Hemophagocytic Histiocytosis: Etiology, Intensity and Effect on Hematological Parameters

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CERTIFICATE

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ABSTRACT

Phagocytsis of mature and developing blood cells by histiocytes is known as hemophagocytosis (HP). Though an uncommon condition, histiocytic hyperplasia with HP is seen in a wide variety of hematological and non hematological conditions, mainly as a reactive phenomenon.

The present study was designed to determine the etiology of hemophagocytosis, its intensity (extent of disease) in the bone marrow and its effect on hematological parameters. The study was conducted in the Department of Pathology, Pakistan Institute of Medical Sciences (PIMS), Islamabad. The period of study spanned from March 2003 to March 2005.

Variable degrees of HP (mild, moderate, severe) were observed in the bone marrow smears of 250 patients having different underlying disorders. Hemophagocytic syndrome (HPS) with clinical and biochemical derangement, was observed in 24 (9.4%) patients. HPS was mostly associated with infection.

The etiological distribution of patients in different groups of disorders was: non malignant hematological conditions (NMHC) (56.80%), infections (24.80%), storage disorders (4.40%), malignant hematological condition (4.40%), autoimmune disorder (1.20%) and miscellaneous group (8.40%).

The distribution of patients in different grades of intensity was: grade I (mild) (35.50%), grade II (moderate) (45.40%), grade III (severe) (19.60%). All of these patients had reactive (benign) hemophagocytosis. We had no patient with primary histiocytic malignancy or malignant histiocytosis.

NMHC was the largest group showing HP. Megaloblastic anemia ranked on the top in this category with peripheral cytopenia and increased degree of HP. Infection associated HP was the second largest group mainly with moderate and severe degree of HP. Eighteen patients had HPS.

There was apparently no effect of age on either intensity of HP or on blood counts in our study. With the increase in the intensity of HP there was steady decline in the

Hb (6.37gm/dl) and platelet count (81.55x103/L) were low compared to other groups. The mean Hb of grade II was significantly decreased compared to the mean of grade I ($P<.01$), the mean Hb of grade III was significantly decreased compared to grade I ($P<.001$), and grade II ($P<.01$). In the case of total leukocyte count, the mean of grade II was significantly decreased compared to grade I ($P< .05$). The mean TLC of grade III was significantly decreased compared to grade I ($P< .01$), while the difference of the means was not significant between grades II and III ($P> .05$).

The mean platelet count of grade II was significantly decreased compared to grade I ($P<.01$). The mean platelet count of grade III was significantly reduced compared to grade I ($P<.001$) and grade II ($P<.001$). The present study thus reveals that the main statistically significant effect of increasing intensity of HP was on Hb and platelet count.

The cellularity of bone marrow apparently had no effect on the intensity of HP, since most of our patients either had increased cellularity (152; 60.80%) or normal cellularity (53; 21.20%) as compared to decreased cellularity, (45; 18%). Pathogenesis of peripheral cytopenia in patients of HP appears to be multifactorial and depends upon the underlying disease process, immunosuppression, immune destruction of cells, drug therapy and above all the exaggerated HP with or without hemophagocytic syndrome, associated with hypercytokinemia particularly Interferon-gamma and TNF-alpha.

Additional investigations are required to elaborate the role of the microenvironment in the pathogenesis and intensity of HP and its effect on blood cell counts.

LIST OF ABBREVIATIONS

Abbreviations	
AA	Aplastic anemia
ANF	Antinuclear Factor
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
CHS	Chidiac Higashi Syndrome
CFU-GM	Colony forming unit granulocyte- monocyte
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
DLC	Differential leukocyte count
FAB	French American British classification
FHL	Familial Hemophagocytic lymphohistiocytosis
G. CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte monocyte colony stimulating factor
GS	Griscelli Syndrome
Hb	Hemoglobin
HD	Hodgkin's disease
HLA	Human leucocyte antigen
HLH	Hemophagocytic lymphohistiocytosis
HMR	Histiocytic medullary reticulosis
HP	Hemophagocytosis
HPS	Hemophagocytic syndrome
IL	Interleukin
INF	Interferon
ITP	Idiopathic Thrombocytopenic purpura
LC	Langerhan's cell
LEo	Lofflers eosinophilia
LFTs	Liver Function Tests
MCSF	Macrophage Colony Stimulating Factor
MH	Malignant Histiocytosis
MHC	Malignant Hematological Conditions
NMHC	Non Malignant Hematological conditions
MDS	Myelodysplastic Syndrome
NHL	Non-Hodgkin's Lymphoma
NHM	Non Hematological Malignancies
PT	Prothrombin Time
RBC count	Red Blood Cell Count
SA	Sideroblastic anemia.
SD	Storage Disorders
TLC	Total leukocyte count
VAHS	Virus associated hemophagocytic syndrome

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INTRODUCTION

Histiocytic hyperplasia with hemophagcytosis (HP) is a relatively uncommon condition that has often been mistaken in the past for a neoplastic disorder. Most frequently it represents a secondary reactive phenomenon due to increased production of various cytokines (Suster et al 1988). Histocytosis comprises a group of disorders of multiple etiologies involving cells of the monocyte/macrophage system (Groopman et al 1981).

The disorders showing histiocytosis can be broadly classified into two categories, reactive (benign) and non reactive (malignant).Although these disorders may have similar clinical features which include pyrexia, wasting, and generalized lymphadenopathy hepatosplenomegaly with or without pancytopenia, the histopathologic findings vary from proliferation of apparently benign histiocytes to cells that are obviously malignant (Fujiwara, 1993).

An element of HP is very often observed in disorders with histiocytic proliferation, (Arya et al 1985). A syndrome of exaggerated histiocytic proliferation and activation with hemophagocytosis has been defined during the mid 1970s. This is known as hemophagocytic syndrome (HPS). The syndrome is usually associated with systemic viral infections (Risdall et al 1979) but it can also occur with bacterial (Risdall et al, 1984), parasitic (Auerbach et al 1986) and fungal infections. (Majluf et al 1993).

In addition, it has also been reported in autoimmune diseases (Moriguchi, 2004), non-neoplastic haematological conditions (Imashuku, 1996) and malignancies (Abe et al 2001). Hemophagocytic lymphhistiocytosis has also been observed in certain inherited and genetic conditions such as familial hemophagocytic lymphohistiocytosis (FHL), X-linked lymphohistiocytosis and Griscielli syndrome, as a reactive phenomenon (Larroche, 2003).

In histiocytic disorders particularly showing hemophagocytosis there is an intricate interplay between macrophages and T lymphoeytes. T lymphocytes produce cytokines

the complement system. Macrophages on the other hand synthesize substances which promote proliferation of T-cells and their activation (Groopman et al 1981). The ultimate result is exaggerated phagocytosis of blood cells along with tissue damage.

In spite of numerous reports of the occurrence of hemophagocytosis and haemophagocytic syndrome particularly in various benign disorders, the literature is scanty on its correlation with bone marrow function and effect of its intensity on hematological parameters. The present study deals with the etiology of HP, its intensity in the bone marrow and its effect on haematological parameters, particularly haemoglobin and platelet count.

Historical Background

The process of histiocytic hyperplasia with prominent HP was first recognized in adults by Scott and Robb-smith (1939). They believed that the process was neoplastic, and used the term histiocytic medullary reticulosis for this disorder. Most of the patients exhibiting this phenomenon were severely ill with a high mortality rate. Early pathological reports described a systemic proliferation of predominantly mature histiocytes showing marked hemophagocytosis in the bone marrow (Suster et al 1985)

In the pediatric age group a similar process was later described and designated as familial erythrophagocytic lymphohistiocytosis (FLH) or familial hemophagocytic reticulosis (Farquahr et al 1958). The diagnosis in these conditions was generally established by the demonstration of proliferation of histiocytes in the bone marrow and lymph nodes exhibiting hemophagocytosis (Perry et al 1976). Rappaport (1966) introduced the term malignant histiocytosis and described differences between cytologically benign and malignant hemophagocytic histiocytes. Warnke et al. (1975) also emphasized on the need for applying standard cytologic criteria of malignancy in the diagnosis of histiocytic proliferations.

A syndrome of exaggerated histiocytic proliferation and activation has been defined in mid 1970's. This is known as hemophagocytic syndrome (HPS). It was reported initially by Risdall et al. (1979) in 19 cases with proven viral infection. The disorder

It is commonly seen in Epstein barr virus (EBV), herpes simplex, cytomegalo virus (CMV), Varicella zoster, adenovirus and human immunodeficiency virus (HIV) infection. Recently it has also been found to be associated with hepatitis A (Watanabe 2002). All of these cases lack cytologic features of malignancy (Imashuku, 1996).

The signs and symptoms of this syndrome include fever, jaundice, coagulopathy pallor, myalgia, hepatic and splenic enlargement, hyperferritinemia and lipid derangement .There may be pancytopenia or bicytopenia with increase in marrow macrophages having prominent hemophagocytosis. Later on proper diagnostic and prognostic criteria were established for this syndrome (Fujiwara 1993).

Risdall et al. (1984) reported patients with histiocytic hyperplasia with hemophagocytosis in association with bacterial infection. Such patients have drawn attention to the fact that the development of a benign, reactive HP is not a phenomenon solely restricted to patients with viral infection, but may also develop in association with other conditions.

It is now apparent that reactive histiocytic hemophagocytosis is an accompanying feature in a variety of systemic disorders such as tuberculosis(Fujiki et al 2003), protozoal infections (Tune 2001) and autoimmune diseases (Sekagava et al 2002), sarcoidosis (Groopman et al 1981) disseminated malignancies, leukemia (Strauss, 2004), Hodgkin's lymphoma (Narimatsu 2004) and storage disorders. It has also been reported in inherited disorders, like FLH, Griscilla syndrome, X-linked lymphoproliferative syndrome, and Chidiak Higashi syndrome with genetic derangement (Larroche, 2004). Previously the pathogenesis of HP was not clearly defined, but it is now apparent that it is mainly because of increased production of cytokines in the presence of excessive proliferation T-lymphocytes and macrophages (Fujiwara et al 1993).

Mononuclear Phagocyte System

The monocyte- macrophage system or mononuclear phagocyte system is now known

(Dougles, 1988). It is a wide spread system of cells originating in the bone marrow from pluripotent stem cell passing through the stages of monocytic progenitor,colony forming unit granulocyte-monocyte (CFU-GM), monoblast, promonocyte and monocyte. Transitory period of 3 days, Monocytes in the blood migrate to the various tissues of the body such as lungs, liver spleen and lymph nodes (Cline, 1987).

The concept of a continuance of the cells from marrow monoblast / promonocyte through monocyte to the larger tissue macrophages and multinucleated giant cells, is critical to the understanding of the development and function of these cells (Groopman et al 1981).

Production and Maturation

The earliest identifiable cells of monocyte/macrophage system are monoblasts and promonocytes in the bone marrow derived from bipotential progenitor cell known as CFU- GM. The growth of granulocyte and monocyte colonies in semisolid culture requires the presence of granulocyte-monocyte colony stimulating factor GM-CSF (Cline,1987).

When these cells develop a complex golgi apparatus and definite granules,they are designated as promonocytes.The promonocyte has poorly developed phagocytic capacity and few receptors for the Fc portion of IgG. It has a relatively large size (10-15um), high nuclear cytoplasmic ratio, basophilic cytoplasm, peroxidose activity and glass adherence capability (Groopman et al 1981).

After two or three cell divisions, promonocytes give rise to monocytes, which are generally smaller than their precursor cells but have highly developed lysosomal system and increased phagocytic activity. They are non dividing under ordinary conditions, but with appropriate stimulation, proliferation may be induced.

In the adult, absolute monocyte count generally ranges between 200-600 cells/ micro liter and in relative terms 1-6% of the total leucocyte count. Monocyte counts of 1000-12000/ul an normal in the first 2 weeks of life. After that in young children a count up to 750cells /microlitre may be taken as normal.

leave the blood vessels and enter the tissues, where they mature into macophages or tissue histiocytes (Cline, 1987).

The tissue macrophages are widely distributed throughout the body and include the alveolar macrophages of the lung, Kupffer cells in liver, the lining macrophages of spleen, histiocytes in the bone marrow and microglial cells of brain, freely migrating macrophages of the pleural and peritoneal cavities, dermal Langerhans cells and the osteoclasts of bone (Van Furth et al 1985).

With maturation certain functional capabilities develop.These include phagocytic ability, protein synthetic capacity, surface receptors for immunoglobulins (IgG) and certain complement components. Ultimately the end stage of development is reached and the mature macrophage is a large fully developed functional cell with little prolifirative capacity (Groopman et al 1981). Figure 1 shows production and maturation of macrophages.

Morphology of Macrophages

As the monocyte begins its evolution into a macrophage, the cell enlarges in size and the lysosomal content is increased, along with the amount of hydrolytic enzymes within the lysosomes. At the same time the size and the number of mitochondria increases, with a concommitent increases in their energy metabolism. The Golgi complex, which packages lysosomes, increase in size and numerous secondary lysosomes are formed by the fusion of primary lysosomes. These are called phagosomes (Dougles, 1991).

On Romanowsky stained film, macrophages are 25 to 50 um in diameter. They have an eccentrically placed fusiform nucleus, with one or two indistinct nucleoli and finely dispersed, loosely stranded nuclear chromatin. A juxtanuclear zone (Golgi complex) is well defined. The cytoplasm shows fine granules and multiple, pink purple, large azurophilic granules. The active pinocytosis in these cells is reflected by the cytoplasmic vacuoles found near the cell periphery (Nelson, 1991).The morphologic specialization of macrophages is dependent upon their location and function.

The fixed macrophages of the spleen are involved in the sequestration and destruction of effete or abnormal red cells and display stages of erythrophagocytosis and intra cytoplasmic aggregates of ferritin (Cline, 1987).

The macrophages of the marrow, the "nurse cells" of the erythroblastic island, play a similar role in erythrophagocytosis and are a source of iron storage and transfer (Groopman et al 1981).

Hepatic macrophages (Kupffer cells), found in liver sinusoids, also phagocytose red cells, and other cellular elements. Macrophages of the pulmonary alveoli, the lamina propria of the gastrointestinal tract and the peritoneal and pleural fluids reflect in their morphology a specific function of phagocytosis of microorganisms, cells, cellular and non-cellular debris (Cline, 1987).

The macrophages of the pulmonary alveoli, peritoneal and pleural cavaties and inflammatory exudates are hypermature cells that have undergone in vivo stimulation and maturation. This results in enhanced bactericidal activity due to augmentation of the number of lysosomes and their acid hydrolase content (Nelson, 1991).

Membrane Antigens and Surface Markers

Monocyte-macrophage system cells display several surface antigens and receptors that have been characterized through their binding to specific monoclonal antibodies. These antigens serve as markers for the origin, differentiation and function of cells of the monocyte-macrophage lineage. These include the Fc receptors (FcRII and FcRI), HLA DR (Ia), Mac1, CD4 and complement receptors.

With the help of these membrane antigens, monocyte-macrophage system cells, perform diverse functions including phagocytosis of microorganisms, killing of antibody coated erythrocytes, other blood cells and tumor cells, spontaneous killing of unsensitized tumor cells, antigen presentation to helper T-lymphocytes and secretion of different biologic substances (Leonard et al, 1983).

Fc – Receptors (FcR)

There are 3 distinct classes of FcR, I, II and III. FcR I and FcR II are expressed on mononuclear phagocytes. All the three Fcrs specifically bind human IgG subclasses

"activation" with increase in phagocytostic activity, super oxide production, and leukotrien release (Leunard, 1983).

Mac – I antigen.

It is expressed exclusively on macrophages.Mac-I antigen is the complement receptor type 3 which is specific for C3bi. AntiMac-I monoclonal antibody (MOAb) strongly inhibits complement receptor mediated rosetting of erythrocytes IgM antibody complex (Wright , 1984).

Complement Receptors

At least two distinct types of complement receptors are recognized in mononuclear phagocytes. One recognizes C3b and another recognizes C3bi (MaC-I Ag). Monocytes and macrophages also synthesize and secrete serum components of the complement system of classic pathway and all the components of the alternate pathway of the complement system (Leunard, 1983).

CD4 Antigen

The CD4 and the corresponding mRNA have been demonstrated on monocytes, macrophages and monocyte-like lines U-937 (Mocicki et al 1984). The CD4 molecule is involved in the induction of T-lymphocyte helper functions (t4) and T-proliferative responses to antigen stimulation (Rogozinski et al 1984). CD4 antigen is an important part of the receptor for the human immunodeficiency virus (HIV) and anti-CD4 MoAb (OKT4a) blocks HIV infection efficiently.

Functions of Mononuclear Phagocytes

Monocyte macrophage system cells perform diverse functions which include phagocytosis of microorganisms, killing of antibody coated erythrocytes or tumor Cells (antibody dependent cellular cytotoxicity), spontaneous killing of un-sensitized tumor cells, antigen presentation to helper T-lymphocytes and secretion of different biologic substances (Groopman et al 1981).

Phagocytosis and Microbial Killing

many aspects of their phagocytic behavior. They kill various species of bacteria, fungi and parasites. Particle ingestion induces increased oxygen consumption, H_2O_2 and super oxide production and stimulates hexose-monophosphate shunt activity. Human blood monocytes contain myeloperoxidase and manifest secondary peroxidase reactions after ingesting the microorganisms (Klebanoff, 1983).

There are certain pathogens which particularly parasitize macrophages and replicate within them. When the macrophage is activated, these intracellular pathogens may be inhibited or destroyed. Salmonella, brucella, listeria, mycobacteria, chlamydia, rickettsia, leishmania, toxoplasma, trypanosomes, etc. have been found capable of invading and inhabiting non activated macrophages. The human immunodeficiency virus (HIV) has also been found to infect and replicate within monocytes and macrophages (Tracy et al 1987).

Mononuclear phagocytes are capable of interferone production, which may aid in protection against viral infection (Groopman et al 1981). Monocyte cationic proteins, other than myloperoxidase, have been shown to have fungicidal activity. Cytokines such as interferone gamma, tumor necrosis factor, or interleukin-1 (IL-I) activate macrophages, resulting in greater microbicidal capacity (Fujiwara, 1993).

Role of Macrophages in Inflammatory Process

The tissue macrophage is a pivotal modulator of inflammation and microbial killing. The processes involved are complex. These include chemotaxis or directed migration of the macrophages to the area of infection, opsonization of the invading organism, attachment of the coated organism to the surface of the macrophage by means of receptors for complement and immunoglobulins, ingestion of the organism by the macrophage and the formation of a phagocytic vacuole (Nathan et al 1984).

Metabolic changes within the macrophage which accompany phagocytosis result in lysosomal degranulation with release of the active enzymes into the phagocytic vacuole containing the ingested particle and ultimately the killing and digestion of the organism (Nathan et al 1984).

