RETROSPECTIVE STUDY ON HIV/ AIDS ASSOCIATED HAEMATOLOGICAL DISORDERS FOUND IN BONE MARROW AT DR. GEORGE MUKHARI HOSPITAL (DGMH), PRETORIA

by

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RESEARCH DISSERTATION

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Declaration

I, **Dr Mariquinha Jose' Manuel Moniz de Carvalho,** declare that this mini-dissertation hereby submitted in partial fulfillment of the M.Med (Haematology) degree in the Department of Haematology at the University of Limpopo, MEDUNSA campus, is my own work and that all references used have been duly listed and that neither the whole work nor any part thereof has been, is being, or is to be submitted for another degree at this or any other university or tertiary institution or examination body.

Signed …….

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Dedication

To my parents, Mr & Mrs Jose' Moniz

In your loving memory for bringing me into this world.

To my Husband, Domingos Carvalho

For being a pillar of strength, a mentor and a great source of inspiration through this journey.

To My Brother, Fernando Moniz

For taking me by hand as I began this journey, thank you.

Acknowledgements

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- I would like to pass my sincere thanks to the Department of Haematology staff for giving the opportunity to study for my M. Med. The continued guidance and support will always be cherished in my heart.

- Last but not least, I would like to thank my husband, son (Keny) and daughter (Mile') and my family as a whole for their prayers, words of encouragement and endurance throughout my studies; I love you.

Abstract

Background. Infection with human immunodeficiency virus (HIV) is associated with a range of haematological abnormalities including: ineffective haematopoiesis, infiltrative disease of the bone marrow, nutritional deficiency and peripheral destruction of blood cells secondary to splenomegaly and immune deregulation.

Aim. To review and describe bone marrow abnormalities and associated peripheral haematological abnormalities, in HIV/AIDS patients.

Methodology. This is a retrospective study. Data was extracted from DISA, the National Laboratory Health Service Laboratory Information System database at the DGMH Tertiary Laboratory from 2003 to 2007. Medical and laboratory records of 80 HIV positive patients who underwent bone marrow examination for investigation of fever and/or cytopenia were reviewed. All statistical analyses were performed on SAS® Release 9.1.3.

Results. Twenty-five patients out of a total of 80 (31.25%), had pancytopaenia. Of the 25, eight (32%) were males and 17 (68%) were females. In this study, pancytopaenia was described as a haemoglobin concentration, granulocyte count and platelet count below normal ranges for age and gender. Among male patients in this study, five (17%) patients had TB out of 30. Among female patients, five (10%) out of 50 patients had TB. The majority of patients with malignancies were males six out of nine (67%). Three of the five patients with non-Hodgkin's lymphoma (NHL) and all of the patients with multiple myeloma (MM) were males.

Conclusions. Haematological abnormalities were present in all patients. Bone marrow involvement by TB was found in 12.5% in the study population. Malignancies were more frequent in males; three patients with NHL, two with MM and one with Kaposi sarcoma (KS). The difference in distribution was not statistically significant (p=0.391002).

Recommendations. It is recommended that health education and health promotion focus on the control of biological carcinogenic agents such as EBV, HPV and HHV-8 by routinely testing for these agents and by promotion of positive reproductive behaviour among people living with HIV/AIDS. The use of non-invasive tests will be helpful in our setting where there is high TB prevalence.

List of Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
B cell	B lymphocyte
BL	Burkitt's lymphoma
BM	Bone marrow
CD	Cluster of Differentiation
CMP	Common myeloid progenitor
CLP	Common lymphoid progenitor
DC	Dendritic cell
DGMH	Dr George Mukhari Hospital
DIC	Disseminated Intravascular Coagulation
DL BCL	Diffuse large B-cell lymphoma
DNA	deoxyribonucleic acid
EBV	Epstein-Barr virus
FBC	Full blood count
Fc	Non variable region
g/l	Grams/litre
G6PD	Glucose 6 phosphatase dehydrogenase
GI	Gastrointestinal
Gp	Glycoprotein
Hb	Haemoglobin
HHV-8	Human herpes virus subtype 8
HIV	Human immunodeficiency virus
HL	Hodgkin's lymphoma
HPC	Haematopoietic pluripontential cell
HSC	Haematopoietic stem cell
HV	Hyaline Vascular
IL-6	Interleukin 6
KSHV	Kaposi Sarcoma associated Herpes virus

LPM-1	Latent membrane protein 1
MAC	*Mycobacterium avium* complex
MCD	Multicentric Castleman's Disease
MSC	Mesenchymal stem cell
NHL	Non-Hodgkin's lymphoma
KS	Kaposi Sarcoma
NK	Natural Killer
PBL	Plasmablastic lymphoma
PC	Plasma Cell
PEL	Primary Effusion Lymphoma
RS	Reed Sternberg
TB	Tuberculosis
TTP	Thrombotic thrombocytopenic purpura
VEGF	Vascular endothelial growth factor
WCC	White cell count

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CHAPTER ONE

1.1 Introduction

A unique human retrovirus, previously termed human T-lymphotropic virus type III (HTLV-III), lymphadenopathy associated virus (LAV), AIDS-related virus (ARV) and now termed human immune deficiency virus (HIV) is the primary aetiological agent in the pathogenesis of the acquired immunodeficiency syndrome (AIDS). Three broad categories of clinical outcome are recognized after infection with HIV. These include full blown AIDS manifested as severe opportunistic infections and/or malignancies (Leonard *et al*, 1987). AIDS syndrome is presently seen primarily among sexually active populations, intravenous drug abusers, individuals who have received blood or blood products, including haemophiliacs treated with factor VIII concentrate, among sexual partners and infants born to members of high–risk groups (Antonio *et al,* 1985).

Previous studies have shown that stromal elements are directly infected and may act as reservoir for the HIV virus. The HIV virus directly infects a variety of stromal elements including monocyte/macrophage elements, micro-vascular endothelial cells, fibroblastic and myeloid cells (Gill *et al,* 1997; Kulkosky *et al,* 2000; Ercoli *et al,* 1996; Moses *et al,* 1996). Infection of stromal elements leads to a loss of the haematopoietic support function (Bahner *et al,* 1997). In addition, megakaryocytes, which play an active role in the maintenance of bone marrow stroma, are also infected by the HIV virus, as demonstrated by the presence of CD4 receptors on their surface (Sato *et al,* 2000). HIV infection of bone marrow elements may lead to

loss of haematopoietic support function and may be responsible for some of the haematological abnormalities. These changes may ultimately lead to cytopenias and susceptibility to infection, which are significant problems in the HIV-positive population (Kulkosky *et al,* 2000). A further study on peripheral blood findings showed lymphopenia, leucopenia and abnormal circulating monocytes were common in patients with AIDS or Aids related complex (ARC) and may result directly from HIV infection (Treacy *et al,* 1987).

1.2 Study Problem

Infection by immunodeficiency virus (HIV) is associated with a range of haematological abnormalities including: ineffective haematopoiesis, infiltrative disease of the bone marrow, nutritional deficiency and peripheral destruction of blood cells secondary to splenomegaly and immune deregulation. HIV infection causes progressive damage of the immune system of individuals making them susceptible to a wide variety of opportunistic (bacterial, fungal, and protozoan) infections.

