	-	-	HM	MA
43	290141	25	M	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	8.6	1600	11000	NH	+	-	NM	MALARIA
44	290537	23	F	+	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	5.2	3500	5000	NH	+	-	HM	MALARIA

Sr. No.	IP No.	Demography		Presenting symptoms											Clinical signs											Investigations							Diagnosis
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45	290325	27	F	+	+	+	-	-	+	-	-	-	-	-	+	+	-	-	-	+	-	+	-	-	+	5.1	1100	20000	NH	-	-	HM	HS
46	288702	43	M	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	+	+	-	7.0	1600	20000	NH	-	+	HM	RVD
47	287044	56	F	+	+	+	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	6.8	3500	10000	NH	-	-	AM	AA
48	293471	20	M	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-	-	-	-	-	-	-	2.8	2400	90000	NH	-	-	HM	MA
49	294019	51	M	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	+	-	-	10.2	2500	14000	NH	-	-	NM	DENGUE
50	293896	41	M	+	+	+	-	-	+	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	6.1	3100	26000	DP	-	-	HM	ITP