Macrophages secrete proteases which are active at neutral pH.These include plasminogen activator, collegenase and elastase. Plamin is capable of lysing fibrin and thereby dissolving and degrading clot at the site of inflammation. In addition, plasmin activates complement components. Break down of complement components yields products which are chemotactic for macrophages and neutrophils. Macrophage collagenese and elastase may act to degrade the structural components of the vessel wall, perivascular tissue and joint surfaces. The macrophage is also capable of turning off destructive inflammatory responses by secreting plasmin inhibitors as well as a macroglobulin, which inhibits plasmin and many other proteases (Horiyschi et al 1987).Complement components synthesized and secreted by macrophages are capable of affecting macrophage migration, endocytosis and secretion as well as help the macrophage in ingestion of target antigens via opsonization. Release of super oxides, hydrogen peroxide, and hydroxyl radicals by activated macrophages may facilitate the destruction of proteins, lipids and nucleic acids (Nathan 1987). Mononuclear phagoytes secrete IL-1 and tumor necrosis factor alpha which act upon temperature regulation centre in the hypothalamus causing fever during inflammatory process (Rosenthal 1980).

Role in immunity

The mononuclear phagocyte is essential for the development of cellular and humoral immunocompetence. There is an intricate interplay between the mononuclear phagocytes and the T-lymphocytes. Recognition of protein antigens by the T-lymphocytes is usually preceded by phagocytosis and processing of antigen by the macrophages. Macrophages and lymphocytes must share genetic identity at some portion of the major histocompatability complex (MHC) if the T-lymphocyte is to recognize the antigenic signal presented by the macrophage. The primary interaction in immune recognition is between the macrophages and the T-lymphcytes, which then allow effective interactions between T and B lymphocytes resulting in antibody production (Larroche 2003).

IL-1, produced by macrophages, stimulates the production of variety of lymphokines, including IL-2, by target T-lymphocytes. This in turn, may induce proliferation of a clone of specifically immunized T-lymphocytes and thereby act in the efferent limb of

Role in Production and Destruction of Blood Cells

Monocytes and macrophages secrete colony stimulating factor CFU-GM, which stimulates granulopoiesis in vivo. Early erythroid development is also regulated by mononuclear phagocytes. They also secrete growth factor for fibroblasts (Nathan 1987).

Erythrocytes, at the end of their life span, are phagocytosed by macrophages during their circulation through the spleen. The membrane of senescent red cells gets coated with immunoglobulins which are recognized by the Fc-receptors of macrophages and the cell is finally phagocytosed. Complement coated aged cells may also by ingested by activated macrophages (Kay, 1975). It is possible that removal of effete leukocytes and platelets is also mediated by a similar mechanism (Golde, 1988).

Histiocytic hemophagocytosis, that is prominent in some histiocytic disorders, may result from activation of tissue macrophages with increase in receptor activity and premature ingestion of blood cells coated with normally sub threshold amounts of immunoglobulin or complement. Studies showing the requirement for IgG in vitro erythrophagocytosis by histiocytes obtained from lesions of eosinophilic granuloma of bone lend credence to this model (Cline et al 1987) Table 1(a) gives secretary products of macrophages. Table 1(b) gives pathophysiology and clinical features of histiocytic disorders.

Table # 1(a) Secretary products of mononuclear phagocytes Groopman et al 1981

- Complement (C) components
- Coagulation factors.
- Polypeptide hormones.
- Other enzymes.
- Inhibitors of enzymes and cytokines.
- Reactive oxygen and intermediates metabolites.
- Reactive nitrogen and intermediates metabolites.
- Proteins of extracellular matrix or cell adhesion.
- Other binding proteins.
- Bioactive oligopeptides.
- Bioactive lipids.
- Sterol hormones.
- Purine and pyrimidine products.
- M-CSF

Table # 1(b) **Pathophysiology of Clinical Features of Histiocytic Disorders (Groopman et al 1981)**

Granuloma formation with multinucleated giant cells
Lability of macrophages plasma membrane and specialized zones of adherence;
Hemophagocytosis
Increased activity of macrophage Fc receptor for IgG present on hemopoietic cell surface and liberation of cytokines
Osteolytic lesions
Macrophage osteolysins
Macrophage-initiated release of osteoclast-activating factor from lymphocytes
Dermal Lesions
Activation of a subset of macrophage with characteristics of dermal Langerhan's cells.
Fever
Macrophage-derived endogenous pyrogens, Lymphocyte cytokines
Destructive arthritis
Macrophage collagenase and elastase
Panniculitis
Macrophage collagenase, elastase, lipase
Tissue eosinophilic infiltration
Macrophage stimulation resulting in release of eosinophilic chemotactic factor from lymphocytes.
Renal tubular dysfunction
Lysozymuria

Histiocytosis with Hemophagocytosis

Histiocytosis is a diverse group of disorders involving cells of the mononuclear phagocyte series. These diseases are usually characterized by proliferation and activation of macrophages caused either by external stimuli or by intrinsic cellular abnormality (Groopmann et al 1981).

Macrophage activation appears to mediate many clinical features of histiocytic disorders, such as hemophagocytosis, fever, and tissue damage. Macrophage may be activated in certain benign and neoplastic histiocytic disorder and the interaction of such macrophages with surrounding normal tissue forms the pathophysiologic basis of many clinical features (Strauss et al 2004).

Macrophages also act as antigen presenting cells. They present exogenous antigens to CD4+ or CD8+ T-cells and produce IL12 which stimulate Tcell activation and proliferation. Macrophages also secrete M-CSF and GM-CSF which can also influence T-cell function. T cells in turn enhance the phagocytic activity and recruitment of macrophages by producing mainly INFgamma and TNF-alpha (Fujiwara 1993).

Histiocytic proliferation and activation is seen in various benign and malignant disorders. A proposed classification of underlying clinical conditions which result in proliferation of histiocytes with hemophagocytosis is given in table 2(a).

Table # 2(a) Classification of histiocytic disorders with hemophagocytosis Groopman et al 1981

A- REACTIVE HEMOPHAGOCYTOSIS

1. **Infection associated**
   a. Viral
   b. Bacterial
   c. Parasitic
   d. Fungal

2. **Inherited Disorders**
   a. X-Linked lymphoproliferative disorder
   b. Familial Erythrophagocytic lymphohistiocytosis.
   c. Gricielli Syndrome
   d. Chidiak Higashi Syndrome

3. **Langerhans cell histiocytosis.**

4. **Associated with pre-existing malignancies**
   a. Acute and chronic leukemia
   b. Hodgkin's disease and Non-Hodgkin's lymphomas
   c. Multiple myeloma
   d. Non-hematological malignancies. e.g. carcinoma stomach.

5. **Non Malignant hematological conditions**
   a. Megaloblastic anemia
   b. Aplastic anemia
   c. Hemolytic anemia

6. **Storage disorders**

7. **Autoimmune disorders**

B -MALIGNANT (NON – REACTIVE)

   a. Malignant Histiocytosis
   b. B-Cell lymphoma
   c. Lymphoma associated HPS

Hemophagocytosis (HP)

Phagocytosis of all blood cells, mature and developing, by activated histiocytes of the mononuclear phagocyte system is known as hemophagocytosis (Larroche 2004). Histiocytic proliferation frequently shows an element of HP. It has been reported in the literature under various diagnostic terms, such as histiocytic medullary reticulosis, malignant histiocytosis and infection associated hemophagocytosis(Imashuku 1997).

Hemophagocytosis with or without histiocytic proliferation has been reported in several clinical situations with fatal course as well as in transitory benign disorders. It may result from immunologic activation of the mononuclear phagocyte system (reactive) or may be due to a neoplastic proliferation of histiocytes (malignant). Sometimes this may occur as a result of genetic or chromosomal derangement (Larroche 2004).

In the past, the vast majority of cases of histiocytic hyperplasia with hemophagocytosis were grouped under the designation of histiocytic medullary reticulosis or malignant histiocytosis and were invariably thought to represent a malignant disorder. But it is now well established that a similar phenomenon may develop in association with a variety of non- neoplastic condition as a reactive process (Risdall et al 1984).

In reactive histiocytosis macrophages are mature, whereas in malignant histiocytosis, proliferating cells are mainly immature. The mature macrophages are characterized by low nuclear cytoplasmic ratio, condensed chromatin pattern, inconspicuous nucleoli and abundant cytoplasm. Distinctive features of the malignant histiocytes include thickening of nuclear membrane, relatively fine chromatin pattern, conspicuous nucleoli. Mitotic figures are common. Hemophagocytosis is present in both reactive and malignant histiocytosis (Imashuku 1996). Table 2(b) shows the differences between reactive and malignant histiocytosis.

Table # 2(b) **Differences Between Malignant Histicoytosis and Reactive Histiocytosis with Hemophagocytosis**

	Malignant Histiocytosis	Reactive Histiocytosis
Predominant cells	Immature Macrophages	Mature Macrophages
Cytologic atypia	Present	Absent
Mitotic figures	Common	Few or absent
Involvement of organs other than MPS*	Common	Uncommon

*M.P.S. Mononucear Phagocyte system.

Hemophagocytic Syndrome (HPS)

Hemophagocytic syndrome is a clinicopathologic entity characterized by increased proliferation and activation of macrophages and T-lymphocytes with hemophagocytosis. It was first identified by Risdall et al. (1979) in association with viral infection. Clinical features and biochemical events of HPS include fever of unknown origin, cytopenias, hepatosplenomegaly, coagulopathy, hyperferritinemia, liver dysfunction and derangement in lipid metabolism. It may be primary as observed in familial hemophagocytic lymphohistiocytosis(FHL), X-linked lymphoproliferative syndrome (XLP), Chediak – higashi syndrome (CHS) and Griscelli syndrome, or secondary to infection, malignancy, autoimmune disease, drugs, non-malignant hematological conditions and variety of other diverse disorders (Larroche 2003) Figure 2 shows the proposed pathaphysiology of HPS.

Clinical features and their biological manifestation in both infection associated and non-infection associated cases, result from hypercytokinemia by activated T-cells and macrophages. Genetic defects involving the perforin dependent cytotoxic process of T-lymphocytes and natural killer cells have been identified in a subgroup of primary

Table # 3(a) Diagnostic Criteria for HPS Larroch 2004

Fever (duration≥7days with peaks ≥38.5°C) Splenomegaly(> 3cm below the costal margin)
Cytopenia (affecting ≥2 of 3 images in the peripheral blood and not caused by a hypocellular or dysplastic bone marrow).
Hb (≤90g/f).PLTs (≤ 100x10 9/l).ANC (≤ 1.0x10 9/l).
Hyperferritinemia and Hyper-LDH-nemia (ferritin ≥ 3 SD of the normal value for age, generally ≥ 1000 ng/ml,
LDH≥ 3 SD of the normal value for age, generally ≥ 1000 IU/l)
Histopathologic criteria : **Hemophagocytosis** in bone marrow , spleen or lymph nodes. Large granular lymphocytes, mature and immature, are often increased in number.

Pathogenesis

Histiocytic proliferation and hemophagocytosis in HPS have been postulated to be due to inappropriate or excessive immunologic responses of proliferating monoclonal or polyclonol lymphocytes along with macrophages in association with infection, non malignant hematological conditions, autoimmune diseases and neoplastic disorders.

Once T-cells are activated in an uncontrolled manner by abnormal immune responses, they produce large amounts of interferon gamma (IFN) and other lymphokines, which promote macrophage infiltration and form cytokine network producing hemophagocytosis. Therefore clinical features and metabolic derangement have been postulated to be due to hypercytokinemia (Fujiwara et al 1993).

Cytokines, Cytokine Receptors and Chemokines in HPS

Patients with active HPS have serum levels of Th1 cytokine IFN-gamma, Interleukin(IL)12 and IL 18 significantly higher than in the remission phase of the disease or in healthy controls. IL –18 seems to play a pivotal role in inducing IFN-gamma and IL12 and it has been found to be particularly elevated in tuberculosis and autoimmune disorders (Tochimoto et al 1999).

Serum levels of proinflammtory cytokines TNF-alpha, IL-IB and IL-6 are also elevated in patients with active HPS as compared to controls, whereas this is not the case with Th2 cytokine IL-4 (Osugi et al 1997). Serum levels of both IL-I and IL-10 are increased in patients with active HPS. However the amount of IL-10 produced is not sufficient to influence Th1 cytokine production (Imashuku et al 1993).

Serum level of M-CSF is elevated in active HPS along with macrophage inflammatory protein –I (MIP-1δ) and mRNA expression in tissue of patients, with HPS. This is as a consequence of increased IL-18 productions (Lin et al 2002). Increased concentration of soluble interlukin 2 receptor (SIL-2R) is found during active disease and correlates with poor prognosis (Fujiwara et al 1993).

Other markers such as CD8, soluble Fas (sFas) and Fas ligand (FasL) are elevated in the active phase of the disease. Fas –Fas ligand interaction contribute to apoptosis of target cells (Emmeneger et al 2000).

Hyperferritinemia with increased iron deposition in macrophages is attributed to increase in acute phase reactant protein (Essumi et al 1988). Role of various cytokine in HPS is given in table No.3(b).

Table # 3(b) Pathogenesis of hemophagocytic syndrome. cytokines and their effects Fujiwara et al 1993

CYTOKINES	EFFECT
ILI,TNF α, IFNγ	Fever through increase in PGE2
ILIβ,TNF-α	Pro coagulant activity through release of tissue factor
TNF α, IFN-γ	Liver dysfunction
ILI ,TNF α	Hyperlipidemia through inhibition of Lipoprotein lipase.
IFN-γ	Bone marrow depression
IL-6	Pro inflammatory effect
SIL -2R	Inhibits normal immune response after binding to IL-2
IL -18	Important in Pathogenesis of SLE and TB

Primary Haemophagocytic Lymphohistiocytosis (HLH)

i) Familial Haemophagocytic Lymphohistiocytosis (FHL)

This is an inherited condition which is autosomal recessive and it is characterized by infiltration of benign macrophages and activated T-cells, mainly CD8+ T lymphocytes following viral infection (Gan et al 2004).

The disease runs in the families and it has a genetic basis. There is involvement of the gene on chromosome 9q (FHLI gene) and on chromosome 10q (FHL 2gene) which codes for perforin membranolytic protein found in cytotoxic T lymphocytes (CTL) and natural killer (NK) cells. There is perforin deficiency and defective T-cell and natural killer (NK) cell function (Goransdotter et al 2001)

Clinical symptoms include, fever, hepatosplenomegaly, meningeal involvement with CNS symptoms and pancytopenia. As a result of defective .T-cell and NK cell function, patients with infection particularly EBV, measles, rubella, suffer a fatal course. Sporadic cases may also occur without familial predisposition (Larroche 2004). Bone marrow, lymph nodes and spleen show the presence of HP, atypical lymphoid cells and histiocytes. FHL is considered to be a non -neoplastic disorder but its treatment requires chemotherapy and bone marrow transplant (Durken et al 1999)

ii) X- linked lymphoproliferative syndrome (XLP)

XLP is a rare inherited immunodeficiency leading to fatal infection following EBV exposure in young boys. The XLP gene has been localized on xq identified by three independent groups and named SH_2DIA, DSHP and SAP, respectively (Coffey et al 1998). Deletions or mutation of the gene result in an absent or a non functioning SAP protein in patients with a XLP family history. The SAP protein is a small adapter molecule that interacts with the CD_2/SLAM family of receptors and with FynT, and Src – related protein tyrosine kinase. In T cells, interaction with SLAM (CD 150) is essential for the modulation of IFN-gamma production and NK cells binding to CD 244 which is crucial for cytotoxicity (Nicholes et al 1999). There is ultimately an inability to sustain anti EBV humoral immune response in XLP (Latour 2004).

iii) Griscelli Syndrome (GS)

It is a rare autosomal recessive disorder characterized by pigmentry dilution of skin and hair, increased susceptibility to infections and an accelerated phase with life threatening HP. There may also be neurological manifestations. Two closely linked genes have been identified on human 15q region (MYO –VA and RAB27A) in GS. These genes encode for proteins involved in intracellular vesicular transport .There is defective cytotoxic lymphocyte (CTL) activity and uncontrolled lymphocyte expansion with hemophagocytosis (Larroche 2004)

iv) Chediak Higashi Syndrome (CHS)

This rare autosomal recessive condition is characterized by hypopigmentation, severe recurrent bacterial infection, bleeding tendency and neurological impairment. In children with severe CHS, mutations of the CHSI gene localized on chromosome. It has been identified causing a complete defect in full length protein. There is reduced number and enlargement of lysosomes (Karem et al 1997).

CHS patients have deficient CTL and NK cells activity due to an inability to secrete giant granules containing lytic proteins. Hemophagocytosis with lymphohistiocytosis is observed in severe cases, in the bone marrow, lymph nodes, liver and spleen (Wang et al 2000).

Infection Associated HP

Infection associated HP was first described in immuno compromised patients by Risdall et al (1979), suffering from viral infection. The used the term virus associated hemophagocytic syndrome (VAHS) Subsequently it was recognized as a reactive process in a variety of other infections that is, bacterial fungal and parasitic (Imashuku 2000).

Infection associated HP is a multisystem disease process. There is usually an illness 2-6 weeks before the manifestation of HP becomes apparent. It may present as a full blown syndrome with all the constitutional signs and symptoms or as histiocytic hyperplasia with hemophagocytosis (Fujiwara et al 1993). Table No.4(a) shows different etiologies of infections associated HP.

Table # 4(a) Infection-associated hemophagocytosis

Infection	*Type*
Viral Infections	Epstein-Barr virus Adenovirus Cytomegalovirus Herpes simplex Varicella zoster Rubella Parvovirus B19 HIV
Bacterial Infections	Tuberculosis Salmonella species and other gram negative organisms Brucella
Fungal Infections	Histoplasma capsulatum Candida tropicalis
Parasites	Malaria Leishmania donovani Babesiosis

Virus Associated Hemophagocytosis (VAHS)

The term virus associated haemophagocyitc lymphohistiocytosis was introduced by Risdall et al. (1979) to describe a disorder characterized by generalized histiocytic proliferation with hemophagocytosis associated with systemic viral infection, having constitutional signs and symptoms and metabolic derangements, described earlier.

Since the first description, several case reports compatible with VAHS have been described .The viral agents commonly implicated include Epstein- barr virus (EBV). cytomegalovirus (CMV), herpes simplex varicella zoster, adenovirus II, HIV, parvovirus B19, Dengue virus, rubella, influenza A, Coxsacki A9 and Hepatitis A virus(Strauss et al 2004).