The purpose of this research is to review previous cases describing the haematological laboratory findings associated with HIV infection including peripheral blood findings, and identification of occult infections or malignancy in the bone marrow. This review will explore the prevalence of cytopenias such as anaemia, granulocytopenia and thrombocytopenia noted in patients infected with

HIV and other abnormalities including opportunistic micro-organisms and malignancies.

1.3 Aim of the Study

To review and describe, bone marrow abnormalities and associated peripheral haematological abnormalities in HIV/AIDS patients.

1.4 Objectives

1. To establish the prevalence of trilineage cytopenia (anaemia, granulocytopenia, thrombocytopenia) in HIV- positive patients undergoing bone marrow examination for investigation of fever and / or cytopenia.
2. To identify the prevalence of infection by opportunistic microorganisms as observed in trephine biopsy:
    - Tuberculosis (TB)
    - Histoplasmosis
3. To determine the presence of other secondary malignancies:
    - Non-Hodgkin's lymphoma (NHL); Hodgkin's lymphoma (HL)
    - Acute lymphoblastic leukaemia (ALL)/Acute myeloid leukaemia (AML)
    - Kaposi sarcoma (KS)
    - Plasma cell leukaemia

CHAPTER TWO

LITERATURE REVIEW

2.1 Human immunodeficiency virus (HIV): Definition

Acquired immunodeficiency syndrome (AIDS) as defined by Abbas (2004) is a disease caused by the retrovirus human immunodeficiency virus (HIV) and is characterized by profound immunosuppression that leads to opportunistic infection, secondary neoplasm and neurological manifestations.

2.2 Background of HIV/AIDS

AIDS was first recognized in 1981 when cases of *Pneumocystis carinii* pneumonia and Kaposi sarcoma were reported in homosexual men in California and New York. By the year 1984, a human retrovirus HIV-1, which had the greatest social and medical impact was identified as the cause of this widespread epidemic of severe immunosuppression.

HIV-1 and HIV-2, have been identified as retroviruses causing AIDS in different geographical regions; HIV-1 causes most cases of AIDS in the Western Hemisphere, Europe, Asia, Central-, South- and East-Africa while HIV-2 is the main agent of AIDS in West Africa and it appears less virulent than HIV-1, but in certain areas of West Africa, both organisms are prevalent (Abbas, 2004).

By the end of 2002 more than 900,000 cases of AIDS had been reported in the USA where it was the second leading cause of death in men aged between 25 and 44 years and third leading cause of death in women in this age group. Although initially recognized in USA, AIDS is a global problem. By the year 2002, HIV had infected 60 million people worldwide, and nearly 20 million adults and children had died of the disease. There are now about 42 million people living with HIV/AIDS, of whom 70% are in Africa and 15% in Asia and over 8% in Sub-Saharan Africa. AIDS has now been reported from more countries around the world, and the pool of HIV infected persons in Africa and Asia is large and expanding (Abbas, 2004).

Studies conducted in Sub-Saharan Africa show that HIV infection is not only strongly associated with AIDS defining cancers but also provides some evidence, of associations with other neoplasia. African countries now need to implement well designed population based studies, in order to improve the description for the spectrum of AIDS associated malignancies and the most effective strategies for their prevention, screening and treatment. (Sasco *et al.,* 2010).

Lymphomas in Sub-Saharan Africa have been poorly studied. Overall prevalence of HIV infection in 709 patients with available results was 37%, with a very high prevalence of >80% in the diffuse large B-cell lymphoma (DLBCL) and Burkitt's lymphoma group. (Cainelli *et al.,* 2010).

In Tanzania, 21.6% of the patients with malignant lymphoma were available for testing by serology for HIV infection during the study period. Nine (23.7%) tested positive including five (55.6%) Non-Hodgkin's lymphoma (NHL) (all B-cell type) and four (44.4%) Hodgkin's lymphoma (HD). (Mwakigonja A. R *et al.,* 2008).

The Kampala Cancer Registry, one of the continent's first and foremost, has shown a significant alteration in the incidence of Kaposi Sarcoma in the era of AIDS. In Uganda, Kaposi sarcoma has accounted for almost half (48.9%) of cancer cases in men and 17.9% in women. The incidence in men (30.1 cases per 100 000) represents a more than 10-fold increase in men since the 1950's and is approximately 3 times the incidence found in women (11 cases per 100 000). For endemic Kaposi sarcoma in Uganda, a progressive rise in incidence peaked in women aged 25 to 29 years and in men aged 35 to 39 years of age. *(*Schwartz MD *et al*., 2010).

Over the past 20 years, HIV has increased TB notification rates, which have increased 3- to 5-fold in many African countries. In 2007, Sub-Saharan Africa accounted for 79% of the global HIV-associated TB rates. The countries worst affected were those in the East and South of Sub-Saharan Africa, where HIV prevalence rates are the highest. Approximately 1% of the population in South Africa and Swaziland develop TB annually.

In some poor South African communities, notification rates have increased to over 2% per year. South Africa alone accounts for a staggering one-in-four, of the world's cases of HIV associated TB. (Lawn. S. *et al.,* 2010).

In Angola, tuberculosis (TB) co-infection with HIV is a major concern. It is the leading cause of death in HIV positive people. After decades of war, the health infrastructure is not adequate enough to cope with the TB epidemic. In 2006 the incidence rate was 127 cases per 100 000 population, according to the World Health Organisation. Nineteen percent of patients recently diagnosed with TB are also HIV positive. As of December 2005, UNAIDS estimated that 320 000 people living in Angola were HIV positive. The National Institute Against HIV/AIDS however recorded that 400 050 people in Angola were living in AIDS in 2006. As of 2006, it was reported that 60 percent of all reported HIV/AIDS cases occurred among people aged 20 to 39. (USAID Angola, 2007).

Two epidemiologic patterns of HIV (type 1 and type 2) transmission are recognized. In USA and Europe, type 1 transmission is primarily homosexual or via blood. It can also be transmitted by drug users who share needles and recipients of transfused blood or blood components that may transmit HIV to women heterosexually. In Africa, South America, and South Asia, type 2 transmission is primarily heterosexual in these areas, with men and women being nearly equally affected. While in countries such as Brazil and Thailand, mixtures of the two patterns have been found (Beers & Berkow, 2004).

2.3 Type and structure

Infection with both subtypes of the Human Immunodeficiency virus, HIV-1/2 causes acquired immunodeficiency syndrome (AIDS), although those infected with HIV-2 tend to progress at a slower rate. HIV-1 and HIV-2 are retroviruses of the lentivirus subfamily and are sufficiently alike to be considered as one in terms of viral properties. HIV, like other retroviruses, replicates by forming a DNA provirus using the viral enzyme reverse transcriptase.