VAHS has been particularly studied in relation to EBV infection .Many cytokines, such as TNF alpha, INF gamma, and IL 6 have been implicated in the causation of the syndrome. There is also CD8+ T cell infiltration in various organs infected with EBV (Sato et al 2002). In another study conducted by Ohga et al. (2001) it was found that fractionated CD3+HLA-DR+ cells from patients with chronic active EBV infection contained higher copy number than did CD3+HLA-DR-cells.Quantitative PCR for cytokines revealed that interferon gamma, IL-2 ,IL10 and TGF beta genes were expressed at higher levels in HLA – DR+ than HLA DR--T cells.

These results suggest that activated T cells in chronic active EBV infection expressed high level of EBV DNA and both Th 1 & Th2 cytokines. EBV infected T-cells may contribute to the unbalanced cytokine profiles of chronic mononucleosis (Ohga et al 2001). Lmashuku et al. (1997) emphasized the importance of IL -6 as proinflammatory cytokine in EBV disease. It was found in high quantity (IL-6 >100pg/ml) preferentially induced by EBV in monocyts and B cells.

Hemophagocytosis in non viral infections

Reactive histiocytic hyperplasia with HP has also been reported in bacterial infection with negative viral studies (Risdall et al 1984). Such cases have drawn attention to the fact that the development of the benign, reactive hemophagocytosis is not a phenomenon solely restricted to patients with viral infection. It may also develop in

fever (Groopman et al 1981), brucellosis (Kurakukcu 2004) and variety of other gram negative and gram positive bacteria.

Risdal et al. (1984) reported four cases of HPS associated with bacterial sepsis. In addition to this there have been several case reports in the literature that have associated hemophagocytic syndrome with fungal and protozoal infections (Tune 2004) Parasitic infection in which haemophagocytosis has been observed include leshmaniasis (Bhutani et al 2002) and malaria (Zulunov et al 2002).

Hypercytokinemia and excessive proliferation of T-cells and macrophages have been implicated in the causation of HPS in such cases. The cytokines most commonly involved are ILI, INF-gamma, TNF- alpha, IL6, IL18, IL-2 (Fujiwara 1993).

Non Malignant Hematological Conditions

Hemophagocytosis is not an infrequent finding in non malignant hematological conditions, but it is not widely reported in the literature. HP was studied in relation to hemolytic anemias in the bone marrow and it was observed that BM is the major site of destruction of cells of erytheroid series (Marton 1975). It has also been observed in megaloblastic anemia and results in peripheral cytopenia (Iqbal et al 2003).

Dyserythropoietic anemias, autoimmune hemolytic anemias,and stem cell disorder like aplastic anemia also give rise to HP in the bone marrow,wih varied underlying mechanisms.It has also been studied extensively in relation to myelodysplastic syndrome (MDS). A case of MDS was recently reported with neutropenia by Wang et al. (2004).The bone marrow aspirate and biopsy demonstrated HP with MDS. Granulocyte colony stimulating factor (G-CSF) and granulocyte-monocyte colony stimulating factor (GM-CSF), were found to be elevated in the patient. Mechanisms of peripheral cytopenia in all such conditions depend upon the underlying disorder and of course exaggerated HP in the bone marrow.

Drug Induced Hemophagocytosis.

A number of drugs have been associated with the causation of HP. It has been

include TMP-SMX, diphenylehydantoin, carbamazaine, minocycine and phenobarbitone (Chinok et al 1994). The mechanism of drug induced HP is however not fully understood.

Langerhans Cell Histiocytosis.

The abnormal Langerhans cell is the characteristic cell of Langerhans cell histiocytosis. It has an irregularly shaped nucleus. Langerhans cells are normally present in the epidermis, mucosa, lymph nodes, thymus and spleen. They originate in the bone marrow and have a common stem cell of origin with all other types of histiocytes. The Langerhans granules (Birbeck bodies) are derived from the cytoplasmic membrane. Under the electron microscope they appear as racquet shaped structures in the cytoplasm (Favara et al 2002).

Langerhan cell histiocytosis embraces the terms eosinophilic granuloma, Letterer – Siwe disease, Hand – Schuller – Christian disease as well as self healing histiocytosis, eosinophilic xanthomatous granuloma, Langerhans cell granulomatosis. Hemophagocyosis is uncommenly associated with these disorders. The exact etiology of Langerhans cell histiocytosis is not known. It may be a manifestation of an atypical immunologic reaction or an autoimmune disease (Favara et al 2002).

Lipid Storage Disorders

Lipid storage disorders include Gaucher's disease, Niemann-Pick disease, Sea blue histiocytosis, gangliosidosis, and Fabry's disease. Genetic abnormalities in the catabolism of complex glycolipids, gangliosides and globosides cause the lipid storage disorders. Accumulation of these lipids result in enlargement and dysfunction of various organs. Enzymatic impairment of macrophage degradation of the membrane remnants of effete cells results in lysozomal accumulation of lipid (Zimran, 1997).

In Gauchers disease there is deficiency of enzyme glucocerebrocidase resulting in accumulation of glucocerebrocide in macrophages (Gaucher cells), giving a fibrillary appearnnce under the light microscope.Hemophagocytosis and sometimes sea blue

Niemann Pick disease results from accumulation of sphingomyelin in histiocytes due to deficiency of enzyme, sphingomyelinase. Some times there is also deposition of cholesterol. The histiocytes give vacuolated appearance in stained bone marrow smears under the light microscope (Kalodeen, 2000).

Hemophagocytosis Associated with Pre- Existing Malignances

Reactive histiocytic hyperplasia with hemophagocytosis has been observed in a large number of hematological and non-hematological malignancies. Hematological malignancies in which this phenomenon has been observed include acute and chronic leukemia, Hodgkin's disease, Non-Hodgkin's lymphomas and multiple myeloma (Strauss et al 2004). It has also been observed in non-hematological malignancies such as stomach carcinoma, breast carcinoma and bronchogenic carcinoma (Suster et al 1987).

Malignant disorders with hemophagocytosis include malignant histiocytosis, lymphoma associated hemophagocytic syndrome(LAHS) and histiocytic lymphoma (Falini et al 1990).

In hematological and non-hematological malignancies showing reactive hemophagocytosis, the proliferating cells have been found to be mature macrophages confined to the mononuclear phagocyte system and the process was determined to be reactive. Possible underlying pathophysiologic mechanisms have been extensively studied by various workers in this condition. It has been suggested that a histiocytic medullary reticulosis-like syndrome in patients with acute and chronic leukemia is probably reactive to underlying viral infection in the presence of immunosuppression with or without chemotherapy (Suster et al 1987) Sepsis was also documented to be an underlying cause of reactive histiocytic hemophagocytosis. Chemotherapy may intensify the histiocytic reaction in these cases. The role of cytokine and T-cell proliferation is crucial in the pathogenesis of such conditions (Strauss et al 2004).

Malignant Histiocytosis

Malignant histiocytosis (MH), previously known as histiocytic medullary reticulosis, is one of the rare malignant hematological disorders and represents a malignant transformation in the monocyte macrophage pathway. In the past, many cases of HP were diagnosed as MH. There has long been confusion regarding the cell lineage of the frequently occurring atypical promonocytoid mononuclear cells (Pritcherd 1994) Based on careful cell lineage studies, the MH-like HPS should be referred to as Lymphoma associated hemophagocytic syndrome(LAHS), when neoplastic cells are identified to be lymphoid with reactive histiocytosis, probably casued by cytokines released from lymphoma cells or as a result of lymphoma triggered immune reactions (Wilson et al 1991).

Byrne and Rappaport (1973) classified MH as systemic neoplastic proliferation of histiocytes and precursors with an acute onset and poor prognosis. The pathological characterization are: (a) proliferation of histiocytes, (b) evidence of cytologic atypia, (c) evidence of phagocytosis, especially by cytologically atypical histiocytes, (d) lack of cohesive cell masses, (e) presence of plasma cells, and (f) absence of capsular invasion. With the advancement in immunological and molecular studies on these tissues, the majority of these cases were found to be LAHS and true histiocytic malignancies were very rare (Strauss et al 2004).

The current discussion by the Histiocyte society group clearly differentiate between histiocytic and lymphoid malignancies as shown in Table No 4(b). The major issue for HPS cases is how to identify LAHS and to differentiate it from virus associated hemophagocytic syndrome, VAHS (Chubachi et al 1992).

Lymphoma Associated Hemophagocytic Syndrome (LAHS)

Reported LAHS cases include peripheni T- cell lymphoma, NK cell lymphoma, adult nasal T- cell lymphoma, large cell lymphoma including both T and B cell type, Ki –I-positive large cell lymphoma, immunoblastic lymphadenopathy like T-cell lymphoma and aggressive NK cell leukemia. Involvement of bone marrow, spleen, liver and intra abdominal lymph nodes is common in LAHS. Patients show evidence of hemophogocytosis during their clinical course and subsequently develop overt fatal

B-Cell lymphoma

Large B-cell lymphoma is associated with initial presentation of HP as a marrow involvement in the absence of peripheral lymphadenopathy. The disease mainly affects the middle aged and elderly and predominantly males. Most patients present with swinging fever, pancytopenia and hemophagocytosis, with increased morbidity and mortality (Baberin et al 1992).

In a case showing progressive hemolysis and serve pancytopenia reported by Takagi et al. (1992), postmortem examination revealed a widespread infiltration of diffuse large cell type infiltration of malignant lymphoma. Hemophagocytosis was found in the bone marrow, liver, spleen and lymph nodes. Barberan et al (1992) reported a B immunoblastic and kappa monoclonal case, with a fatal outcome, and the diagnosis was only possible after necropsy.

Table # 4(b) Markers for histiocytes in malignant lesions to determine true Histiocytic Malignancy Imashuku 1997

Most specific	**Less specific**
M-CSF receptor	MAC-387
Lysozyme	CD11c
Ki-M8	CD14
S100 + large cells	CD68
Ki-M4	LN5
Cathepsin D and E	

Hemophagocytosis and Effect On Hematological Parameters

Peripheral cytopenia is an important finding in hemophagocytosis particularly when it presents as a hemophagocytic syndrome. There may be various underlying mechanisms resulting in pancytopenia or bicytopenia during this phenomenon (Strauss et al 2004)

In hematological disorders pancytopenia or bicytopenia is a common occurrence. It could be due to bone marrow suppression as a whole for example in aplastic anaemia , or due to bone marrow infiltration by abnormal cells such as leukemia, lymphoma secondaries, parasitic disease, storage disorders or myelofibrosis (Iqbal et al 2003). Sometimes peripheral pancytopenia may occur due to peripheral destruction of cells which is mostly antibody mediated in immunological disorders such as systemic lupus erythematosus, HIV infection or drugs (Iqbal et al 2001).

The process of erythrophagocytosis was studied for the first time in detail in the bone marrow by Marton (1979) in patients with hemolytic anemia. This study showed that bone marrow is the main site for destruction of cells of erythroid series in healthy individuals as well as in pathological states such as hemolytic anemia (Marton 1975). The study however failed to correlate mechanism of peripheral cytopenia with destruction of hemopoietic cells in the bone marrow through phagocytosis.

Risdall and his coworkers (1979) reported for the first time hemophagocytosis in relation to viral infection in 19 patients. The bone marrow smears of the patients showed histiocytic hyperplasia with prominent hemophagocytosis and clinicopathological derangements attributed to hemophagocytic syndrome. In addition to other findings peripheral blood cytopenia was striking.

According to his belief, the cytopenia described in patients of histiocytic medullary reticulosis was attributed to massive phagocytosis, did not seem adequate in the present study. The severe cytopenia observed in virus associated hemophagocytic syndrome (VAHS) appear to be due in part to bone marrow failure. Decreased

The mechanism responsible for this finding was unclear. It is possible that the viral agent exerts a direct effect on hemopoietic cells. CMV can be isolated from peripheral blood leucocytes and is known to be pantropic. Infectious hepatitis virus is also known to be pantropic (Weller 1981).

Purtilo et al (1999) have reported agranulocytosis and aplastic anemia in a familial lymphoproliferative reactive syndrome associated with EBV infection. The abnormal coagulation parameters suggest that intravascular consumption may play a role in the pathogenesis of the thrombocytopenia in addition to the platelet phagocytosis.

Risdall et al. (1984) described three cases of bacterial infection, presented as HPS with constitutional symptoms, coagulopathy, organomegaly and peripheral cytopenia. The bone marrow smears showed marked hyperplasia of mature-appearing histiocytes and their monocytic precursors. Most of the histiocytes contained ingested erythrocytes and platelets. The sections in all three cases showed moderately hypocellular marrow due to acute cell destruction. There was granulocytic and erythroid hypoplasia. A follow up marrow biopsy in the surviving patients showed hypercellular marrow with normal hematopoiesis. There was however persistent moderate histiocytic hyperplasia with hemophagocytosis.

Sullivan et al. (1985), described EBV associated HPS in two children with peripheral cytopenia but the bone marrow aspirate and biopsy showed a hyperplastic marrow with infiltration by histiocytes. Erythrophagocytosis and leukophagocytosis were also present.

Tracy et al. (1985) described peripheral blood and bone marrow abnormalities in patients with HIV infection. Peripheral blood abnormalities included thrombocytopenia, neutropenia, lymphopenia and monocytopenia apart from low hemoglobin. The most frequent bone marrow abnormalities were dyserythropoiesis, megaloblastic change and erythroid hypoplasia .Histiocytic hyperplasia with prominent hemophagocytosis were other important findings in the bone marrow smears. They suggested that causes of these abnormalities were probably multiple and

Campo et al. (1986) presented three cases of HPS associated with miliary tuberculosis. Benign histiocytic proliferation with striking hemophagocytosis was present in a disseminated, multi systemic pattern in all three patients. The phagocytosed cells were mainly erythrocytes and lymphocytes and less frequently granulocytes and platelets. There was marked peripheral cytopenia in all patients. They believed that this was mainly because of exaggerated phagocytosis of cells and disseminated pattern of disease in the marrow (Campo et al 1986).

Chandra et al. (1985) described two patients with infection-associated HP. According to their study hemophagocytosis in reactive HP might be due to either a primary alteration in the phagocytic cells or the ingested material. The mechanism responsible for pancytopenia is not clear. It is possible that viral or infectious agents exert a direct effect on hemopoietic cells. CMV and hepatitis virus can be isolated from peripheral blood leucocytes.

Luppi et al. (2004) described a case of HHV8 primary infection with severe pancytopenia and hemophagocytosis in a renal transplant patient. The bone marrow was however normocelluar in that patient. They believed that peripheral pancytopenia could be attributed to direct effect of virus on blood cells in the presence of exaggerated HP.

Karakukcu et al. (2004) described eight children with brucellosis having pancytopenia. Bone marrow aspiration smears showed hypercellularity or normocellularity, with histiocytic hyperplasia and HP. Bone marrow aplasia and granulomas were not detected. All patients recovered completely and their peripheral blood counts returned to normal after antibiotic treatment for brucellosis. In this study the increased cellularity of bone marrow in patients indicated that bone marrow failure was not the cause of pancytopenia. The pathogenesis of pancytopenia is not clear but it seems that more than one mechanism could be responsible in brucellosis, such as hypersplenism, granulomas and hemophagocytosis.

normal with hyperplasia of mature histiocytes, ingesting erythrocytes, platelets and nucleated cells. The levels of cytokines, 1L-6, soluble 1L-2 receptor, TNF-alpha and IFN-gamma were markedly increased. Thrombocytopenia was particularly striking. It may be caused by increased platelet consumption associated with disseminated intravascular coagulation, decreased production in the bone marrow, hypersplenism, autoimmune mechanism and hemophagocytosis. They suggested that when patients with acute hepatitis reveal thrombocytopenia with hyperferritinemia, and hypertriglyceridimia, hemophagocytic syndrome should be considered.

Ohga et al. (2001) studied the role of cytokines in six patients with EBV infection having HPS. Role of CD3+HLA- DR cells, and cytokines INF-gamma, IL-2, 1L-10, was described. They suggested that peripheral cytopenia and hemophagocytosis in the B.M could be mainly attributed to hypercytokinemia, particularly of INF-gamma and TNF-alpha.

Peripheral cytopenia in relation to malignancy-associated HP has been observed in conditions such as malignant histiocytosis, lymphomas, acute and chronic leukemia. Pathogenesis of pancytopenia in these disorders have been found to be multifactorial, that is, infiltration of abnormal cells in the BM, chemotherapy, immunosuppression, underlying infections, with hypercytokinemia and prominent hemophagocytosis (Larroche 2004).

Suster et al. (1985) studied the risk factors in reactive histiocytic hyperplasia with hemophagocytosis in hemopoietic organs. The important risk factors identified were multiple blood transfusions, bacterial sepsis, malignancy, candida sepsis and viral infection. Majority of the patients had moderate to severe degree of HP. The histiocytic proliferation consisted of mature histiocytes. In all patients phagocytosed red cells were present in association with nucleated red cells and platelet structures. The marrows were hypercellular in 25, hypocellular in 35 and normocellular in 42 patients. Erythroid, granulocytic and megakaryocytic series were not depressed in the majority of the patients .Depression of cell counts according to this study could be multifactorial depending upon the underlying risk factors, immunosuppression, and

Fujiwara et al. (1993) extensively studied the role of cytokines in hemophagocytic syndrome. According to their study, pancytopenia is an essential sign in HPS and it is induced by a large number of cytokines with overlapping functions acting in synergy. They concluded that it is mainly IFN-gamma which acts as a potent suppressor of hemopoiesis. Similar findings were observed by Imashuku in 1996, in his work on HP. in which he studied underlying disorders and selection of the most effective treatment.

Strauss et al. (2004) studied the risk factors of BM histiocytic hyperplasia with hemophagocytosis in critically ill patients. The multiple risk factors identified, included malignancies, cardiovascular disease and sepsis. Most of the patients presented with peripheral cytopenia. The histologic examination of the BM revealed that activated and enlarged macrophages were diffusely distributed throughout the bone marrow. The macrophages appeared mature with low nuclear/cytoplasmic ratio and inconspicuous nucleoli. Within the cytoplasm of the macrophages, mainly erythrocytes, less frequently platelets and neuthophils and their precursors were present.

In the BM, small aggregates of phagocytosing macrophages were observed, but with no preferential localization. A significantly increased number of diffusely distributed T cells with small lymphoid aggregates were seen in BM with HP. The extent of HP was not associated with BM cellularity that is majority of the patients either had normocellular or hypercellular marrow. They concluded that frequency and grade of HP were independent of BM cellularity and this latter has apparently no influence on the pathogenesis of HP resulting in pancytopenia. Similar findings were observed by Wong et al. (1992) and Florena et al. (2002). In common with other studies they found that BM can be hypercellular as well as normo or hypocellular irrespective of intensity of HP. The influence of BM microenvironment however should be more thoroughly studied. Role of aggregates of macrophages and CD3 positive T lymphocytes should be emphasized. The T-cells may play a key role in the causation of HP particularly by elaborating IFN-gamma and TNF-alpha resulting in the depression of cell counts. So T-cells infiltrations but not BM cellularity may play a

PATIENTS AND METHODS

Patients

All bone marrow aspirates performed at the Department of Hematology/Patholgy Pakistan Institute of Medical Sciences (PIMS), Islamabad were examined for the presence of hemophagocytosis. In the present study variable degrees of hemophagocytosis were observed in the bone marrow smears of 250 patients having different underlying disorders. The period of study spanned March 2003 to March 2006.

Methods

The patients were interviewed and a thorough physical examination was performed. The findings were recorded in a Performa attached as Annex-A.

In addition to bone marrow aspiration, the following investigations were performed:

1. Bone marrow trephine biopsy in selective patients
2. Complete blood counts, reticulocyte count, RBC morphology in all patients.

In the light of the history, physical findings and findings in blood and bone marrow one or more of the following investigations were carried out to determine the etiology:

1. Viral screening.
2. Widal Test.
3. Liver Function Tests.
4. Coagulation profile.
5. Lipid profile
6. Serum ferritin, triglyceride, LDH
7. Anti Nuclear Factor.
8. Bone marrow culture for microbes.

Sample collection

Blood

a. EDTA anticoagulated specimen with EDTA to a concentration of 2.5 mg/ml of blood. Three ml of blood was collected.
b. Ten ml of blood was aseptically collected in a clean sterile glass tube and allowed to clot at room temperature. When the clot had contracted, the clear serum was aseptically transferred to another sterile tube. This specimen was used for other tests as and when required.
c. For coagulation tests 1.8 ml of blood was collected in 0.2 ml 3.31% trisodium citrate solution. The specimen was mixed and centrifuged at 2500 rpm for 5 minute. Supernatant plasma was transferred to another clean tube and tests were performed within half an hour.

Complete Blood Counts

Total leukocyte count, red cell count, platelet count and absolute values were performed on an automated hematology analyzer (Sysmex K1000). The equipment was calibrated daily and quality control was checked with high, normal and low commercial controls provided by corresponding manufacturers daily. Low and high platelet counts were confirmed by manual method and by scanning peripheral smears.

Reticulocyte Count (Decie and Lewis, 1991)

Reticulocytes were stained with by brilliant cresyl blue. Equal quantities of blood and stain were taken in a test tube (i.e, 2 drops of anticoagulated blood and 2 drops of reticulocyte stain). After proper mixing, the solution was kept in an incubator at 37°C for 20 – 30 minutes. After incubation smears were prepared from a drop of the mixture (stain and blood) on a clean glass slide which was dried. Reticulocyte counting was done by the standard method described by Dacie and Lewis (1991).

Bone Marrow Aspiration

Bone marrow aspiration was performed using Salah's needle. The posterior superior iliac spine was the site used in adults and in children above the age of two years, Sternal puncture was only done in adults if they were obese, in cases of dry tap or when repeated attempts at post iliac spine failed to yield any aspiration material. The sternum was punctured opposite the second or third inter costal space slightly to one

done more than 0.5cm. In the case of children below two years of age, the site of the biopsy was tibia.

When the site was the posterior superior iliac spine, the patient was comfortably laid in the prone position. The site was cleaned with spirit and then painted with tincture iodine. In order to prevent infection an area larger than required was cleaned. Two percent lignocane was used as a local anaesthetic. About 3-5ml was injected into the skin, subcutaneous tissue and in the periostium. After 4-5 minutes of local anesthetic injection the aspiration needle was introduced with a gentle boring movement for up to about 1 cm inside the iliac spine. About 2 ml of marrow was sucked into a 10 ml plastic syringe.

The smears were made immediately to avoid clotting of the marrow. The wound was sealed with a sterile adhesive dressing after ensuring hemostasis by firmly pressing the puncture site for 5-10 minutes.

Preparation of Bone Marrow Smears

For direct preparation of bone marrow smears, marrow was poured over slides placed in a slanting position. The fragments were picked with the edge of a spreader and smears were made. For each patient at least 10 smears were made.

The following staining procedures were performed on bone marrow smears.

1. Leishman stain:
2. Iron staining (Perl's stain)
3. Acid phosphatase, PAS and specific esterase stain only when required.

Leishman Staining of Bone Marrow Aspiration Smears

Principle

Leishman's stain is a Romanowsky stain. The acidic elements in the cells, like nucleic acids and proteins of the cell nucleus bind the basic dye ,that is, azure-B and stain blue. The basic elements like hemoglobin stain red with acidic component, that is, eosin. Alkaline pH accentuates the basic dye staining, therefore an optimum pH of 6.8

Requirements

1. Leishman's stain powder 0.2g.
2. Methanol (acetone free) 100 ml.
3. Funnel with filter paper.
4. Conical flask.
5. Mortar and pestle.
6. Potassium dihydrogen phosphate 9.1g.
7. Disodium hydrogen phosphate 9.5g.
8. Distilled water.

The staining constituents used belonged to Medline diagnostics (arr.

Preparation

Stain Solution

Powder stain was weighed (0.2g) and transferred to a mortar. It was ground with about 25 ml of methanol and allowed to settle. The supernatant was transferred through filter paper to a flask. Grinding was repeated with 25ml of alcohol each time until all alcohol was finished and all powder was dissolved.

The flask was placed in a water bath at 50°C for 15 minutes. It was then filtered in a clean brown bottle. Mixture was left for at least 2-3 days to mature. Required amount for daily use was filtered into a smaller dropping bottle every morning.

Buffer pH 6.8

Potassium dihydrogen phosphate was dissolved in one litre of distilled water (Stock buffer solution -A) and stored at 40°C.Disodium hydrogen phosphate was dissolved in one litre of distilled water (Stock buffer solution -B) and stored at 4°C.Working buffer solution was made by mixing 50.8 ml of stock solution A with 49.2 ml of stock solution –B and diluting it upto 2 liters with distilled water.

Procedure

Leishman stain was poured on the smears for 5 minutes. After 5 minutes buffer was poured on the slides, mixed, and allowed to stand for 10 minutes. Finally the stain lying over the slides, was drained off and the slides were washed in running tap water.

Iron staining (Prussian blue Staining)

Iron staining (Prussian blue reaction) was done in all case according to the standard procedures described by Decie and Lewis (1991) using Medline Diagnostics stains.

Principle

The method of staining is based on pearl's reaction, in which water insoluble hemosidrin acquires a blue color when exposed to an acidic solution of potassium ferrocyanide. Iron stains as bright blue to blue green granules.

Acid Phosphate Staining

Acid Phosphatase staining was done by the standard method described in the manufacture kit (Sigma Diagnostic cataloge No. 386-1).

Principle

Acid Phosphatase activity is present in lysosmes of almost all cells of hemopoietic origin including cells of the mononuclear phagocytes system (monoblasts, promonocytes, monocytes and macrophages). A substrate is provided which is converted to a colored product by the enzyme.

Significance

The most common application of the acid Phosphatase reaction is in the classification of lymphoproliferative disorders. In acute myeloid leukemia (AML), blasts of the monocytic lineage react more strongly than those of the granulocytic lineage. The reaction may be of value in the distinction of the various types of AML. Malignant histiocytes are also acid phosphatase positive.

Alpha-Napthol Acetate, Estrase (Non-Specific Estrase)

Non-specific estrase staining was done by the standard procedure described in the manufactures kit (Sigma Diagnostic catalogue number 91-A).

Principle

Significance

The cytochemical reaction for ANAE is of practical value because it gives distinct patterns in lymphocytes (dot like reaction) and in monocytes (diffuse positive reaction). The localized reaction in lymphocytes is resistant to sodium fluoride (NaF), where as in monocytes, it is NaF sensitive. Malignant histiocytes may be ANAE sensitive (Decie and Lewis 1991).

Bone Marrow Trephine Biopsy

Bone marrow trephine biopsy was performed with a Jamshidi needle. The posterior superior iliac spine was the site for bone marrow trephine. Initial preparation was the same as for aspiration biopsy. After making a small crisscross incision at the site of the biopsy with a sharp sterilized scalpel, the needle was introduced, with boring movement applying gentle pressure forward.

After fixing the needle, the stillet was removed and the needle was moved further to a depth of 1.5-2 cm. Clockwise and anticlockwise movements were then performed several times to detach the internal portion of the marrow. The needle was then withdrawn.

The biopsy specimen was then carefully removed from the inside of the needle with a finer stillet from in front and trephine imprints were made on clean glass slides (to be stained by leishman stain later on). The biopsy specimen was then put in a small bottle containing 10% buffered formal saline. These were left for overnight fixation, Decalcification of the specimen was carried out in 5% nitric acid for 24 hours.

Dehydration of biopsy material was in absolute alcohol of increasing concentrations (70%, 80%, 90%) for 2 hours (in each concentration), followed by block making. Cutting of the block was done by rotary microtome. Thickness of each section was 5 microne. Finally hematoxalin and eosin staining was carried out by standard methods (Steven, 1990).

- Cellularity in general.
- Cellularity and maturation of erythropoiesis and myelopoiesis.
- Abnormal cells including abnormal infiltrate.
- Lymphocytes and plasma cells.
- Granuloma.
- Hisriocytes and haemophogocytosis.
- Reticulin stained sections were examined to assess the degree of fibrosis.

Examination of Bone Marrow Smears

Bone marrow aspiration slides (Leishman stained) were examined under low power as well as under oil immessian lens. The following parameters were examined:

- Cellularity of bone marrow in general.
- Cellularity and maturation of erythropoiesis.
- Cellularity and maturation of myelopoiesis.
- Megakaryocytes, number and maturity.
- Lymphocytes.
- Plasma cells.
- Abnormal Cells.
- Histiocytes / macrophages and hemophagocytosis.
- Parasites.

Grading of Intensity of Hemophagocytosis

Grading of intensity of hemophagocytosis was done on bone marrow aspiration smears. The slides were mounted with cover slips. The mounted slides were first scanned under low power (X10) in 10 random fields, and presence or absence of hemophagocytosis was noted. If hemophagocytosis was seen then it was graded in the following manner. The number of hemphagocytic cells were counted in 10 random fields and the average of such cells per high power field were calculated. The following criteria was used to grade the intensity:

Grading	No. of Hemophagocytic cells /HPF
Grade I (Mild)	1 – 2
Grade II (Moderate)	3 – 5
Grade III (Severe)	6 – 8

Viral Screening

The serological methods used were complement fixation tests and ELISA.

Viral screening was done for the following viruses using commercial kits (Ismunit, verion, Behring).

- Epestein barr virus(EBV)
- Cytomegalo virus (CMV)
- Human immuno def. virus(HIV)
- Adenovirus
- Herpes simplex
- Varicella zoster virus.

For EBV the monospot test was also carried out.

The widal test was done in relevant cases only by agglutination (tube method) using commercially prepared reagents (Bio kit).

Liver Function Tests

The following tests were performed:

Total Bilirubin

Direct and indirect

ALT

AST

Alkaline Phosphatase

Serum Albumin

The tests were performed on an automated chemistry analyzer microlab 200 using commercial reagents (Merck).

Coagulation Screening

In patients in whom coagulation abnormality was suspected, coagulation screening was carried out. The following tests were performed by standard procedures described by Dacie and Lewis (1991) using kits of Sigma diagnostics:

1. Bleeding time (Ivy's method)
2. Clotting time
3. Prothrombin time
4. Partial thromboplastin time.

Antinuclear Factor

Antinuclear factor in suspected cases was done in the immunology department by fluorescent antibody test according to the method described in manufacturer's kit.

Bone marrow Culture for Microbes

At the time of bone marrow aspiration, 2ml of the aspirated material was mixed with Brain heart infusion (BHI medium) for routine culture in relevant cases. Lewnstein Janson (L.J.) medium was used for acid fast bacilli (AFB) and inoculated with 0.5 ml of bone marrow aspirate. The culture bottles were sent to the microbiology department for incubation and further processing.

Statistical Analysis

Mean values and standard error were determined. T tests were applied to determine statistically significant difference between mean of cell counts in different grades of HP. Chi square analysis was used to determine significant difference in the number of male and female patients.

RESULTS

In the present study total number of patients showing variable degree of hemophogocytosis in the bone marrow smears was 250 .These patients were diagnosed in the Department of Pathology Pakistan Institute of Medical Sciences Islamabad. The period of study spanned from March 2003 to March 2006.

Age and Sex Distribution

Age of the patients ranged between 03months to 76 years. Their mean age was 30.8±1.20 years. Male patients in the study were 137, while female patients were 113. The difference between number of male and female patients was not significant ($p>0.05$) according to chi square $x=2.304$.

Male to females ratio was 1.21:1 (Table No. 5a).

Clinical Features

Symptoms

Fever was the most common symptom at the time of presentation in patients of HP. It was documented in 163 (65.2%) patients, with variable degree. Generalized weakness was the next common symptom occurring in 139 (55.60%) patients, followed by diarrhea, in 78 (31.20%, cough, in 62 (24.80%) and bleeding in 57 (22.80%) patients in the form of epistaxis, purpuric spots, bruises and bleeding per rectum (Table No. 5b).

Physical Findings

Pallor was found in majority of patients, (219, 87.60%). Splenomegaly was present in 93 (37.20%), hepatomegaly in79 (31.60%) and lymphadenopathy in 54 (21.62%) patients. Jaundice was found in 31 (12.40%), bruises and purpuric spots in 29 (11.60%) and ascites in 15 (6%) patient (Table No. 5c).

Table # 5(a): Age and sex distribution

Age range	3 Months – 76 Years.
Mean age (years)	30.80±1.20
Number of Males	137
Number of Females	113
Sex Ratio	1.21:1

Table# 5(b): Symptoms in patients of HP

SYMPTOMS	Number	Percentage
Fever	163	65.2
Generalized Weakness	139	55.6
Diarrhea	78	31.2
Cough	62	24.8
Bleeding	57	22.8

Table # 5(c): Physical signs in patients of HP

Features	Number	Percentage
Pallor	219	87.6
Splenomegaly	93	37.2
Liver	79	31.6
Nodes	54	21.62
Jaundice	31	12.4
Bruises		

Hematological Parameters in Patients of HP

Hemoglobin

Mean Hb in all the patients with HP was 7.60 ± 0.16gm/dl while its range was between2.7 -17.6.gm/dl .Out of 250 patients 74 had Hb less than or equal to 6 gm/dl and thus they were severely anemic.165 patients had Hb between 7 -12 gm/dl, showing mild to moderate anemia. Only 11 patients had Hb in the normal range, that is, between 13-18 g/dl No patients had Hb more than18 gm/dl .In other words anemia was more prevalent in our patients (Table No. 6).

Total leucocytes Count

Mean TLC in all the patients was $6.42\pm0.37x10^9$/L, while its range was between 1.1 – $78x10^9$/L.Eighty five patients had leucopenia having TLC less than or equal to 4.0 $x10^9$/L. One hundred and thirty nine patients had TLC between $5\text{-}12x10^9$/L,that is, in the normal range and only 26 patients had TLC above $12x10^9$/L showing leucocytosis. In other words most of the patients had normal white cell count (Table No. 6).

Platelet Count

Mean platelet count was $127.42\pm6.54x10^3$/ul,while its range was between 3.0-$619x10^3$/ul.Six patients had platelet counts below or equal to $10x10^3$/ul showing severe thrombocytopenia. One hundred and seventy five patients had Platelet count between 11-150.$x10^3$/ul, showing mild to moderate thrombocytopenia. Sixty four patients had platelet count between $150\text{-}450x10^3$/ul, that is, in the normal range. Only 5 patients had Platelet count more than 450 $x10^3$/ul showing thrombocytosis. In other words thrombocytopenia was more prevalent in our patients (Table No. 6).

RBC Morphology

Microcytic hypochromic blood picture was present in 19 (7.6%) patients. Hypochromia alone was present in 15 (6%). Macrocytosis was seen in 71 (28.4%) patients Normocytic normochromic blood picture was observed in 145 (58%) patients. Anisocytosis, poikilocytosis, nucleated RBCs, polychromasia, RBC fragmentation, basophilic stippling, were other important findings in peripheral blood

Table # 6 **Hematological parameters in patients of HP**

Hemoglobin : gm/dl			
Mean	7.60 ± 0.16		
Range	2.7 – 17.6		
	Hb:	No. of patients	%
Severe Anemia	≤ 6	74	29.60
Mild to Moderate	7 – 12	165	66.0
Normal	13 – 18	11	4.40
Leucocyte Count : x 10^9 / L			
Mean	6.42 ± 0.37		
Range	1.1 – 78		
	TLC:	No. of patients	%
Leucopenia	≤ 4	85	34.0
Normal	4 – 12	139	55.60
Leucocytosis	> 12	26	10.40
Platelet count: x10^3/ul			
Mean	127.42±6.54		
Range	3.0 – 619		
	Platelets:	No. of patients	%
Severe	≤ 10.0	06	2.40
Mild to Moderate	10 – 150	175	70.0
Normal	150 – 450	64	25.60

Etiological Break down in 250 Patients of Hemophagocytosis

Non malignant hematological conditions (NMHC) constituted the largest group and was comprised of 142(56.80%) patients. The next largest group was that of infections 62(24.80%) followed by storage disorders 11 (4.4%) and malignant hematological condition MCH 11 (4.4%). In the miscellaneous group, HP was present 21 (8.4%) patients. Three patients with SLE were also dignosed in our study. All of these patients had reactive (benign) haemophagocytosis. None of the patient had malignant histiocytosis. Etiological breakdown is given in (Table No.7) Figure 3.