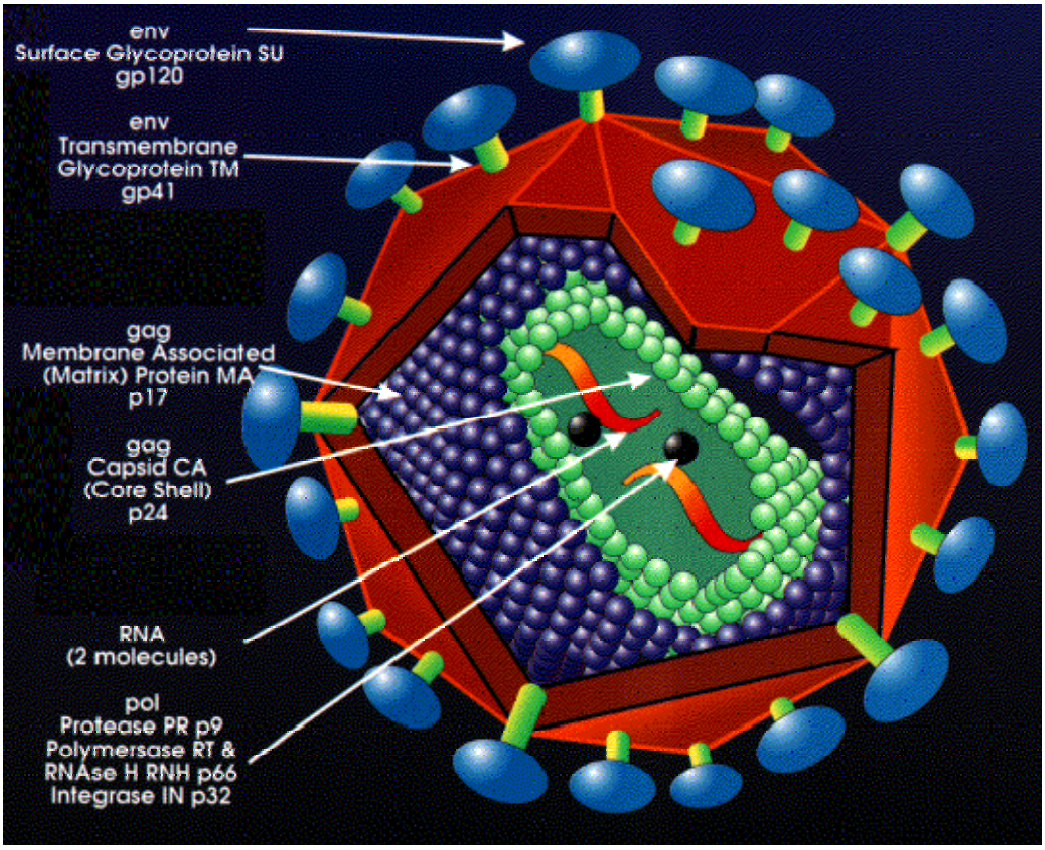

Figure 1 Structure of the Human Immunodeficiency virus

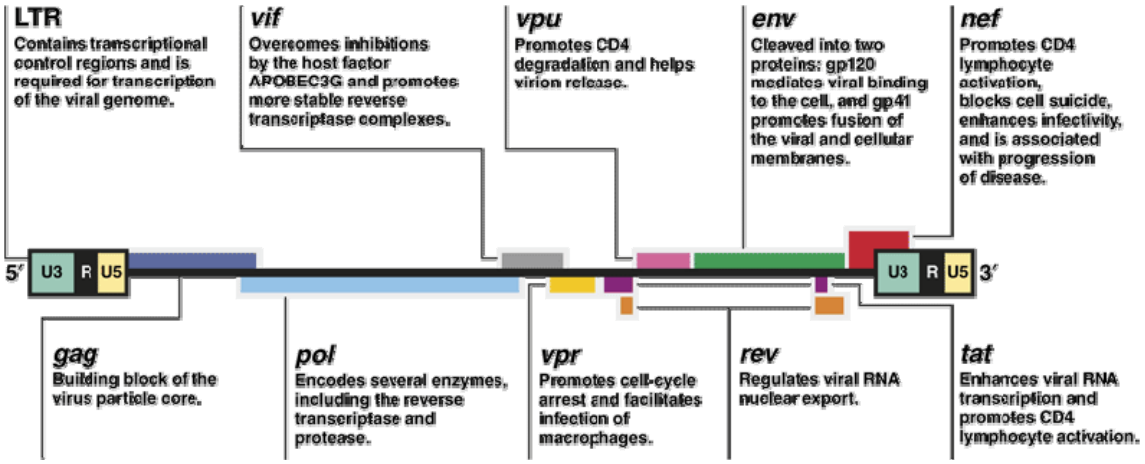

Figure 2. Map of the HIV genome

Retroviruses contain RNA in two subunits and have three genes central to their action: *gag* (group-specific-antigen) coding for proteins within the viral particle; *pol* coding for reverse transcriptase, which converts the RNA into DNA within the host cell, and *env* which codes for envelope glycoproteins. In addition to these genes, the HIV genome contains several regulatory genes that modulate the rate of viral gene expression and infectivity of the virus. The *tat* and *rev* genes act in positive feedback to enhance the processing and translation of RNA encoding the structural genes and proteins, whereas the *nef* gene appears to down regulate HIV replication (Costello, 2005).