Table# 7 Etiological break down of HP

Etiology	Number	%
Non Malignant Hematological Conditions	142	56.8
Infections	62	24.8
Storage Disorders	11	4.4
Malignant Hematological Conditions	11	4.4
Miscellaneous	21	8.4
Auto immune Disorders (SLE)	3	1.2

In the NMHC group megaloblastic anemia ranked on the top having HP in 61(24.40%) patients .Two patients presented as HPS. In infection associated HP, viral infections ranked on the top, 17 (6.8%).Nine patients presented as HPS with depression of cell lines. Two patients with malaria, three patients with visceral leishmaniasis, two patients of tuberculosis, one patient with enteric fever also presented with hemophagocytic syndrome. Among the patients of malignant hematological conditions one patient of NHL presented as HPS, with severe degree of hemophagocytosis resulting in peripheral cytopenia. Detailed etiological break down

Table # 8 Etiological distribution with grades of intensity of HP

	Grade I		Grade II		Grade III	
	No.	%	No.	%	No.	%
NMHC 142	51	20.4	64	25.6	27	10.8
Aplastic anemia	06	2.4	3	1.2	—	
IDA	10	4.0	4	1.6	—	
IDA ,MB	6	2.4	8	3.2	2	0.8
E.M.Hypo	2	0.8	2	0.8	—	
Hemolytic Anemia	8	3.2	4	1.6	—	
ITP	6	2.4	5	2.0	3	1.2
MB	9	3.6	31	12.4	21	8.4
MDS	1	0.4	3	1.2	1	0.4
Mega Hypo	1	0.4	—		—	
Polycythemia	2	0.8	—		—	
SA	—		1	0.4	—	
TTP	—		1	0.4	—	
LEo	—		1	0.4	—	
CDA	—		1	0.4	—	
MHC 11	7	2.8	3	1.2	1	0.4
ALL	1	0.4	—		—	
AML		0.4	—		—	
CML	1	0.4	1	0.4	—	
H.D	1	0.4	1	0.4	—	
MM	1	0.4	—		—	
NHL	2	0.8	1	0.4	1	0.4

Table # 8 continued

	Grade I		Grade II		Grade III	
	No	%	No	%	No	%
INFECTIONS 62	10	4.0	31	12.40	21	8.40
STORAGE 11 DISORDERS	8	3.2	3	1.2	—	
Gaucher's disease	6	2.4	2	0.8	—	
Niemann Pick disease	2	0.8	1	0.4	—	
MISCELLANIOUS 21	12	4.8	9	3.6	—	
Carcinoma stomach	1	0.4	—		—	
CHS	—		1	0.4	—	
CUD	6	2.4	4	1.6	—	
Hypersplenism	4	1.6	3	1.2	—	
Lead Poisoning	1	0.4	—		—	
Sarcoidosis	1	0.4	—		—	
SLE 3	—		3	1.2	—	

Infection Associated Hemophagoytosis

This was the second largest group showing HP in 62(24.8%) patients. Detailed etiology of infection associated HP with different grades of intensity is given in Table 9. Viral infections were on top of the list 17 (27.41). In this group, EBV, CMV, and adenovirus were the main etiological agents. Nine patients presented as hemophagocytic syndrome with peripheral cytopenia, liver dysfunction, coagulopathy and organomegaly. Other etiological factors in this group were enteric fever, tuberculosis, malaria, visceral lieshmaniasis,and brucellosis.

Most of the patients with infection associated HP fell into either grade II, 31 (50%) or grade III, 21 (33.87%) HP with increased intensity .Only 10(16.12%) patients showed grade I or mild intensity of HP. In other words severity of HP was more in infection associated cases.

Table # 9 Infection Associated HP=n 62 (24.80%)

Etiology	No	%	Grade I	Grade II	Grade III
Viral	17	27.41	3	10	04
Enteric	13	20.96	2	05	06
T.B	09	14.51	2	04	03
Malaria	08	12.90	-	03	05
Visceral leishmaniasis	09	14.51	01	05	03
Brucellosis	03	4.83	01	02	-
Miscellaneous	03	4.83	01	02	-
Total	62		10	31	21

Bone Marrow Findings in Patients of HP

In the majority of patients, 152 (60.80%), bone marrow was hypercellular. In 45 (18.20%) the cellularity was reduced. It was normocellular in 53 (21.2%) patients. Erythropoiesis showed megaloblastic features in 79 (31.60%) patients. The rest of the patients had normoblastic bone marrow, 171 (68.4%), Erythropoiesis was increased in 122 (48.8%) and decreased in 73 (29.2%) patients. In the rest of the patients it was active. Myelopoiesis was normal in 79 (31.60%) while it was decreased in 61 (24.4%) patients; 110 (44%) had increased cellularity. Megakaryocytes were normal in 131 (52.4%) patients while they were increased and decreased in 49 (19.6%) and 70 (28%) patients, respectively. Iron overload was found in the majority of the patients, 207 (82.8%), while it was absent and decreased in 19 (7.6%) and 14 (5.6%) patients, respectively. In the rest of the patients, 10 (4%), it was normal (Table No.10).

Intensity of Haemophagocytosis in the Bone Marrow

Out of 250 patients, 88 (35.2%) patients showed mild or grade I haemophacytosis by criteria described in material and methods. Moderate or grade II HP was observed in the majority of patients 113 (45.2%) while 49 (19.6%) patients had grade III of H.P. None of our patients had grade IV HP (Figure 4).

The histiocytic proliferation consisted of mature-appearing cells with low nuclear cytoplasmic ratio, inconspicuous nucleoli and coarse chromatin pattern. In the majority of the patients, phagocytosed red cells were present, often in association with nucleated red cells or normoblasts, with or without platelets and cells of myeloid series. Ingested pigment for example, hemosiderin was also present in same of the patients. Aggregates of histiocytes and lymphocytes were also found in bone marrow smears in some of the patents.

Table # 10 Bone marrow findings in patients of HP

Cellularity	Patients	%	Myelopoiesis Patients	%	Megakaryocytes	Patients	%
Increased	152	60.8	110	44	Normal	131	52.4
Decreased	45	18	61	24.4	Increased	49	19.6
Normal	53	21.2	79	31.6	Decreased	70	28

Erythropoiesis	Patients	%	Iron staining	Patients	%
Normoblastic	171	68.4	Increased	207	82.8
Megaloblastic	79	31.6	Absent	19	7.6
Increased	122	4808	Decreased	14	5.6
Decreased	73	29.2	Normal	10	4

Features in Bone Marrow Trephine Biopsy Sections

The bone marrow trephine biopsy was performed in 85 cases with variable degree of haemophagocytosis and fibrosis . Abnormal infiltrates was found in 50 (20%) patients. The trephine biopsy sections confirmed the findings of aspirate smear examination including HP.

Variable degree of fibrosis was observed in sections stained for reticulum stain. No fibrosis was observed in 16(18.82%) patients while the majority of the patients had moderate (grade 2) fibrosis 37(43.52%). Mild fibrosis was observed in 29(34.11%) Only 03(3.52%) patients showed grade III fibrosis. Tuberculous granulomas were present in 3 patients of disseminated tuberculosis (Table No.11).

Table # 11 Bone Marrow Fibrosis in Patients with Hemophagocytosis.

Grade of	Grade	Number	%
Fibrosis	0	16	18.82
Mild	I	29	34.11
Moderate	II	37	43.52
Severe	III	03	3.52

Grade I (Mild) Hemophagocytosis

This was the second largest group showing haemophagocytosis 88 (35.20%). The majority of the patents with this grade of HP belonged to Non-malignant hematological conditions (NMHC) 51 (20.4%). Iron deficiency anemia cases were on top of the list with mild intensity seen in 10 (4%) cases followed by megaloblastic anemia, 9 (3.6%), hemolytic anemia 8 (3.2%), Aplastic anemia 6 (2.4%) IDA together with megaloblastic anemia 6 (2.4%).

Infection associated HP was the second largest group in this category, 10 (2.8%) followed by Malignant hematological conditions 7 (2.8%) and Storage disorder 8 (3.2%) Other important diseases showing mild degree of HP were hypersplenism, and chronic underlying disorders (Table No.8).

Mean, HB, platelet count and total leucocyte count in this group were 8.61gm/dl 7.99.x10.9/L, 168.07x103/ul respectively. In other words anemia prevailed in patients with mild HP.

Grade II (Moderate) HP

This was the largest group showing moderate degree of HP, 113 (45.2%). Non-Malignant Hematological Conditions again constituted the bulk of cases, 64 (25.6%). Magaloblastic anemia ranked on the top occurring in, 31 (12.4%) patients. One patient of MB presented as HPS other important conditions were IDA with MB, 8 (3.2%), IDA alone 4 (1.6%) and ITP, 5 (2.0%). There were 3 (1.2%) patients of MHC that is, CML, H.D and NHL. The next largest group was that of infections 29 (11.6%) with varied etiological factors. Other important etiologies in this group were storage disorders, 3 (1.2%), and SLE 3 (2.11%) (Table No.8).

Mean Hb, TLC and platelet count in this group was 7.34 gm/dl ,5.74x10 9/L, and 115.64x103 /ul respectively . In other words anemia and thrombocytopenia was prevalent in patients with moderate hemophagocytosis.

Grade III (Severe) HP.

Severe degree of HP was found in 49 (19.6%) patients. Non malignant haematological conditions (NMHC) again constituted the largest group, 27 (10.8%) with severe HP. Patients with megaloblastic anemia were on top of the list, 21 (8.4%) in NMCH cases. Two patients of MA and one patient of MDS were associated with HPS. The second largest group was that of infections, 21 (33%) with varied etiology. Fourteen patients of infection presented as HPS. There was one patient of malignant hematologic conditions, NHL presented as HPS at the time of initial diagnosis. No patient of storage disorder or autoimmune disease was found in this category (Table No.8).

Mean Hb, TLC, and Platelet count was 6.37gm/dl, 5.09×10^9/L & 81.55×10^3/ul, respectively. In other words anemia and thrombocytopenia were common in this group with increased severity. Phenomenon of HP depicted in various diseases in Fig.5(a-v).

Hemophagocytic Syndrome=n=24(9.6%)

Out of 250 cases of HP in the present study, 24 (9.6%) presented as Hemophagocytic syndrome having varied underlying disorders. Most of these patients had severe (grade III) degree of HP, that is, 19 patients, followed by patients with moderate intensity (grade II). None of the patient had mild (grade I) intensity (Table No.11).

Infection associated HPS, ranked on the top with 18(7.2%) patients. Mostly viral infections, 9 (3.6%), gave rise to HPS, having severe intensity of HP, in the majority of patients. Enteric fever, tuberculosis, malaria and visceral leishmaniasis were other disease associated with HPS.

In the NMHC group, 03 patients of megaloblastic anemia had HPS at the time of initial presentation, while one patient of MDS, showed HPS. All of these patients had pancytopenia as initial presentation. A case of NHL also presented as HPS, prior to initiation of chemotherapy. In the autoimmune diseases group, one patient of SLE, presented as HPS at the time of initial diagnosis.

All of these patients of HPS had pancytopenia/bicytopenia, organomegaly, and most of the clinicopatological and biochemical derangements attributd to HPS, such as liver dysfunction, hyperferritinemia, coagulopathy, with bone marrow histiocytic hyperplasia and hemophagocytosis.

Table # 12 Etiological Break down of HPS -n = 24 (9.6%)

Etiology	Number	%	Grade I	Grade II	Grade III
Infections	18	7.2			
Viral	9		--	2	7
Enteric fever	3		--	1	2
Tuberculosis	3		--	--	3
Malaria	2		--	--	2
V. Lesihmaniasis	1		--	1	--
NMHC	4	1.6	--		--
M.A	3		--	1	2
M.D.S	1		--	--	1
MHL (NHL)	1	0.4	--	--	1
Autoimmune	1	0.4	--	--	1

Age Groups with Different Grades of Intensity of Hemophagocytosis

In the present study only eight (3.2%) infants between 0-2 years age group were present, the majority of them having moderate intensity of HP. Patients of 3-15 years age group, 53 (21.20%) children were included, mostly showing grade II, 26 (49.05%), intensity. In adult age group, that is, 160-76 years, there were 189 (75.60%) patients, mostly showing moderate intensity of HP. In other words number of patients in the pediatric age group (0-15 years), were less (24.40%) as compared to adults patients. One of the reasons could be that the majority of the patients subjected to bone marrow examination were adults. The pattern of intensity of HP in the BM however was the same in both the groups, mostly showing moderate degree of HP.

The phenomenon of HP in children has been mostly documented either in the inherited conditions such as Chidiak higashi syndrome, Gricielli syndrome, X–Linked lymphoproliferative disorder and familial hemophagocytic lymphohistiocytosis (Larroche 2004) or in patients with different underlyng infections (Imashuku 1996). In the case of adults, it has been mostly reported in infections as well as in malignancies and non-malignant hematological conditions (Strauss et al. 2004).

There is, however, overlapping of age groups as far as etiology is concerned. In the present study most of the patients in the pediatric age group were infection-associated. No patient of inherited HP was found in the present study There was a mixed pattern in adult age group as far as etiology is concerned.

In the present study it was found that the intensity of HP was dependent upon the underlying etiology rather than the age groups. Most of the patients had grade II intensity of HP. Cases of HP reported in the past having increased intensity either had infections (Risdall et al 1984) or malignancies (Suster et al 1984, Strauss et al 2004) . In the present study too, increased intensity of HP as shown in the results is by and large not age-dependent, but mostly etiology related, such as infections and megaloblastic anemia (Table No.12).

Table # 13 Age groups with grades of intensity of HP

Age Group Years	Grade1 No	%	Grade II No	%	Grade III No	%	Total No	%
0-2	5	62.5	3	35.5	--	--	8	3.2
3-15	18	33.96	26	49.05	9	16.98	53	21.2
16-35	33	33.67	48	48.97	17	17.34	98	39.2
36-55	21	36.2	25	43.1	12	20.68	58	23.2
56-76	11	33.33	11	33.33	11	33.33	33	13.2

Mean Hb, TLC, and Platelet count in different age groups of HP

Mean Hb and platelets count were low in children 0 - 5 years) and adults more than 15 years of age. There was however no fixed pattern of fall of Hb and platelets according to the different age groups. Total white cell count was however in the normal range in all age groups (Table No.13).

In other words anemia and thrombocytopenia were prevalent in all age groups, and it was not dependent upon particular age group. As far as our knowledge goes, no study has been conducted in the past .in which phenomenon of HP causing peripheral cytopenia has been investigated with reference to multiple age groups.

Investigation on a larger scale is required to elicit the correlation of HP with different age groups and their effect on hematological parameters.

Table # 14 Mean Hb, TLC and platelet count in different age groups of HP

Age Groups years	Hb gm/dl	TLCx10^9/L	Platelets x 10^3/ul
0 – 2	7.65±0.54	7.70±0.92	102.78±23.49
3 – 1	7.14±0.28	7.21±1.54	148.73±18.23
16 – 35	7.59±0.26	6.21±0.35	120.68±10.00
36 – 55	7.44±0.35	6.13±0.54	126.19±1.63
56 – 76	8.38±0.53	5.88±0.58	113.76+11.12

Mean Hb, TLC, Platelet Count according to grades of intensity HP

Mean Hb(gm/dl) in grade I, II and III was 8.613±0.31, 7.34±0.19 and 6.37±0.31, respectively. Mean TLC ($x10^9$/L) was 7.99±0.92, 0.92, 5.74±0.31 and 5.09±0.51, respectively, according to grades of intensity. Mean platelet ($x10^3/\mu l$) count was 168.07±14.32, 115.64±7.74 and 81.55±5.39, respectively.

As a result of increased intensity of HP in grade III, group mean Hb (6.37gm/dl) and platelet count (81.55x103/L) were low as compared to other groups. The mean Hb of grade II was significantly decreased as compared to mean of grade I (P<.01), the mean Hb of grade III was significantly decreased as compared to grade I (P <.001), and grade II (P<.01) .

In the case of TLC, the mean of grade II was significantly decreased as compared to grade I(P< .05) .Mean TLC of grade III was significantly decreased as compared to grade I(P< .01),while the difference of mean was not significant between grade II and III (P> .05).

The mean platelet count of grade II was significantly decreased as compared to grade I (P<.01), the mean platelet count of grade III was significantly reduced as compared to grade I (P<.001) and grade II (P<.001).

The present study thus reveals that the main effect as a result of increasing intensity of HP was on Hb and platelet count which has been proved statistically (Table No.14).

Table # 15 Mean Hb, TLC, Platelet count in different Grades of HP

GRADES	Hb gm/dl	TLC x 10^9/L	PLT.Count x 10^3/µl
I	8.61±0.31	7.99±0.92	168.07±14.32
II	7.34±0.19a**	5.74±0.31a*	115.64±7.74a**
II	6.37±0.31b***c**	5.09±0.51b**	81.55±5.39b***c***

Grade I vs II (a) P<.05*

Grade I vs III (b) P<.01**

Grade II vs II (c) P<.001***

DISCUSSION

In the present study variable degrees of hemophagocytosis were observed in 250 patients subjected to bone marrow examination having diverse underlying etiologies. Hemophagocytic histiocytosis, although an uncommon condition, has been reported in several clinical situations mainly as a reactive phenomenon, sometimes with fatal outcome (Larroch 2003). This phenomenon was first recognized by Scott and Robb-smith (1939).The term initially used for this process was histiocytic medullary reticulosis and it was invariably believed to be neoplastic.

Rappaport (1966) introduced the term malignant histiocytosis and described morphological difference between benign and malignant histiocytes. The cytological features described by Rappaport in malignant histiocytosis were characterized by atypical cells, with pleomorphism, large irregular nucleoli, mitotic figures, isolated phagocytosis by poorly differentiated neoplastic histiocytes.

Histiocytic hyperplasia with prominent hemophagocytosis was described by Risdall et al. (1979), as a benign, reactive phenomenon for the first time in viral infections. They used the term hemphagicytic syndrome to describe clinical and biochemical derangements in their patients. The macrophages found in the bone marrow smears of these patients were mature, showing cytologically benign appearance with a striking phagocytic activity. Later on this phenomenon was reported in several non-viral infections such as tubrculosis, leishmaniasis, fungi, and gram positive and negative infections (Suster et al 1985).

Similarly it has been described in a diverse group of disorders with varied etiologies such as gastric carcinoma, non- Hodgkin's lymphoma. Hodgkin's disease, acute and chronic leukemia and non-malignant hematological conditions (Larroche 2004).
Strauss et al. (2004) extensively described the risk factors underlying the causation of this condition and tried to establish the correlation of bone marrow cellularity with depression of blood cell counts.

Inspite of many case reports on existence of hemophogocytosis in various benign and malignant disorders, the literature is however scanty on correlation between intensity of HP and its effect on hematological parameters. The present study deals with etiology of HP and the effect of intensity of this phenomenon on hematological parameters.

The present study showed variable degrees of reactive hemophagocytosis that is, mild, 88 (35.20%), moderate, 113 (45.20%) and severe, 49 (19.60%). In other words, the majority of our patients had moderate or grade 11 intensity of HP. The study done by Strauss et al. (2004) however shows that patients with mild HP were more than moderate intensity patients.

Most of the patients belonged to non malignant hematological conditions 142, (56.6%) followed by infections 62(24.8%), storage disorders 11 (4.4%) malignant hematological conditions 11 (4%), autoimmune disorders 3(1.2%), chronic underlying disorders, 10 (4.0%) and hypersplenism, 7 (2.80%). These patients showed variable degrees of HP in the bone marrow, that is, mild, moderate and severe, as a reactive phenomenon .None of these patients had malignant histiocytosis.

As mentioned earlier non-malignant hematological conditions (NMHC) ranked on the top in the list of etiology showing hemophagocytosis in the bone marrow. Megaloblastic anemia was the commonest cause in this category 61 (24.4%), followed by patients of mixed deficiencies, 16 (6.4%), ITP, 14 (5.6) hemolytic anemia, 12 (4.8%) IDA, 14 (5.6%). Aplastic anemia, 9 (3.6%) and MDS, 5 (2.0%). Other important conditions in this group were polycythemia, megakaryocytic hypoplasia, sideroblastic anemia and thrombotic thrombocytopenic purpura (TTP).

Wang et al. (2004) reported a case of hemophagocytosis associated with MDS and exacerbated by GM-CSF treatment. They proposed that myeloid growth factors could be detrimental in patients with MDS associated hemophagocytosis. Another case of MDS with HP was reported by Nakada et al. (2001). The patient also had nontuberculous atypical mycobacterium infection and developed progressive

Kfoury et al. (2002) described a case of reactive hemophagocytic syndrome associated with TTP during therapeutic plasma exchange and proposed that cytokine-induced reactive HPS can develop in TTP patients due to lymphocyte activation during treatment. As mentioned earlier in the present study there was also one case of TTP, showing mild intensity of HP.

Most of the patients of the NMHC group showed grade II (moderate) intensity of HP 64(25.6%). The rest of the patients showed either mild 51 (20.4%) or severe 27(10.8%) intensity.

Megaloblastic anemia deserves special mention. It was the most frequent underlying disorder in the NMHC group .Patients with megaloblastic anemia (MA) either showed moderate 31(12.4%) or severe 21(8.4%) degree of HP. Three patients presented as HPS. Only 9(3.6%) patients had mild intensity of phagocytosis.

In megaloblastic anemia there is frequently an element of dyserythropoiesis with hemophagocytosis but most of the studies do not emphasize the importance of this phenomenon in the bone marrow along with dyserythropoiesis, ineffective myelopoiesis and intramedullary destruction of marrow cells.

Iqbal et al. (2003) studied the morphological features of megaloblastic anemia in the bone marrow and emphasized the importance of HP along with dyserytheropoiesis as an important cause of pancytopania. In another study regarding the etiological break up of pancytopenia, Iqbal et al. (2001), highlighted the significance of HP in the bone marrow in patients of MA. We suggest that MA with HP should be included in the list of causes of Pancytopenia and HPS in the standard text books of hematology and other scientific literature which is so far quite scarce.

What triggers this phenomenon particularly in cases of megaloblastic anemia is still not known. Perhaps dyserythropoiesis with exaggerated intramedullary destruction of bone marrow precursor cells stimulates monocyte macrophage system, along with T-lymphocytes resulting in elaboration of cytokines. It is suggested that the role of

increased frequency of this phenomenon in the bone marrow particularly in patients of megaloblastic anemia.

In the present study patients with immune thrombocytopenic purpura (ITP) also showed mild 6 (2.4%) or moderate 5 (2.0%) degrees of H.P. Only 3 (1.2%) patients had the severe degree of HP. In most of these patients underlying viral infection with antibody formation was presumed to be the cause of platelet destruction and HP.
We also had one patient with megakaryocytic hypoplasia and 03 patients with erythro- megakaryocytic hypoplasia. It is presumed that in these patients the underlying infection, particularly viral infection, may be the cause of bone marrow depression and HP.

Patients with iron deficiency anemia (IDA) alone or IDA with megaloblastic anemia also showed variable degrees of HP. Most of the cases of IDA fell in the grade I category showing a mild degree of HP. The literature is again scanty regarding the presence of HP in such cases.

Twelve patients with hemolytic anemia mostly belonging to thalassemia syndrome showed either mild or moderate degrees of HP. Marten et al. (1975) studied erythrophagocytosis in five patients with hemolytic anemia. According to their study, the major site of destruction of aged red cells is bone marrow. They compared the bone marrow findings of healthy individuals with that of hemolytic anaemia and arrived at the conclusion that the normal phenomenon of erythrophagocytosis is enhanced by hemolysis and dyserythropoiesis.

Nine patients with plastic anaemia were also diagnosed showing either mild, 6 (2.4%), or moderate 3 (1.2%), intensity of HP. Underlying infection because of neutropenia and immunosuppression was thought to be the cause of HP in aplastic anemia. Five patients with MDS were also diagnosed in this group. One patient with grade III intensity presented as HPS. Wang et al. (2004) recently reported a case of MDS associated with HPS. The patient had neutropenia and the bone marrow aspirate demonstrated hemophagocytosis. GM-CSF and G-CSF were found to be elevated in

It should be noted that literature is quite scanty as far as the occurrence of this phenomenon in NMHC is concerned. In our study it was the largest group showing HP. In future, research should be focused to highlight the pathogenesis and effect on hematological parameters of this phenomenon in NMHC particularly in megaloblastic anemia patients.

In the present study, patients with different underlying infections were the second largest group resulting in reactive histiocytic hemophagocytosis 62 (24.80%) with different grades of intensity. In the present study, 17 patients with viral infection showed variable intensity of HP. Risdall et al. (1979) studied 19 patients with active viral infection. The bone marrow smears in those cases showed histiocytic hyperplasia with prominent HP. Most of those patients presented as hemophagocytic syndrome. Most of those patients recovered with supportive treatment.

Before the study of Risdall et al. (1979), the association of viral infections with reversible histiocytic proliferation showing HP was not established, although it was suggested as a cause of transient histiocytosis by Chandra et al. (1975). Such cases were mostly considered to be essentially neoplastic with fatal outcome. Apart from the study of Risdall et al, other workers have also isolated different viral agents including herpes simplex (Kalderon et al 1979), Epstein Barr virus, (Sullivan et al 1985) CMV (Weller 1971) HIV (Tracy et al, 1987) Parvovirus B19 (Boroucroff et al 1990) as a cause of virus associated HPS.

Virus-associated HPS has been extensively studied, particularly in relation to EBV. Sullivan et al. (1985) described VAHS in two children. Neither of these patients had underlying immunodeficiency and both recovered from the disease by giving supportive therapy and antiviral agents. In each patient, evidence for primary EBV infection was documented with a typical humoral immune response, including IgM antibody directed against virus capsid antigen. EBV was demonstrated in lympho-reticular tissue by electron microscopy and molecular hybridization studies. That study however did not throw light on possible role of cell of immune system and cytokines in the causation of HP.

Ohga et al. (2001) studied the role of T-Cell in chronic active EBV infection. EBV and cytokine gene expression was quantified by use of real time PCR among 6 patients. Fractionated CD3+HLA DR+ cell from patients with chronic active EBV infection contained higher copies of EBV DNA than CD3 + HLA –DR- cells. Quantitative PCR for cytokines revealed that interferon gamma, IL 2 , IL -10 ,and TGF beta genes were expressed at higher levels in HLA-DR+ than in HLADR- T-cell. These results suggested that activated T-cells in chronic active EBV infection expressed high levels of EBV DNA and both Th1 and Th2 cytokines. EBV-infected T-cells may contribute to the unbalanced cytokine profiles of infections mononucleosis.

Sato et al (2004) described a case of EBV-associated HPS in a 19 year old female with a fatal outcome. They found that HPS resulted from hyper-activation of T cell, and macrophages with overproduction of cytokines, including INF gamma, TNF - alpha and, IL -6. EBV genomes were detected by the PCR. Theoe results suggested that EBV-infected CD8+T cells in tissues like peripheral blood lymphocytes, lung, kidney, brain and liver may have an integral role to play in the pathophysiology of the HPS. They further suggested that in order to cure this fatal disease, it is necessary to target and control the mature EBV infected T-cell.

Imashuku (2002) studied the clinical features and treatment strategies of EBV-associated HPS. According to his study, patients with EBV having HP, the EBV infected T-cell or natural killer (NK) cells are mostly mono-or oligoclonally proliferating, with hypercytokinemia resulting in HP and cellular damage and dysfunction of various organs In terms of treatment, special therapeutic measures are required to control the cytokine storm generated by EBV and to suppress proliferating EBV genome containing cells, because the clinical coarse is often fatal and results in a poor outcome.

In the present study, out of 17 patients with viral infection, 10 patients showed grade II and 4 had grade III intensity of HP. Only 3 patients had mild HP. In other words, the majority of the cases with viral infection had increased severity of HP. Viral

Hemophagocytic syndrome was found in 18 patients. All of these patients presented with bicytopenia or pancytopenia .Hepatosplenomegaly, liver dysfunction, hyperferritinemia, lipid derangement, coagulation disturbance were other findings of HPS. The bone marrow was mainly hypercellular or normocellular in these cases of HP. The macrophages in the bone marrows of all these patients were benign looking with low N/C ratio, inconspicuous nucleoli and abundant cytoplasm .Erythrophagocytosis was mianly present along with phagocytosis of leukocytes and platelets in some of the patients.

Hemophagocytosis has been observed in other viral infections as well. Takeoka et al (2001) presented a case of VAHS caused by rubella and Varicella zoster virus as dual infection in patients with idiopathic thrombocytopenic purpura. The had liver dysfunction, coagulopathy, hyperferritinemia and thrombocytopenia. Bone marrow examination revealed many atypical lymphocytes and histiocytes with hemophagocytosis. On laboratory examination they were seropositive for both the viruses IgM. The simultaneous rubella and VZV infection might have been related to VAHS pathogenesis. She was treated with prednisolone and gamma globulin therapy and recovered completely.

Isobe et al (2004) reported hemophagocytosis in a 40 year old patient suffering from lymphoma having parvo B 19 virus infection. Sokanotu et al (2002) presented a case of SLE complicated by Cytomegalovirus induced hemophagocytic syndrome and colitis. In this patient fever, progressive pancytopenia and intestinal bleeding were observed. Bone marrow aspiration showed an increase in mature histiocytes with HP. A large number of CMV antigen positive leukocyte were detected, suggesting an active CMV infection.

Luppi et al (2002) reported the occurrence of human herpesvirus (HHV)-8 primary infection in an adult male kidney recipient with HP. Four months after transplantation the patient developed kaposi sarcoma with progressive and severe peripheral cytopenia in the presence of hypercellular marrow and hemophagocytosis. HHV-8 nuclear antigen was detected in immature proginator cells from the bone marrow

and co-workers ,mostly having hemophagocytosis in the bone marrow with peripheral cytopania .(Karti et al 2004).

A number of cases have been reported with hemophagocytosis in relation to hepatitis A. Watanabe et al. (2002) reported hepatitis A with hemophagocytosis in an 18 year old female. The bone marrow smears revealed proliferation of mature histiocytes ingesting platelets and erytherocytes. Gluocorticoid pulse therapy was started with favorable out come.

A case of Hepatitis A with co existent Hepatitis C virus infection presenting as VAHS was diagnosed by Shyong and coworkers (1995). The 23 year old patient had fever, jaundice with progressive anemia, thrombocytopenia and hepatosplenomegaly. Bone marrow biopsy revealed hemophagocytosis. Despite aggressive supportive treatment with parallel steroids the patients died of disseminated intravascular coagulopathy with gastrointestinal bleeding. Necropsy of the liver showed histiocyte aggregation in the portal area with hemophagocytosis. In the present study however, there was no patient with hepatitis A.

Most of the patients with viral infection reported so far presented with HPS. In the present study, also the majority of the patients with viral infection fulfilled the criteria of HPS. Peripheral cytopenia with increased intensity of HP was an important finding in these patients, apart from other clinical and biochemical derangements.

Nine patients with tuberculosis were diagnosed in the present study. Six patients had pulmonary and three patients had miliary tuberculosis. Two patients with disseminated TB had granuloma in the BM trephine biopsy specimens. AFB culture in the BM was positive in 2 patients. Three patients presented as hemophagocytic syndrome.

Campo et al (1986) reported three patients of HPS associated with tuberculous sepsis. Benign histiocytic proliferation with striking hemophagocytosis was present in all three patients. Fujiki et al (2003) reported a case of HP in a 70 year old female caused

bacilli by acid fast stains .Histiocytic hyperplasia and hemophagocytosis was also present. 1L -18, sICAM, Svcam-1 were elevated. They concluded that 1L -18, and adhesions molecules mentioned above play important roles in the pathogenesis of tuberculosis associated hemophagocytic syndrome and correlate with the disease activity.

Goto et al. (2001) reported a case of disseminated tuberculosis-associated HPS in a 40 year old man. Peripheral blood counts decreased rapidly and bone marrow aspiration revealed HP by macrophages. After plasma exchange and continuous hemodilution, hypercytokinemia and vital signs improved dramatically.

In the present study, patients with tuberculosis with increased intensity, grade III (3cases) had depression of cell counts, including a cases of miliary tuberculosis. We believe that 1L -18 as seen in previous studies plays an important role to induce peripheral cytopenia particularly by causing secretion of cytokines such as INF - gamma and TNF alpha.

Thirteen patients with enteric fever were diagnosed in the present study, with variable degrees of HP. Three patients presented as HPS with peripheral pancytopenia, organomegaly and liver dysfunction. One patient had coagulopathy. Erythropoiesis and granulopoiesis were depressed in these patients with histiocytic proliferation and prominent hemophagocytosis in the B.M. Blood culture was positive in 5 patients and bone marrow culture for salmonella typhi in 4 patients of enteric fever.Association of erythrophagocytosis with enteric fever is well known (Serck – Hanssen et al 1986). Risdall et al. (1984) also reported three patients with enteric having HPS.

Nine patients with visceral leishmaniasis were also present in the present study, mostly showing either grade 11 (5 cases) or grade III (3 Patients) HP. One patient presented as HPS. Patient having HPS had severe peripheral cytopenia. Erythropoiesis and myelopoiesis were depressed in this patient. Although an uncommon occurrence, association of visceral leishmaniasis with HPS is well known. Bruekaert et al. (1979) reported a case of fatal leishmaniasis causing severe hemophagocytosis in a renal

Tune (2004) reported a case of HPS as a life threatening complication of visceral leishmaniasis in a young boy. The 4 year old patient had high fever, hepatosplenomegaly and pancytopenia BM showed histiocytic hyperplasia with prominent hemophagocytosis, consistent with HPS. Another case was reported by Bhutani et al. (2002) in a 28 years old man who presented with fever, organomegaly and pancytopenia with reactive histiocytosis and severe hemophagocytosis. Erythropoiesis, granulopoiesis and megakaryopoiesis were markedly depressed.

Peripheral cytopenia in visceral leishmaniasis could be multifactorial. Hypersplenism, parasitic infiltration, bone marrow fibrosis and above all exaggerated HP are the important factors resulting in pancytopenia.

Eight (3.2%) patients with malaria were diagnosed in the present study. Three patients showed grade II and five patients had grade III HP. Four patients had vivax malaria, two had falciparum infection and two patients had mixed infections. Two patients with falciparum infection with grade III intensity presented as HPS with peripheral cytopenia, hepatosplenomegaly, liver dysfunction and hyperferritinimia. BM in both the patients was hypercellular with depression in erythroid series.

Hemophagocytic syndrome in association with malaria was reported for the first time in Pakistan by Anwar et al (1994). Presence of HP in malaria has been sparingly reported in the literature, perhaps because of its uncommon occurrence in the west. Zvulunov et al (2002) reported a case of falciparum malaria with hemophagocytosis resulting in pancytopenia. They proposed that systemic parasitic infections, particularly falciparum malaria, should be included in the differential diagnosis of pancytopenia and infection associated HPS. In the present study also, 2 patients of falciparum malaria had HPS and pancytopenia.

We also had three adult patients with brucellosis, one having mild HP and two patients had moderates HP. Serology was positive for burcella melitensis and abortis. None of the patient presented as HPS. Peripheral pancytopenia was found in one patient.

viral infection could be related to over production of cytokines such as TNF-alpha and INF gamma Bone marrow failure, hypersplenism, granulomas and hypercytokinemia, may result in peripheral cytopenia in such patients.

In the present study, 11 patients with malignant hematological conditions (MHC) were diagnosed. Most of the patients had grade I HP, 7 (2.8%). Only 3(1.2%) had moderate and, 1 (0.4%) had severe degree of HP. The need for applying standard cytologic criteria of malignancy in the diagnosis of histiocytic proliferations was emphasized by Byrne and Rappaport (1973) and Warnke et al (1975) in their studies of MH.

Esseltine et al (1983) described clinicopathological features in 10 patients with malignant histiocytosis. The value of bone marrow aspiration for the diagnosis was also discussed. All of 10 patients had anemia and thrombocytopenia. Mostly patients were neutropenic with peripheral monocytosis. In the bone marrow phagocytic macrophages were present and the cellular element phagocytosed generally corresponded to the peripheral cytopenia. In the present study however no case of malignant histioytosis was diagnosed.

Two patients with acute leukemia (ALL and AML) were diagnosed in the present study showing reactive HP of mild intensity in the bone marrow .Four patients with NHL with variable degree of HP were also diagnosed in the study .A case of NHL with grade III intensity presented as HPS. Two patients of CML and HD each were also present in this category.

Liang et al (1986) reported 03 patients with ALL and 2 patients with NHL showing reactive hemophagocytic histiocytosis. Monaharen et al (1981) reported four patients with HPS characterized by proliferation of mature macrophages and HP as a terminal complication of CLL. There are also reports in the past showing HP in NHL (Jaffe et al, 1983) and HD (Korman et al 1979), T-cell lymphoma (Chan et al 1983, Inove et al 1999) Imashuku et al, (1999), reported 7 paients of AML showing HP by leukemia blast cells with translocation, t (16,21) (p11, q22). These hemophagocytic blasts had abundant cytoplasm with irregular margins in addition to vacuoles and

Abe et al (2001) described two patients with B-Large cell lymphoma with HPS. One patient, BM showed proliferation of large CD 20 positive T cells and HP at presentation. The other patient had swelling of the kidneys and adrenals and HP in the bone marrow. The phagocytic cells were benign with low N/C ratio .In the present study cytology of patient of NHL with HPS, showed B-Large cell lymphoma. Four patients of intravascular lymphoma were diagnosed by Narimatsu and coworkers, (2004) in which bone marrow showed prominent HP, mainly erythrophagocytosis.

A case of NK/T-cell lymphoma of the larynx with HPS was reported by Nishi et al. (2004). BM examination showed severe HP. The hemophagocytic cells were benign and mature. A case of HPS associated with C8 positive T-cell chromic lymphocytic leukemia was described by Ando et al 2005. Bone marrow showed infiltration by CD8 positive T-lymphocyts along with increase in histiocytes and prominent HP. Serum concentrations of IL – 6, soluble 1L -2 receptor were elevated in the present study however there was no case of either CLL or NK/T-cell lymphoma. No patient of either intravascular lymphoma or N/K cell lymphoma was diagnosed in the present study.