2.4 Viral transmission and replication

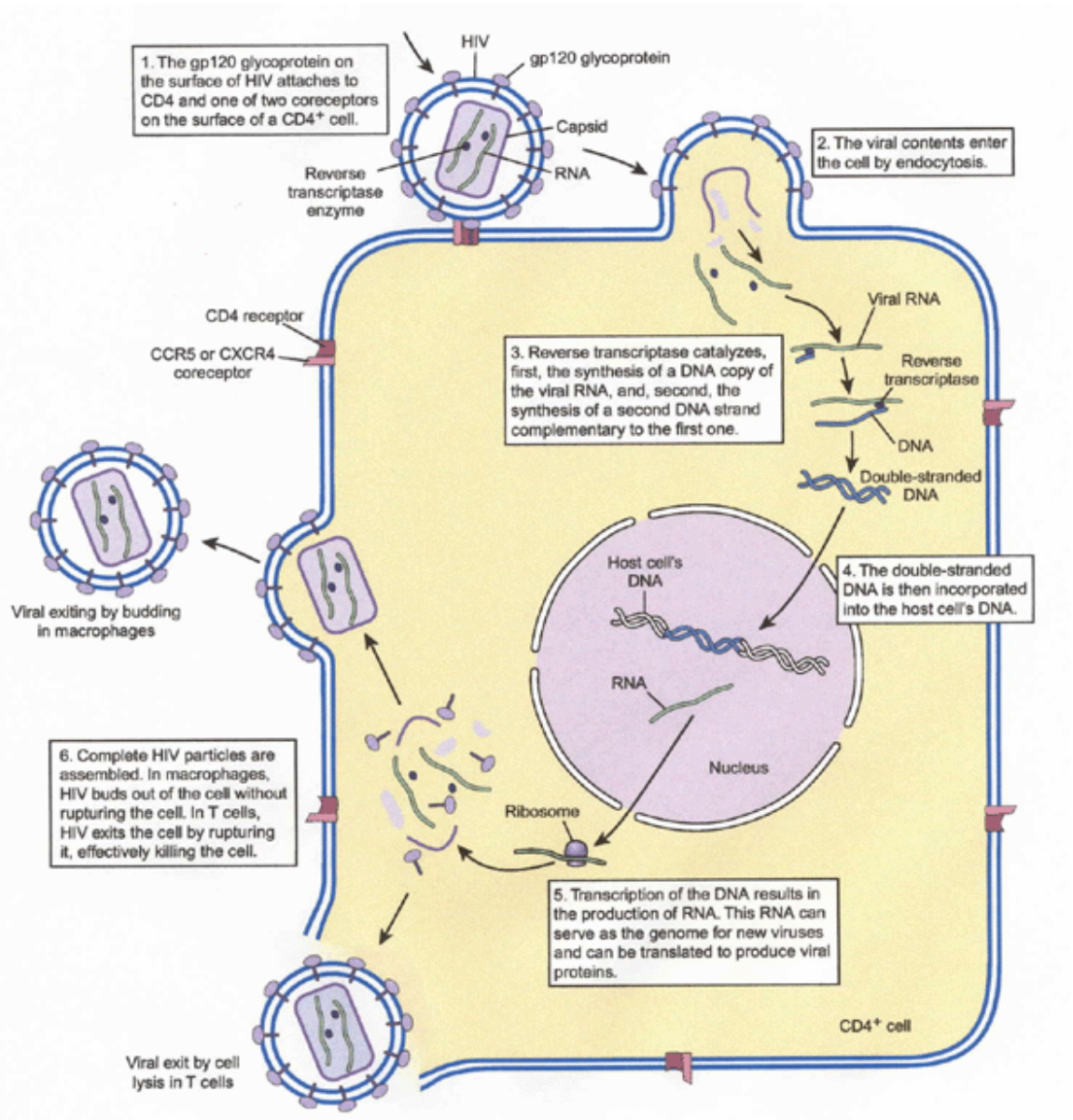

Figure 3. HIV replication cycle

Both HIV-1 and HIV-2 viruses mutate rapidly, so that an infected person may have several different strains. Transmission of the virus takes place via three routes: (1)

Mucosal: through sexual contact which is associated with other sexually transmitted diseases; (2) Vertical: from mother to child during the birth process and in breast milk; and (3) Exposure to infected blood: intravenous drug abusers, blood transfusions, needle-stick injuries (Griffin, 2003).

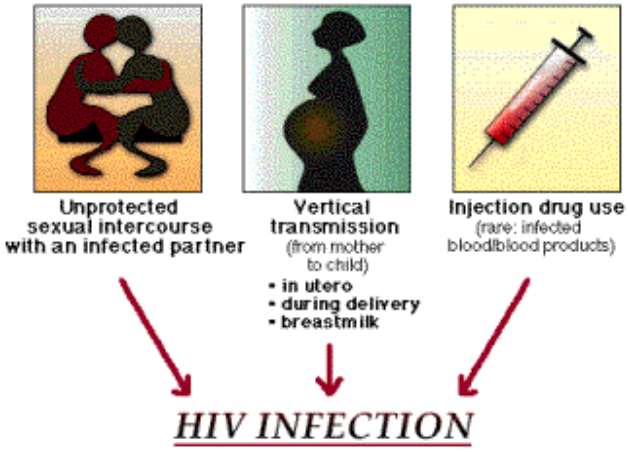

Figure 4. Modes of HIV transmission

It has been suggested that infected lymphocytes and macrophages are particularly effective at transmission (Costello, 2005 b). Monocytes and macrophages are important reservoirs of this virus in tissues. Differentiation of monocytes into macrophages leads to cell attachment and susceptibility to infection and replication of HIV. Among other cell surface molecules, integrins are over expressed during monocyte/macrophage differentiation and may play a role in the replication cycle of envelope virus including HIV (Ballana *et al.,* 2009).

Integrins are a family of transmembrane cell adhesion receptors that recognize cell surface and extracellular matrix ligands (Hynes, 2002). All integrins are heterodimers composed of noncovalently linked α and β subunits. In humans, at

least 18α and 8β subunits have been identified, forming more than 20 heterodimers (Takada *et al.*, 2007).

Integrins function as mediators for cell to cell interaction and adhesion but may also elicit signal transduction events leading to cell activation and response to extracellular stimuli (Hynes, 2002). Different enveloped and non-enveloped viruses use integrins to enter and replicate in host cells (Stewart & Nemerow, 2007). Integrins may act as viral receptors that mediate virus attachment to the cell, a process that may also promote signalling responses influencing viral replication at a later step (Wang *et al.* 2005).

Precursors in the bone marrow give rise to mature macrophages which are distributed ubiquitously in all tissues (Metcalf, 1989; Gordon & Taylor, 2005). Adhesion molecules, particularly integrins, have been shown to up-regulate when monocytes differentiate into macrophages suggesting that integrins play an important role in macrophage adhesion, migration and tissue infiltration (Ammon et al.2000; Andreesen *et al,* 1990; Shi & Simon, 2006). Blood monocytes and tissue resident macrophages are important *in vivo* cell targets of HIV-1 infection (Meltzer et al, 1990; Gartner *et al,* 1986; Cassol *et al.,* 2006). Macrophages may become chronically infected or serve as reservoirs of latent virus. Furthermore, HIV may sequester macrophage immuno-regulatory function that is inducing secretion of pro-inflammatory cytokines that lead to recruitment and activation of new target cells for HIV including CD4 T cells (Wahl *et al.* 2003; Wahl *et al.* 2006).

2.5 Target Cells

HIV produces its dominant effects on CD4 T cells which are lysis or formation of syncytia with adjacent CD4+ cells. CD4+ antigen presenting cells can harbour and release high titres of active virus. The CD4 antigens are the main receptor for HIV. However, CD4 alone is not sufficient for attachment but a second receptor (CXCR4 or CCR5) is acquired. CD4- (negative) cells including brain and haematopoietic cells may also be infected using such alternative receptors. CD4 molecule is a high affinity receptor for HIV.