Patients with acute and chronic leukemia and multiple myeloma in the present study had depression of normal hemopoietic tissue with mild to moderate hyperplasia of mature looking histiocytes having low N/C ratio, coarse chromatin pattern, inconspicuous nucleoli and abundant cytoplasm. The phagocytosed cells were mostly red cells, nucleated RBCs, with or without granulocytes and platelets. None of the patients showed cytological atypia in histiocytes. The process of histiocytosis with HP was essentially reactive. We did not have any patients of malignant histiocytosis.

A case of HP in non-secretary multiple myeloma was described by Savage et al (2004) A 70 year old women presented with pancytopenia associated with plasma cell infiltration of her bone marrow. The plasma cells showed phagocytosis of erythroid and granulocytic cells with progressive pancytopenia. In the present study one patient of multiple myeloma had mild intensity of HP.

Most of the investigators whose findings have been cited above suggested that histiocytic medullary reticulosis-like syndrome developing in patients with hematological malignancies was reactive to an underlying opportunistic infection (viral, bacterial, fungal) in the presence of immunosuppressive state due either to disease process, itself and or chemotherapy. It is presumed that infiltration of malignant cells in the bone marrow, hypersplenism, underlying infections with immunosuppression, hypercytokinemia with histiocytic proliferation and prominent HP could be the cause of peripheral cytopenia in MHC. The relatively few MHC patients in this study could be due to the fact that not many patients with underlying malignancies are referred to the hospital where the study was conducted, for bone marrow examination.

We also had one patient with adenocarcinoma of the stomach having HP of mild intensity in the bone marrow. Reactive HP with histiocytic hyperplasia has been observed in gastric carcinoma (James et al 1979) bronchogenic, and breast carcinoma (Marria – Padilla 1977) and its occurrence is believed to be the result of underlying immunosuppression.

It is important to distinguish reactive histiocytosis with HP from malignant histiocytosis complicating a pre existing malignancy, because chemotherapy is deleterious in former patients, while specific antimicrobial, anticytokine therapy, plasmapheresis and supportive treatment may save them (Jaffe et al 1983).

In the present study, 03 patients with autoimmune disease were included, all diagnosed as SLE. These patients were already diagnosed prior to bone marrow examination. One patient had signs and symptoms attributed to HPS. These patients showed moderate degree of HP.

Mori et al (2001), reported a case of SLE with bacterial salivary gland swelling, associated with HPS in which serial determination of cytokines were made. The patients revealed pancytopenia, high serum LDH, and ferritin with hypercytokinemia and prominent HP in the bone marrow. They concluded that aberrant production of cytokines 1L – 6, 1L – 1B and IL 18, might be involved in HPS in autoimmune

It is possible that association of HP with autoimmune disease is due to hypercytokinemia in the presence of immune deregulation and identification of this phenomena is important because steroid therapy alone will not result in the amelioration of underlying disease.

Age groups with different grades of Intensity of hemophagocytosis

In the present study only eight (3.2%) infants between 0- 2 years age group were present, the majority of them having moderate intensity of HP. Patients of 3-15years age group, 53 (21.20%) children were included, mostly showing grade II 26 (49.05%) intensity. In adult age group, that is, 16—76 years, there were 189 (75.60%) patients, mostly showing moderate intensity of HP. In other words the number of patients in the pediatric age group (0-15years) was less (24.40%) than the adult patients. One of the reasons could be that the majority of the patients subjected to bone marrow examination were adults. The Pattern of intensity of HP in the BM however was the same in both the groups, mostly showing moderate degree of HP.

The phenomenon of HP in children has been mostly documented either in inherited conditions such as Chidiak higashi syndrome, Griscielli syndrome, X–Linked lymphoprolipherative disorder and familial hemophagocytic lymphohistiocytosis (Larroche 2004) or in patients with different underlyng infections (Imashuku 1996). In the case of adults it has been mostly reported in infections as well as in malignancies and non malignant hematological conditions (Strauss et al 2004).

There is, however, overlapping of age groups as far as etiology is concerned. In the present study most of the patients in the pediatric age group were infection-associated. No patient of inherited HP was found in the present study. There was a mixed pattern in adult age group as far as etiology is concerned.

In the present study it was found that intensity of HP was dependant upon the underlying etiology rather than the age groups. Most of the patients had grade II intensity of HP. Cases of HP reported in the past having increased intensity either had infections (Risdall et al 1984) or malignancies (Suster et al 1984, Strauss et al 2004).

large not age dependent, but mostly etiology related, such as infections and megaloblastic anemia.

Mean Hb, TLC, and platelet count in different age groups of HP

Mean Hb and platelet count were low in children (0 - 15 years) and adults more than 15 years of age. There was, however, no fixed pattern of fall of Hb and platelets according to the different age groups. Total white cell count was, however, in the normal range in all age groups.

In other words, anemia and thrombocytopenia were prevalent in all age groups, and it was not dependent upon any particular age group. As far as our knowledge goes, no study has been conducted in the past in which the phenomenon of HP causing peripheral cytopenia has been investigated with reference to multiple age groups.

Investigation on a larger scale is required to elicit the correlation of HP with different age groups and there effect on hematological parameters.

Effect on hematological parameters and mechanism of peripheral cytopenia in patients of HP with different grades of intensity and etiology.

The present study reveals that the majority of the patients had increased severity of HP in the bone marrow. Grade II and Grade III patients together were (64.80%) as compared to grade I HP (35.50%) patients, with mild intensity. Most of the patients of grade II and III either belonged to NMHC or infection. According to the study of Strauss et al. (2004), to identify the risk factors of HP, the majority of the patients had mild (35.2%) degree of HP, followed by moderate (27.9%) intensity. In the present study however, patients with moderate intensity of HP were in the majority (45.2%).

Among the cases of NMHC, megaloblastic anemia ranked on the top, causing pancytopenia in majority of patients. In infection-associated HP, underlying viral infections, followed by enteric fever and tuberculosis, malaria and visceral

identified by Strauss et al (2004) were mainly various infections, followed by cardiovascular disease, and bleeding disorders.

HPS was found to be associated with 24 (9.6%) patients, mostly with different underlying infections showing grade III intensity in majority of patients. Such patients presented with peripheral cytopenia in addition to other clinicopahological and biochemical derangement.

Pancytopenia was present in 78, while bictyopenia was found in 92 patients Patients showing pancytopenia or bicytopenia mostly belonged to grade II or grade III groups of HP.

As a result of increased intensity of HP in the grade III group mean Hb (6.37gm/dl) and platelet count (81.55x103/L) were low compared to other groups. The mean Hb of grade II was significantly decreased compared to the mean of grade I (P<.01), the mean Hb of grade III was significantly decreased as compared to grade I (P <.001), and grade II (P<.01).

In the case of TLC, the mean of grade II was significantly decreased as compared to grade I (P< .05). The Mean TLC of grade III was significantly decreased as compared to grade I (P< .01), while the difference of means was not significant between grade II and III (P>.05)

The mean platelet count of grade II was significantly decreased compared to grade I (P<.01). The mean platelet count of grade III was significantly reduced as compared to grade I (P<.001) and grade II (P<.001).

The present study thus reveals that the main effect as a result of increasing intensity of HP was on Hb and platelet count, which has been proved statistically.

The mechanism of peripheral cytopenia has been studied by various workers in HP patients with reference to different underlying disease processes (Risdall et al 1979, Risdall et al 1984, Suster et al 1985, Karakukcu et al 2004, Strauss et al 2004). Risdall et al (1979) reported 19 patients with viral infection with HPS. According to them, the

finding was unclear. They suggested that viral agents could exert a direct effect on hemopoietic cells. CMV can be isolated from peripheral blood leucocytes and is known to be pantropic and same is true for infection hepatitis virus.

Agranulocytosis and aplastic anemia have been reported by Purtilo et al. (2002) in a familial lympho proliferative reactive syndrome associated with EBV infection. The abnormal coagulation parameters according to them were due to intravascular consumption resulting in thrombocytopenia in addition to platelet phagocytosis.

In the present study, 17 patients with viral infection showed HP, either having grade II or grade III intensity. Viral screening was positive for EBV, CMV, adenovirus and rubella virus. Eighteen patients with HPS had a profound effect on hematological parameters resulting in the depression of cell counts particularly hemoglobin and platelets. Bone marrow in the majority of these patients was however, either normocellular or hypercellular.

Risdall et al. (1984) described 03 patients with bacterial sepsis having HPS and peripheral cytopenia. The BM showed marked hyperplasia of mature macrophages with moderate erythroid and granulocytic hypoplasia.

HPS associated with EBV in two children was reported by Sullivan et al (1985). There was peripheral cytopenia and BM examination showed hyperplastic marrow with Erythro and leuko phagocytosis.

Peripheral blood and bone marrow abnormalities in HIV disease were described by Tracy et al. (1985). These included low Hb, thrombocytopenia, neutropenia, lymphopenia and monocytopenia.The most frequent abnormalities in the bone marrow were dyserythropoiesis, megaloblastic change and erythroid hypoplasia, apart from histiocytic hyperplasia and prominent HP. Possible mechanisms suggested for these abnormalities were multiple and included opportunistic infections, immune mediated destruction of cells, drug therapy and direct insult by HIV virus. In the present study there was no patient of HIV infection.

Similar findings of peripheral cytopenia in relation to HP in bone marrow were

the consistent finding.The bone marrows in these patients were hypercellular, normocellular or hypocellular depending upon the disease process. In other words there was no fixed pattern of marrow cellularity and it did not correlate with the peripheral cytopenia and intensity of HP. It was also observed in the present study that degree of intensity of HP and depresson of cell counts were mostly independent of marrow cellularity.

Suster et al (1985) studied the risk factors in reactive histiocytic hyperplasia with HP in hemopoietic organs. The important risk factors identified were, multiple blood transfusions, bacterial sepsis, malignancy, candida sepsis and viral infections. Most of the patients had moderate to severe degree of HP. The marrow was hypercellular or normocellular in majority of the patients. Erythroid, myeloid and megakaryocytic series were not depressed in most of the patients .They suggested that the depression of cell counts in those patients was multifactorial depending upon the underlying risk factors, infections with immunosupprenion and phagocytic activity of macrophages. In the present study as mentioned earlier mostly patients of megaloblastic anemia and infection had depression of cell counts.

In the present study, one patient with NHL presented as HPS with grade III hemophagocytosis and depression of cell counts before the initiation of chemotherapy. It is presumed that peripheral cytopenia in this patient was mainly because of hypercytokinemia due to HPS.HP in relation to malignancy showing peripheral cytopenia has been observed in malignant histiocytosis, lymphomas , acute and chronic leukemia and non hematological malignancies such as stomach carcinoma and bronchogenic carcinoma. Pathogenesis of pancytopenia in these disorders has been found to be due to various reasons such as, infiltration of marrow by abnormal cells, immunosuppression with underlying infections chemotherapy and hypercytokinemia with exaggerated phagocytic activity of macrophages (Larroche 2004).

Ohga et al (2002) highlighted the role of cytokines in EBV infection resulting in HP and pancytopenia. They found that INF-gamma, IL-10 along with CD_3 + HLA –DR cells play an important role in bone marrow HP and suppression causing peripheral

The role of cytokines in the causation of HP resulting in peripheral cytopenia was extensively studied by Fujiwara and his coworkers .According to them, pancytopenia is an essential sign in HPS and it is induced by a large number of cytokines with overlapping functions particularly IFN-gamma along with 1L-18 acting as a potent suppressor of hematopoiesis (Fujiwara et al 1993). Imashuku et al (1996) observed similar findings in patients of HP with pancytopenia.

Florena et al (2002) evaluated morphologically and immunophenotypically, BM biopsies of 26 patients with HPS. Most of the patients had neoplasia, followed by infection cases. In all these patients there was marked histiocytic hyperplasia with hemophagocytosis and T cell infiltrates .According to them BM biopsies proved to be an essential and reliable diagnostic tool for HPS and its underlying etiology. They suggested that when HPS occurs, the first diagnosis to investigate is neoplastic disease which sometimes can be hidden or latent .In the present study however, most of the patients belonged to non-malignant hematological conditions followed by infections.

The role of bone marrow function in relation to intensity of HP causing peripheral cytopenia was studied by Strauss et al (2004). Most of the patients of HP in their study presented with pancytopenia. The histologic examination of the BM revealed that activated macrophages were diffusely distributed throughout the BM .The macrophages appeared mature with low nuclear /cytoplasmic ratio and inconspicuous nucleoli. Mainly erythrocytes and less frequently platelets and neutrophils and their precursors were phagocytosed.

A significantly increased number of diffusely distributed T-cells with small lymphoid aggregates were seen in BM with HP. The extent of HP (intensity) was not associated with BM cellularity, that is, majority of the patients either had normocelluler or hypercelluler marrow. Thus according to there study the frequency and grade of HP were independent of BM cellularity and this apparently had no influence on the pathogenesis of HP resulting in pancytopenia. Most of the patients of HP in the present study too, either had hypercellular 152 (60.8%) or normocelular 53 (21.2%) bone marrow. Only 45(18%) patients had decreased cellularity.In other words

180 (72%). So bone marrow cellularity apparently had no effect over the peripheral cell counts and it did not correlate with the degree of intensity of HP as most of patients had increased intensity of HP with depressed blood cell counts.

In the present study too it was observed that the pathogenesis of peripheral cytopenia in patients with HP is multifactorial and largely depends upon the underlying disease process. In viral infections the antigens may directly target the cells .Other possible mechanisms include hypersplenism, immune destruction of cells immunosuppression with underlying infections, drug therapy and above all exaggerated hemophagocytosis with hypercytokinemia, particularly INF-gamma, TNF alpha. In the light of present study and previous studies by other workers, it is believed that cellularity of bone marrow apparently does not correlate with the degree of intensity of HP and severity of peripheral cytopenia.

Conclusion

Hemophagocytosis (HP) is seen in a wide variety of hematological and non-hematological conditions, mainly as a reactive phenomenon. It mainly results from immunological activation of the mononuclear phagocyte system. Macrophages and T-lymphocytes play a major role through elaboration of various cytokines. Reactive hemophagocytic histiocytosis is seen mostly in non-malignant hematological conditions and infections. Among the non-hematological conditions, it is typically seen in megaloblastic anemia with increasing intensity and usually results in the depression of blood cell counts. Large number of viral and non-viral infections can give rise to this phenomenon.

It may present as hemophagocytic syndrome due to hypercytokinemia with varied etiology showing pancytopenia/bicytopaenia, liver dysfunction, coagulopathy, hyperferritinemia. Mechanism of peripheral cytopenia is multifactorial but mainly occurs because of exaggerated HP with hypercytokinemia. The extent of intensity of HP and depression of cell counts is independent of bone marrow cellularity. Age of the patient apparently has no effect on either intensity of HP or peripheral cell counts.

Increase intensity of hemophagocytosis has a significant effect on hematological parameters, particularly hemoglobin and platelet counts, resulting in depression of these hematological parameters.

Future Scope

Additional studies are required to clarify the possible role of the bone marrow microenvironment in the pathogenesis of hemophagocytosis and peripheral cytopenia, to characterize the activation status of the macrophages in HP and to investigate in detail the role of T-lymphocytes and their associated cytokine profile (Th1/Th2 immune response).

REFERENCES

- Abe Y, Hara K, Shiratsuchi M, Matshusima T, Muta K. Two cases of B celllymphoma associated with HPS. Rinsho-Ketsueki, 2001; 42(1): 35-40.

- Aksu G, Kuturcules N, Genel F, Omowaire, B.Griscelli syndrome with Rab27 A mutations in human. Am.j. Med.Genet 2003; 116(4) : 329-33.

- Annistra JM. Studies of the macrophages complement receptors. Alterations of receptor function upon macrophages activation. J.Exp Med .1988; 141 : 1278.

- Arico M, Imashuku S, Clementi R, Hibi S, Nicholas K. Hemophagocytic lymphohistiocytosis due to germline mutations in SH2DIA, the X-linked lymphoproliferative gene. Blood, 2001; 97(4), 1131-3.

- Arya, S. , Hong, R, Fauci, A. Reactive Hemophagocytic syndrome. Pediat. Path. 1985 ; 3 : 129-141.

- Audo K, Miyazawa K, Kuriyama Y, Ohyashirik. Hemophagocytic syndrome associated with CD8 Positive T-cell chronic lymphocytic leukemia. Leuk-lymphoma. 2004; 45(1) : 195-8.

- Auerbach M, Haubenstock A, Soloman G : Systemic Bebesiosis. Another cause of haemophagocytic syndrome. Am. J .Ded, 1986; 80 : 301.

- Barbean J, Pastor JM, Menendez MA. Reactive hemophagocytic syndrome associated with immunoblastic B-cell lymphoma. Rev. Clin Esp. 1992; 190: 308 – 310.

- Bhutani V, Dhutta U, Singh K, hemophagocytic syndrome as a presenting manifestation of visceral leishmaniasis. J. of Phys. of India. 2002, 50 : 838-9.

- Blatt, J, Weston B, Belorn, T. Childhood lymphoma presenting as hemophagocytic syndrome. Pediatr.Hematol.Oncol. 2002;19(1):45-9

- Borouchoff SE, Woda BA, Pihan GA, Durbin WA Burstein D,Black NR. Parvovirus B 19 associated hemophagocytic syndrome. Arch .Intern Med.1990; 150 (4) : 897 – 9

- Broeckaert-van Orshovem A, Mihielsen P, Vandepitte J. Fatal leismaniasis in renal transplant patient. Lancet 1979; 2 : 740 – 741.

- Byrne GE Jr, Rappaport H : Malignant histiocytosis : Gann Mono Cancer Res 1977; 15:145.

- Campo E, Condon E, Miro MJ, et al: Tuberculosis associated hemophagocytic

- Chan CS, Cheng DN et al. Nasal T- Cell lymphoma, associated with hemophagocytic syndrome. Cancer.1986; 58: 67.

- Chandra PE, Chandlert SA, Rosner F, et al : Transient histiocytosis with striking phagocytosis of platelets, leukocytes and erythrocytes. Arch .Int .Med 1975 ; 135 : 989.

- Chanok SJ, Lugiubuhi LM, Mcintosh K. Life threatening reactions to trimethroprim / sulfamethoxazole in HIV Infection Pediatics 1994; 93: 519 – 521.

- Chubachi A, Miura R. Review of adult lymphoma associated HPS. Rinsho Ketsueki 1994; 35: 837 – 847.

- Cline MJ, Golde DW. A review and reevaluation of the histiocytic disorders. Am .J .Med. 1973 ; 55 : 49-60.

- Cline MJ. Monocytosis and macrophages: Functions and diseases. Ann .Intern Med. 1987 ; 88 : 78-88.