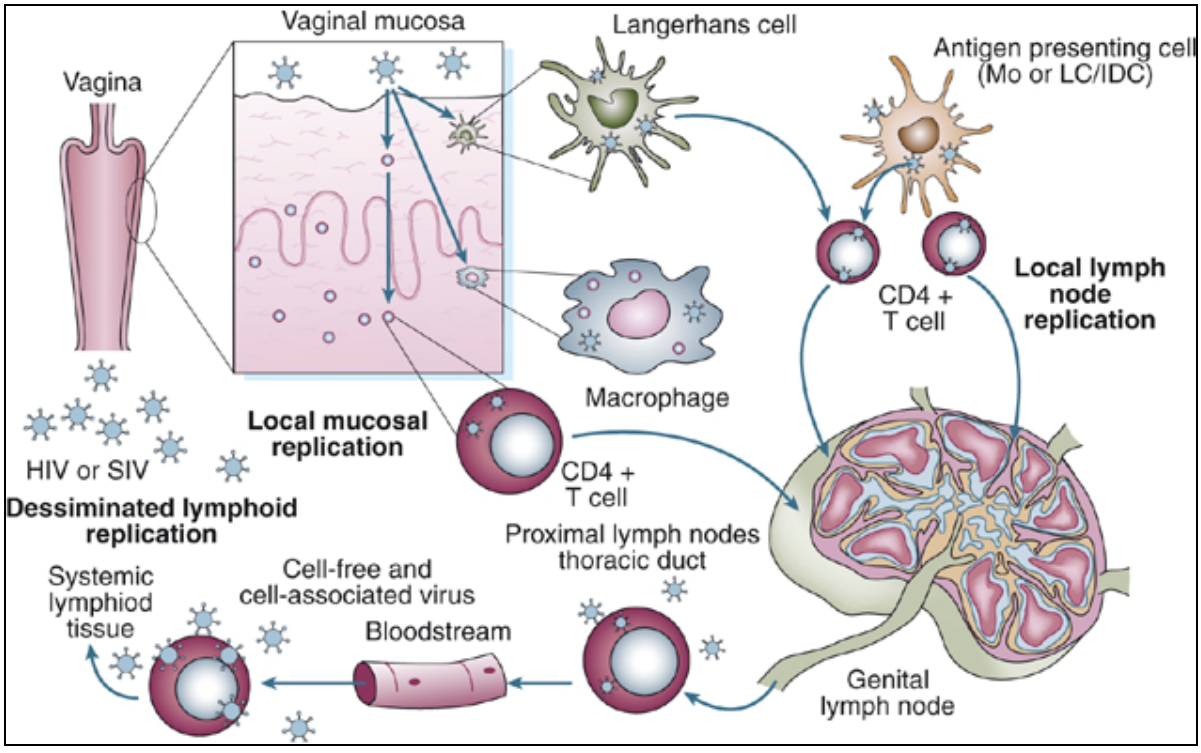

Figure 5. HIV target cells

2.6 HIV pathogenesis

The HIV envelope contains a glycoprotein surface (gp120) that is noncovalently attached to transmembrane protein (gp41). The initial step in infection is the binding of the gp120 envelope glycoprotein to CD4 molecules, this binding leads to conformational changes that result in the formation of a new recognition site on gp120 for the coreceptors CCR5 or CXCR4.

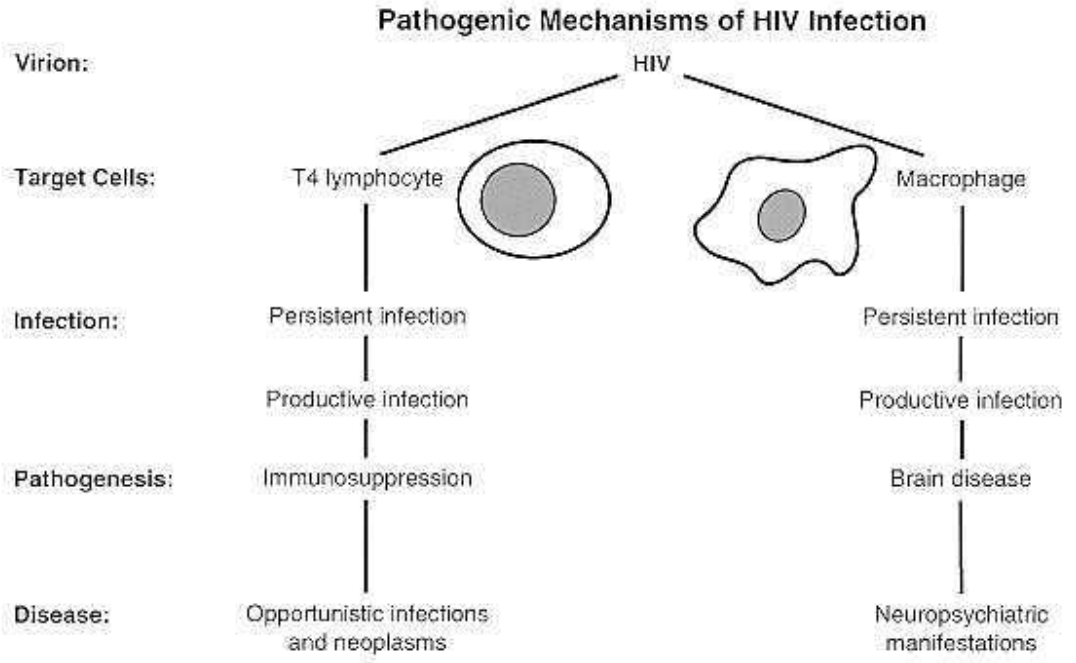

Figure 6. HIV mechanism of pathogenesis

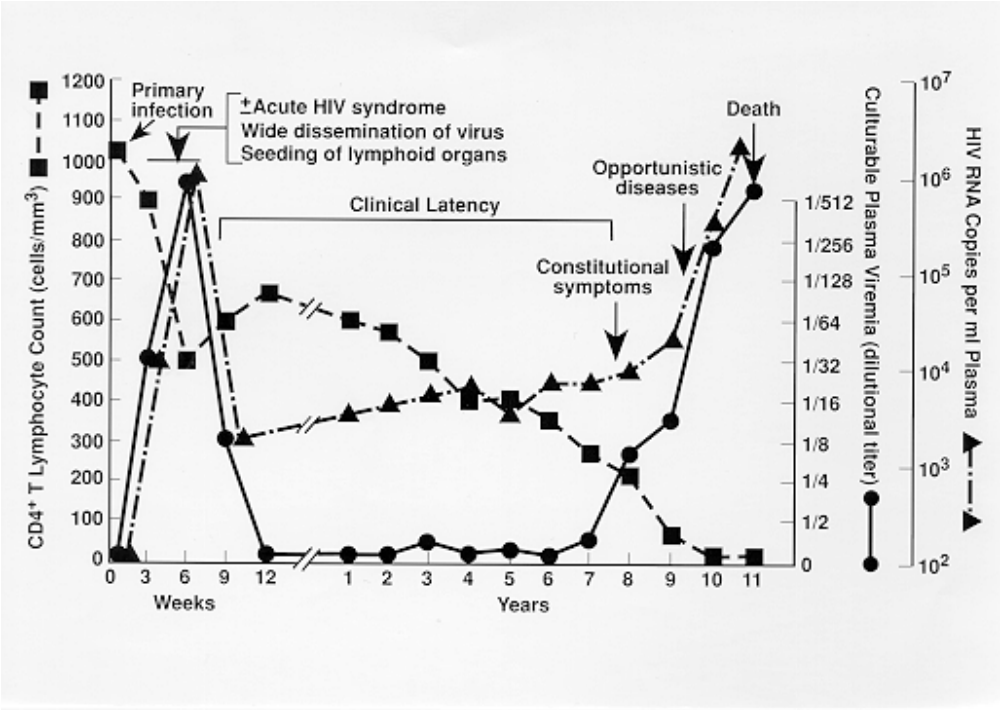

Figure 7. HIV course of infection

The next step involves conformational changes in gp41. These changes result in the insertion of a fusion peptide at the tip of gp41 into the cell membrane of the target cell (T cell or macrophage). After fusion, the virus core containing the HIV genome enters the cytoplasm of the cell. Primary T cells express both CCR5 and CXCR4 and hence can be infected by either of two viral types. In approximately 90% of cases the R5 (M tropic) type of HIV is the dominant virus found in blood of acutely infected individuals and early in the course of infection. However, over the course of infection, T tropic virus gradually accumulates; this type is especially virulent and causes the final rapid phase of disease progression.

The final ability to bind the co receptor resides in the gp120 glycoprotein of the

viral envelope, it follows that there must be molecular differences between gp120 molecule of M tropic and T tropic HIV. First, dendritic cells within the mucosal epithelium richly express CCR5 but do not express CXCR4 thus making them susceptible to infection by M tropic virus. Secondly, binding of M tropic strains to CCR5 on T cell may signal these cells to make chemotactic factors for other T cells, thus increasing the population of potential target in the vicinity of an infected T cell.

Once internalized, the RNA genome of the virus undergoes reverse transcription leading to formation of cDNA (proviral DNA). After this integration the provirus may remain locked into the chromosome for months or years and hence the infection becomes latent. Alternatively, proviral DNA may be transcribed with the formation of complete viral particles that bind to the cell membrane, such productive infection when associated with extensive viral binding, leads to cell death. Completion of the viral life cycle in latently infected cells occurs only after activation, and in the case of most CD4+ T cell virus activation results in cell lysis.

2.7 Bone marrow

The primary site of haematopoiesis in adult humans is the bone marrow. The BM is composed of cells including fibroblasts, endothelial cells, adipocytes, macrophages, osteoblasts, osteoclasts, reticular cells, and mesenchymal stem cells (MSCs), which are collectively called stromal cells (Alexaki & Wigdahl, 2008), megakaryocytes, and haematopoietic progenitor cells (HPCs). This is illustrated in Figure 8 below:

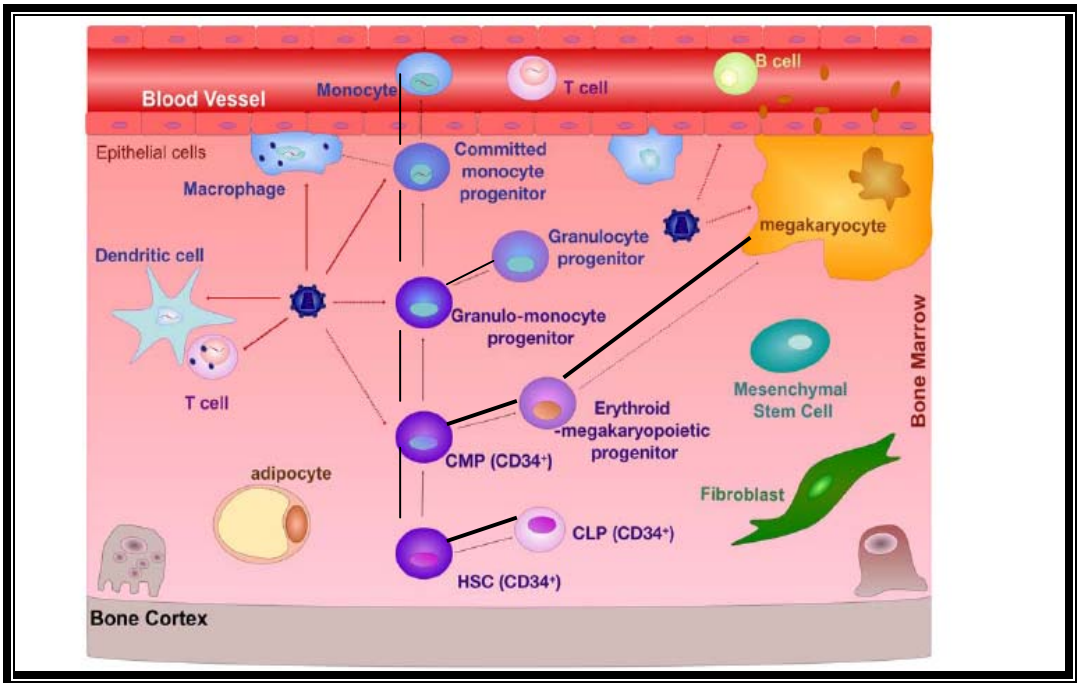

Figure 8. Cells of the BM that are susceptible to HIV-1 Infection.

The cellular components of the BM include HPCs at all stages of differentiation, megakaryocytes, fibroblasts, endothelial cells, adipocytes, macrophages, osteoblasts, osteoclasts, and MSCs, while DCs, T cells, and B cells may also migrate from the blood into the BM. The arrows in the figure above mean the following:

A black line - differentiation from one cell type to another.

A grey line - one or more intermediate cell types have been omitted from the respective differentiation process.

A red line - cells that are known to be infected by HIV-1.

A light red line - cells that have been shown to be infected by HIV-1, but the extent of their infection and their role in HIV-1 pathogenesis is questionable.

HPCs consist of a heterogeneous population of cells that includes the haematopoietic stem cells (HSCs), which are the most primitive HPCs, capable of producing of blood cell lineages. As a result of loss of the potential to develop, HSC become HPC committed to any number of specific haematopoietic cell lineages. Besides losing pluripotency, committed haematopoietic progenitors display a number of characteristics that distinguish them from their parents, namely the lack of capacity for self-renewal, a higher fraction of cells traversing the cell cycle, and a change in their surface protein profile (Alexaki & Wigdahl, 2008). HSCs are able to give rise to common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs). Part of lymphoid progenitor differentiation, and particularly T cell differentiation, occurs in the thymus. CLPs are capable of differentiating into T cells, B cells, natural killer (NK) cells, and plasmacytoid dendritic cell progenitors (Galy *et al.,* 1995). T cells and NK cell progenitors also share a common progenitor often referred to as the T/NK cell progenitor (Ikawa *et al.,* 1999). Myeloid progenitor differentiation occurs exclusively in the BM.

The CMPs, which are also referred to as erythromyeloid progenitors, can differentiate into either erythroid/megakaryopoietic progenitors or granulo/monocyte progenitors. Further differentiation leads to commitment to a single lineage such as red blood cell, megakaryocyte, granulocyte, and monocyte-macrophage lineage. Of these cell types, only the megakaryocyte remains in the BM following maturation and generates platelets by fragmentation of the exvaginations of their membrane, while all others emigrate into the blood. Leukopenias and dysplasias that may affect any of the haematopoietic lineages are

commonly observed within the population of patients with HIV-1, even in the absence of opportunistic infections of the BM, neoplasias, or chemotherapeutic treatment, clearly suggesting that HIV-1 infection is associated with dysfunction within the process of haematopoiesis and that these alterations may very well be due to the direct effects of infectious virus and viral proteins in the BM.