- Coffey AJ, Brooksbauk RA, Braudau O, et al. Host response to EBV infection in X- Linked lymphoproliferative disease, mutation in SH_2 - domain. Natl Genet. 1998; 20 : 129 – 35.

- Coulthard S, Chase A, Orchard K. Two cases of inv (8) (p11, q 13) in AML with erythrophagocytosis: a new cytogenetic variant. Br .J . hematol. 1998; 100: 561-3

- Douglas SD: Kay NE, mononuclear phagocytes. NY J Med 1991; 77 : 327.

- Emmenegger U, Zehnder R, Frey U. Elevation of soluble Fas ligand in reactive macrophage activation syndrome. Am . J .Hematal 2000; 64: 116 – 9.

- Esseltine DW, Deceum NKM, Berry GR: Malignant histiocytosis. Cancer 1983; 52 : 1904.

- Farquahr JW, McGregor AR, Rechmond J: Familial hemophagocytic reticulosis. British Medical Journal 1958;2 : 1516.

- Favara BE Jaffe, R, Egeler, RM. Macrophage activation and hemophagocytic syndrome in langerhans cell histiocytosis. Pediatr-Dev-Pathol. 2002; 5(2): 130-40

- Florena A, Iannitto E, Quintini G. Bone marrow biopsy in hemophagocytic syndrome. Virchows.Arch. 2002 ; 441 (4) : 335-44

- Fujiki R, Shiraishi K, Noda K, Oshita Y, Fukahori S, Aizawa H. A case of Hemophagocytosis associated with miliary tuberculosis. Kekkaku. 2003; 78 (6): 443-8.

- Fujiwara F, Hibi S, Imashuku S. Hypercytokinemia in Hemophagocytic syndrome. Am .J.Pediat Hematol. Oncol, 1993; (15) : 92-98.

- Gabrilove JL, Gakubowsri A, Scher H. et al . Effect of granulocyte colony stimulating factor on neutrophils and associated morbidity due to chemotherapy for Transitional cell carcinoma of the Urothelium. N .Eng .J Med. 1988; 318-144.

- Gan G, Eow I, Tec A. Familial hemophagocytic lymphohistiocytosis in two brothers. Med - J- Malaysia.2004 ; 59(1): 100-2

- Golde DW. Disorders of mononuclear phagocyte proliferation, maturation and function. Clin Haematol. 1978; 4 : 705-21.

- Goto S, Aoike I, Shibasaki Y, Morita T, Miyazaki S, Suzuki M. Disseminated tuberculosis associated hemophagocytic syndrome. Am.J.Kidney disorders. 2001; 38(4): E19.

- Groopman J, Golde DW, Cline M.J; The histiocytic disorder. A pathophysiologic analysis. Ann. Int. Med. 1981; 94 : 95.

- Gurandolter E, Fadeel B, Nilsson S, etal. Spectrum of perforin gene mutations in familial hemphagocytic lymphohistiocytosis. Science.2001; 286: 1957-59.

- Hashimoto H, Maruyama H, Fujimoto K. Hematologic findings associated with thrombocytopenia and hemophagocytosis in primary herpesivirus-6 infection. J-Pediatr.Hematol.oncol. 2002; 24(3): 211-4.

- Horiyschi J, Warren MK, Kufe D. Expression of the macrophage specific colony stimulating factor in human monocytes treated with granulocyte macrophage colony stimulating factor. Blood 1987; 69 : 1259.

- Huang, DB wu, J. Reactive hemophagocytosis associated with the initiation of highly active antiretroviral therapy (HAART) in a patient with AIDS. Scand J. Infection. Dis. 2004; 36 (6-7): 516-9.

- Imashuko S, Hibi S. Cytokines in hemophagocytic syndrome (Correspondence) Br .J .Hematol 1991; 77 (3) : 438 – 9.

- Imashuku S, Hibi S, Sako M, Nakata Y, Trunematsu Y. Hemophagocytosis by Leukemic Blasts in 7 Acute Myeloid Leukemia Cases with t (16, 21) (p11, q22). American cancer society 2000 ;1911-75.

- Imashuku S. Differential diagnosis of hemophagocytic syndrome. Underlying disorders and selection of the most effective treatment. Int. J. of Hematol. 1997;(66)135-151.

- Inone Y, Johno M, Matuka M, Ono T. A case of Adult T-cell Lymphoma Leukemia with Hemophagocytic syndrome. J. of derm. 2000; (27) : 280-82

- Iqbal W, Hassan K, Ikram N.Etiological break up in 208 cases of Pancytopenia:JRMC.2001;5(1):7--10

- Iqbal W, Mehmood H,Akhter H:Morohological features in 42 patients of megaloblastic anemia.JRMC.2003;8(3)22--26

- Isobo Y, Sugimoto K, Shiraki Y, Nishitani M,. Persistent Parvovirus B 19 infection in a lymphoma patient. Am-J- Hematol .2004; 77 (4): 370-3.

- Jaffe ES, Costa J, Fauci AS. et al: Malignant lymphoma and erythrophagocytosis simulating malignant histiocytosis. Am .J. Med. 1983; 75 : 714.

- James LP, Stress SA, Peterson V, et al . Abnormalities of bone marrow simulating histiocytic medullary reticulosis in patients with gastric carcinoma. Am .J. Clin .Pathol .1979; 71 : 600.

- Kadin ME, Kamoux M, lawberg J. Erythrophagocytic T gamma lymphomaa N. Eng J. Med.1981; 304: 648

- Kakkar N , Vasisita K, Banerjee K, Maswana Y, Familial hemophagocytic lymphohistiocytosis. an autopy study. Pediatric.Pathol.Mol.Med. 2003; 22(3) : 229-42.

- Karakukcu M, Patiroglu T, Gummus H. Pancytopenia a rare complication of brucellosis in children. J-Pediatr. Hematol-Oncol. 2004; 26 (12) : 803-6.

- Karman LY, Smith JR, Landaw SA, et al . Hodgkin's disease: Intramedullary phagocytosis with pancytopaenia. Ann .Int .Med. 1979; 91 :60.

- Kay MB: Mechanism of removal of senescent cells by human macrophages in situ. Proc Natl Acad Sci NSA 1975; 72 : 3521.

- Kelbanoff SJ, Hamon CH. Myeloperoxidase halide hydrogen peroxide antibacterial system. J .Bacterial 1963; 95 : 2131-2138.

- Kfonry Baz E-M, Mikati AR, Kanj NA. Reactive hanophagocytic syndrome associated wit thrombotic thrombocytopenic purpura. Ther-Apher. 2002; 6(2): 159-62

- Kolodny EH. Niemann - Pick desease. Curr Opin. Hematol 7; 48, 2000.

- Krim MA. Nagle DL, KandilH. Mutation in CHS (CHSI) indicate requirement for the complete 3801 amino acid CHS protein. Hum. Mol. Genet. 1997; 6:1081- 9.

- Lambotte O, Costedoat N, Amoura Z. Drug induced hemophagocytisis. Am.J. med. 2002; 112 (7): 592-3

- Latour S, Veillette A. Molecular and immunological basis of X – Linked lymphoproliferative disease. Immunal. Rev. 2003; 192: 212 – 24.

- Lee J, Clung I, Shin H, Dong W, Hyeoung J, Viral Hemorrhagic fever with renal Syndrome presenting with hemophagocytic lymphohistiocytosis. Emerg. Infec. Dis.. 2002; 8(2): 209-10.

- Liang DC, Chu ML, Shih CC. Reactive histiocytosis in acute lymphoblastic leukaemia and non-Hodgkin's lymphoma. Cancer 1986; 58 : 1289.

- Lieberman PH, James CR, Dragcon HWR, Begg CF. An appraisal of eosinophilic granuloma of bone , Hand Schuller Christian syndrome and Letterer Sive syndrome. Medicine 1969; 48 : 375 – 400.

- Majluf Cruz AS, Hurtado R, Souto Meiririo C. Hemophagocytic syndrome associated with histoplasmosis in the acquired immuno deficiency syndrome. Sangre Burc 1993; 38: 51 – 55.

- Manoharan A, Castovasky D, Lampart IA, Al-Mashadhani, Gordon Smith EC, Galton DAG. Histiocytic medullary reticulosis complicating chronic lymphocytic lekaemia. Malignant or reactive. Scand. J. Hamatol . 1981; 26 5-13.

- Manoharan A, Catousky D. Histiocytic medullary reticulosis revisited. Springer.verlog. 1981; 205-210.

- Marin- Padilla M: Erythrophagocytosis by epithelial cells of a breast carcinoma. Cancer 1977; 39 : 1085-1089.

- Martin PF: Erythrophagocytosis in the human bone marrow as disclosed by iliacal bone biopsies. Scand .J. Hematol.1975; 14 : 153.

- Matzner Y. Behar A, Beri E, et al. Systemic leishmaniasis mimicking malignant histiocytosis. Cancer, 1979; 43 : 398.

- Mori Y, Sugiyama T, Chiba R, Sugimoto T, Furukawa M, Sueishi M, Tanabe E. A case of systemic lupus erythrematosus with hemophagocytic syndrome. Ryumachi : 2001; 40(1) : 31-6

- Nakada S, Sikikawa T, Takahara S, Yamazaki Y, Yamada J, Iwase S, Kobayashi M. Nontuberculosis atypical mycobacterial infection with progressive Pancytopenia in a patient with MDS. Rinsho-Ketsueki. 2001, 42(7): 543-8.

- Nathan CD: Secretory products of macrophages. J .Clin. Invest. 1987; 79 : 319.

- Nathan CF, Karnovsky IM, David JR Alternations of macrophage functions by mediators from lymphocytes. J Exp. Med. 1984; 1356-1376.

- Nathan CF, Murrey HW, Cohn ZA: Current concepts: The macrophage as an effecter cell. N Engl. J. Med 1980; 303 : 522-626.

- Nelson DM .Morphology of Mononuclear Phagocytes. J .Clin. Invest 1991; 84 : 210

- Nishi Y, Shirane M, Date T, Nishura T, Fujimuto J. Case of NK/T cell lymphoma of the larynx with Hemophagocytic syndrome. Nippon-Gakkai-Kaiho. 2004; 107(10): 966-9.

- Ohga S, Nomura A, Takada H, Yanai F, Hara T. Epstein-Barr Virus (EBV) load and cytokine gene expression in activated T cells of chronic active EBV infection. Journal of infectious diseases. 2001; 183(1) 1 -7.

- Osugi Y, Hara J, Tagawa S, etal. Cytokine protection regulating Th1 and Th2 cytokine in HPS. Blood 1997; 89: 4100 – 3.

- Pellegrin JL, Merho JP, Lawste D etal. Syndrome of macrophage activation with hemophagocytosis in HIV infection. Rev. Med .Intern. 1992; 13: 438 – 440.

- Perry MC, Harrison, EG JR, Buryet ED et al: Familial erythrophagocytic lymphohistiocytosis. Report of two cases and clinicopathologic review. Cancer 1976; 38 : 209.

- Rappaport H: Tumors of the hemopoietic system.In Atlas of Tumor pathology.Sec 3.Fas 8.Washigton.DC.Armad Forces Institute of Pathology.1966;p 49.

- Risdall RJ, Brunning RD, Hernandez JI, et al. Bacteria associated hemophagocytic syndrome. Cancer 1984; 54 : 296.

- Risdall RJ, RJ, Mckenna RW, Nesbit ME, et al : Virus associated – Hemophagocytic syndrome. Cancer 1979; 44 : 993.

- Rogozinski L, Bass A, Glickman E, et al : The T4 surface antigen is involved in the induction of helper function. J. Immunol. 1984; 132: 735.

- Rosenthal AS. Regulation of the immune response: Role of the macrophage N.E ng .J. Med. 1980; 303 : 1153.

- Saribeyoglu T, Anak S, Boral O, Unuvar A. Secondary hemophagocytic lymphohistiocytosis induced by malaria infection in a child with langerhans cell histiocytosis. Pediatr.Oncol.Hematol. 2004; 21(3) : 267-72.

- Saribeyogl ET, Anak S, Agaoglu L. Secondary hemophagocytic lymphohistiocytosis induced by malaria infection in a child with Langerhans histiocytosis. Pediatr-Hematal .Oncol. 2004; 21 (3): 2 67-72

- Sato T, Terui T Kogawa K, NagaokaY. A case of true malignant histiocytosis, identification of histiocytic origin with use of immunohistochemical and immunocytogenetic methods. Ann.Hematol. 2002; 81 (5) : 285-8

- Savage G, Zipin D, Bhagat G, Alobeid B. Hemophagocytic syndrome in a non-secretory multiple myeloma. Leuk .Lymphoma. 2004; 45(5) : 1061-4.

- Scott RB, Robb Smith AHT. Histiocytic medullary reticuosis. Lancet 1939; (2): 194-6.

- Sekigava I, Suzuki J, Nawata M. Hemophagocytosis in autoimmune disease. Clin.Exp.Rheumatol. 2001; 19 (3):333-8

- Sekiuchi M, Nakabayashi K Marumo T. Hemophagocytic Syndrome associated with hypercytokinemia in a patient with rheumatoid arthritis. Ryumachi. 2003; 43(4): 696-702

- Severe Pancytopenia and Hemophagocytosis after HH8 Primary infection in a renal transplant patient. Transplantation. 2002; 74 (1): 131-2.

- Shyong C,Chang K, Dunn P. Acute hepatitis A with Coexistent Hepatitis C virus infection presenting as a virus associated HPS. Am. J- Gastroentrology; 1995; (90) 1002 – 1005.

- Sproat L, Pantanowiz L, Lu CM. Human immunodeficiency virus associated hemophagocytosis with iron def. anemia . Clin. Infect.Dic. 2003; 37(11): 170-3

- Strauss R, Neureiter D, Westenburger B, Hahn G. Multifactorial risk analysis of bone marrow histiocytic hyperplasia with hemophagocytosis in critically ill medical patients. A postmortem clinicopathlogic analysis. Crit. Care. Med. 2004; (32): 1316-21.

- Sullivan JL, woda BA, Herrod HG; Koh G; Rivara FP, Muldar C: Epstein-Barr Virus-associated haemophagocytic syndrome. Virological and immunopathological studies. Blood 1985; 65 (5) : 1097-1104.

  Takada H, Ohga S, Mizuno Y et al: Oversecretion of IL-18 in Hemophagocytic lymphohistiocytosis. A noval marker of disease activity. Br. JHaematol (106): 1999; 182-89.

- Takeoka Y, Hina M, Oiso N, Nishi S, Koh KR, Yamane T, Ohta K, Nakamae H. Virus associated HPS due to rubella virus and varicella-zoster virus dual infection in a patient with adult idiopathic thrombocytopenic purpura. Ann.Hematol 2001; 80(6): 361-4.

- Tracy M. Leslie L. Cestello C. Clark A. Peripheral blood and bone marrow abnormalities in patients with HIV related disease (Correspondence) Br. J. Haematol. 1987; 65 : 289-294

- Tune B, Ayata A . Hemophagocytosis as a rare life threatening complication of visceral leishmaniasis.Journal of pediatric.Hematol.Oncol.2001;18(8):531-6

- Van Furth R, Langevoort HL, Schaberg A. Mononuclear phagocytosis in human pathology Proposal for an approach to an improved classification. In: Van Furth R. ed. Mononuclear Phagocytes in immunity infection and pathology. Oxford Blackwell Scientific Publication, 1975; 1-15.

- Van Furth R, Racburn JA, Van Zwet TL. Characteristics of human mononuclear phagocytes. Blood. 1985; 54 : 485 – 500.

- Wang X, Herberg FW, Laue MM, et al. Neurobeactin: a protein kinase A anchoring, beige/CHS Protein. J. Neuro science. 2000; 20 : 8551 – 65.

- Wang,-S; Degar, B-A; Shafi; N-Q. Hemophagocytosis exacerbated by GM-CSF treatment in a patient with myelodysplasia. Am.J. Hematol. 2004; 77(4): 391-6

- Warnke RA, Kim H, Dorfman RF: Malignant histiocytosis. Clinicopathologic study of 29 cases. Cancer. 1975; 35 : 215.

- Weintraub N, Siegman Igra Y, Yosiphow J, et al: Histiocytic hemaphagocytosis in miliary tuberculosis. Arch. Int. Med.1984; 144 : 2055.

- Weller, TH, The cytomegalovirus Ubiquitous agents with protean clinical manifestations. N .Engl. J .Med .1971; 285 : 203 – 214, 267 – 274.

- Wilson ER, Malluh A, Stagno S, Crist WM. Fatal Epstein Barr Virus associated hemophagocytic syndrome. Ann. Intern .Med. 1991; 114 (5) 387-90.

- Wolfdorf J1, et al: Glycogen storage disease. Endocrino Metab. Clin. North Am.1991; 28: 801.

- Wong CK, Li KE, Ho CY. Elevation of plasma interlukin - 18 with disease activity in SLE. Rheumatology (Oxford) 2000; 39: 1078 – 81.

- Wong KF, Chan JK, Chu YC. Large cell lymphoma with initial presentation in the bone marrow. Hematol. Oncol. 1992; 10: 261 – 271.

- Wong KF, Chan JK: Reactive hemphagocytic syndrome-a clinicopathologic study of 40 Patients in an oriental population. Am .J.Med; 1992;93:177-180

- Zvulunov; A; Tamary, H; Gul, N. Pancytopenia resulting from hemophagocytosis in malaria.. Pediat.Infect.Dis-J. 2002 ;21(11): 1086-7

Annexure A

HISTORY SHEET AND PHYSICAL EXAMINATION

Name	Age Y Sex		Dated.
Ward Bed	OPD	Contact No.	
Ref By .		Address.	

Complaints

	Yes	No	Duration
Fever	☐	☐	
Pallor	☐	☐	
Jaundice	☐	☐	
Weakness	☐	☐	
Cough	☐	☐	
Diarrhea	☐	☐	
Bleeding	☐	☐	
Weight loss	☐	☐	
Any other complaint	☐	☐	

Physical Examination.

	Yes	No
Pallor	☐	☐
Jaundice	☐	☐
As cites	☐	☐
Bruises/Purpura	☐	☐
Lymph nodes.	☐	☐
Liver	☐	☐
Spleen	☐	☐
Any other abnormality.	☐	☐

ANNEXURE C

BONE MARROW FINDINGS

S. No.	Cellularity	Erythropoeisis	Myelopoieosis	Megakaryocytes	Hemophaocytosis Mild, Moderate, Severe	Iron Stain	Misc.

ANNEXURE B

HEMATOLOGICAL PARAMETERS

SR. NO.	PATIENT	Hb gm/dl	T L C X10$^{9/L}$	Platelet countX10^{3}/ul	Pancytopenia / Bicytopenia	Mis