Mechanisms of HIV-1-induced myelosuppression direct infection of HPCs, inability of stromal cells to carry out their normal functions, toxic effects of HIV-1 proteins, and changes in the cytokine milieu are the most plausible mechanisms for the deleterious consequences of HIV-1 replication shown in the BM (Figure 9).

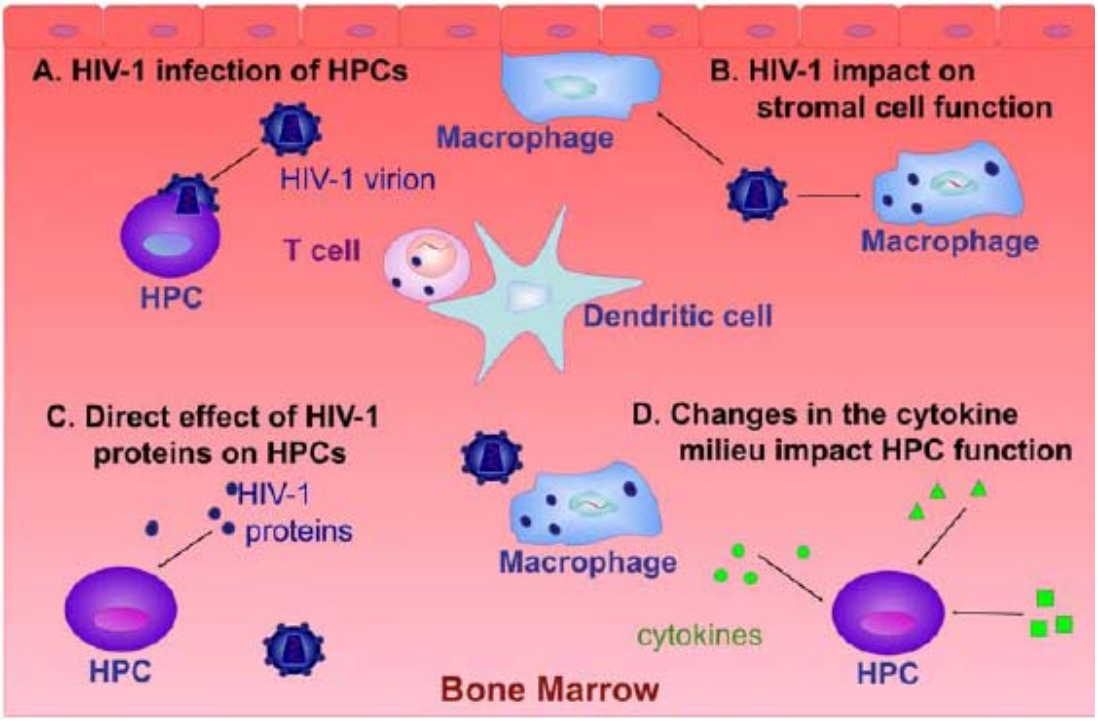

Figure 9. Mechanism of HIV-1-Induced Myelosuppression.

Several mechanisms may be involved in HIV-1-induced impairment of haematopoiesis. (A) HPCs may be infected by HIV-1. (B) The interaction of HIV-1 proteins with HPCs may have a direct effect on haematopoiesis. (C) HIV-1 may indirectly affect HPCs by interacting and/or infecting BM stromal cell populations, making them unable to support normal HPC functions. (D) HIV-1 replication in the BM may lead to changes in the cytokine milieu, which may in turn profoundly impact HPC function.

The BM stroma provides structure, cell-to-cell interactions, and a complex network of inhibitory and stimulatory cytokines that are crucial for the maintenance, differentiation, and proliferation of HPCs. Changes in the BM stromal structure have been observed in patients with HIV-1 and are characterized by a decrease in the fibroblastic population paralleled by an increase of macrophage-like cells. Changes in the clonogenic capacity of BM mesenchymal stem cells, which are responsible for the generation of heterologous stromal lineages, including fibroblasts, have been shown and may be responsible for the altered cellular composition of the BM stroma. It has also been suggested that BM microvascular endothelial cells are involved in BM stromal impairment in individuals with HIV-1, exhibiting a decreased capacity to respond to regulatory signals that would normally augment blood cell production (Hoffbrand *et al.,* 2005; Greer *et al.,* 2009).

2.8 Haematologic complication of HIV

Cytopenia is common in HIV disease and is often associated with morphological abnormalities in peripheral blood and bone marrow cells suggestive of myelodysplasia.

Anaemia is the most frequent cytopenia observed in HIV 1 infection and is often a complication of antiviral drug therapy (Greer *et al.*, 2009). Reduced vitamin B12 levels have been reported 10-35% of patients infected with HIV and many of these patients have gastrointestinal symptoms, or are in the later stage HIV disease. The mechanism underlying the reduced serum cobalamin in HIV disease is malabsorption. The major cause of anaemia in HIV disease is impaired erythropoiesis secondary to marrow dysfunction and impaired response to erythropoietin. The anaemia characteristic of chronic disease is usually normochromic, normocytic with a low reticulocyte count. As with others cytopenias, inhibitory cytokines play a central role. Occasionally, patients develop chronic red cell aplasia following parvovirus infection.

Increased red cell destruction may be seen in HIV infected patients with glucose-6-phosphatase dehydrogenase (G-6-PD) deficiency who are exposed to oxidant drugs; in patients with disseminated intravascular coagulation (DIC) or thrombotic thrombocytopenic purpura (TTP).

Involvement of the gastro intestinal tract (GIT) by various infections or tumours may lead to chronic blood loss with eventual iron deficiency anaemia.

Another prominent cause of hypoproliferative anaemia in HIV infected patients is exposure to radiation, which may cause marrow and/or red cell suppression. Drugs may also cause or exacerbate anaemia *(zidovudine, ganclovir and chemotherapeutic agents)* all of which lead to marrow suppression (Hoffbrand *et al.,* 2005). Infection of BM by Parvovirus B19 is another cause of hyperproliferative anaemia in HIV infected patients resulting in a specific infection of pronormoblasts. Thus, although marrow failure affecting all three cell lines has been described in association with Parvovirus B19 infection, a pure red cell aplasia is the usual consequence (Beutler *et al.,* 1995)

Neutropenia increases as incidence in HIV disease progresses although may be present in asymptomatic individuals (Hoffbrand *et al.,* 2005). Anti-neutrophil antibodies have been detected in some patients with HIV. The risk of infection increases when neutrophil count falls to below 0.5 x 10^9/l (Hoffbrand *et al.,* 2005). One relatively specific neutrophil abnormality is the presence of detached nuclear fragments (Bain, 2008).

Mild to moderate thrombocytopenia is common in patients with HIV and is rarely associated with bleeding. Thrombocytopenia is generally associated with decreased survival except in patients with advanced disease, where bone marrow failure is more prominent (Greer *et al.,* 2009).

Opportunistic infections, spenomegaly and fever reduce platelet survival. However, the HIV disease itself, as well as the toxicity of antiretroviral drugs may result in marrow impairment. The cause of reduced platelet production in HIV infection may be due to direct infection of the megakaryocytes by HIV. Human megakaryocytes bear CD4 receptors, which are capable of binding HIV-1 and can be internalised by human megakaryocytes. The HIV-1 receptors CCR5 and CXCR4 are present on megakaryocytes and platelets.

Several mechanisms for development of platelet associated antibodies have been described (HIV related ITP). Presence of immunochemically characterised platelet specific antibodies against GPIIb and /or GPIIIa has been described.

A further mechanism of antibody induced destruction of platelets arises from absorption of immune complexes against HIV, onto the platelets' Fc receptors, thus providing a free Fc portion for subsequent macrophage binding and phagocytosis. High levels of platelet bound IgG and complement are seen in HIV related Immune Thrombocytopenic Purpura (ITP) than in classic ITP.

The commonly observed dysplastic changes in the megakaryocyte series suggest that dysplasia is an important cause of ineffective platelet production and may be the dominant factor in the thrombocytopenia of advanced HIV disease (Hoffbrand *et al.,* 2005).

Patients who are infected with HIV also show progressive lymphopenia. This is due to the fact that the primary target cells in HIV infection are T lymphocytes and when the T cells are infected, they are destroyed as the HIV progeny are released, resulting in reduced T cell sub population. T cells constitute 60-80% of the peripheral blood lymphocytes and 10-30% B cells and 2-10% non-T/ non-B cells. Productive infection of T cells and viral replication in infected cells is the major mechanism by which HIV causes lysis and subsequent destruction of CD4+ T cells. The selective loss of CD4+ helper cells results in the inversion of CD4: CD8 ratio in HIV infected patients.

2.9 Bone marrow examination in patients with HIV

Morphologic changes tend to be more pronounced in more immunosuppressed patients and are increased in frequency as the disease progresses; all cell lines can be involved. Megaloblastic changes and ring sideroblasts are frequent and dysplastic features involving at least one lineage increases with disease progression. There are significant differences in the numbers of erythroid precursors and in the morphology of megakaryocytes, which clearly differentiates patients with AIDS, from those with myelodysplastic syndrome. (Greer *et al.,* 2009 a).

2.9.1 Cellularity

The true marrow cellularity is better assessed on trephine biopsy, which is hypercellular in a majority of the patients. The difficulty in aspiration may in part be due to increased reticulin fibrosis, seen especially in hypercellular marrows. Hypercellularity of the bone marrow in the face of peripheral cytopenia is a very

common finding in HIV disease and is likely to reflect myeloid dysplasia and ineffective haematopoiesis. There is a correlation between observed marrow dysplastic changes and the peripheral blood finding of anaemia and leucopenia (Greer *et al.,* 2009).

2.9.2 Erythropoiesis

Erythropiesis may show mild dysplastic features, such as nuclear irregularity or fragmentation, large and poorly organised. Megaloblastic changes may be present in patients taking Zidovudine (AZT) (Kumar *et al.,* 2004).

2.9.3 Granulopoietic series

Myelodysplastic changes such as nuclear fragmentation and giant metamyelocytes are quite common (Orazi *et al.,* 2006).

2.9.4 Megakaryocyte series

Megakaryocytes are usually present in normal or increased numbers. Dysplastic features such as bare nuclei are a frequent finding. Clustering of megakaryocytes is also seen (Orazi *et al.,* 2006).

2.9.5 Plasma cells

Increased plasma cells and macrophage activity are seen in one third of the patients. Plasma cells represent a physiological response to antigenic stimulation by virus or other ineffective agents, or may be secondary to dysregulated B cell proliferation, due to HIV. The plasma cells are often morphologically abnormal and appear in

clusters. The marrow plasmocytosis is not confined to those patients with advanced HIV disease, in whom opportunistic infection could be implicated but is seen also in patients at an early stage, who have no recurrent infection (Greer *et al.,* 2009a).

2.9.6 Histiocytes

Reactive haemophagocytes have been described in patients suffering from wide variety of fungal, viral, bacterial and parasitic infection in patients with HIV. HIV is the trigger to histiocyte proliferation and phagocytosis, probably by initiation of cytokine production, resulting in macrophage stimulation (Greer *et al.,* 2009).

2.9.7 Opportunistic infections

Granulomas may be seen in marrow of patients secondarily infected with *Mycobacteria*. Bone marrow examination and culture have a contribution to make in the diagnosis of opportunistic infections (Greer *et al.,* 2009).

2.9.8 Acquired immunodeficiency syndrome (HIV) related malignancies

The development of malignancies is related to a number of factors including immunosuppression and concurrent infection with other viruses, such as human herpes virus 8 (HHV-8) and Epstein-Barr virus (EBV) or human papillomavirus (HPV), which foster malignant transformation (Engels, 2007; IAS, 2008; Greer *et al.,* 2009). These agents are considered members of gamma herpes virus family (Ballana *et al.*, 2009 a). Among the most common HIV associated lyphomas are Burkitt's lymphoma (BL) and diffuse large B cell lymphoma (DBCL) with immunoblastic-plasmacytoid differentiation (also involving the central nervous

system). Lymphomas occurring especially in HIV positive patients include primary effusion lymphoma (PEL) and its solid variants, plasmablastic lymphoma (PBL) of the oral cavity type and large B cell lymphoma arising in the Kaposi Sarcoma associated multicentric Castleman's disease (Ballana I. 2009b).

HIV associated lymphoproliferative disorders are a heterogenous group of diseases that arise in the presence of HIV immunosuppression. In most cases lymphomas developing in HIV positive patients are usually of the aggressive type (Bain *et al.,* 1996).

2.10 Gamma-herpesvirus associated Lymphomas

2.10.1 Hodgkin's Lymphoma

The EBV proteins EBNA-1, LMP-1 and LMP-2A are expressed in the RS cells, the malignant cell population of this tumour (Young & Murray, 2003). RS cells are derived from B cells that have passed through the germinal centre, as shown by the presence of somatic mutation in the rearranged Ig variable region of the immunoglobulin genes (Küppers *et al.,* 1994). Many of the hypermutations are incompatible with the expression of the functional B cell receptor (BCR) suggesting that RS cells may have developed from germinal centre B cells that should have been eliminated by apoptosis but managed to survive (Kanzler *et al.,* 1996; Bräuninger *et al.,* 2006).

LMP-2A interferes with normal B cell development, allowing BCR negative B cells to leave the bone marrow/ colonise peripheral lymphoid organs (Caldwell *et al.*, 1998) and induces transcription patterns in B cells which resembles that of HL RS cells (Portis *et al.,* 2003). LMP-2A is essential for continued proliferation of germinal centre B cells lacking a functional B cell receptor (Mancao *et al.,* 2005; Mancao and Hammerschmidt, 2007). Therefore, LMP-2A promotes the survival of germinal centre B cells and could therefore aid the development into RS cells.

LMP-1 may also induce an HL like transcriptional program in germinal centre B cell (Vockerodt *et al.*, 2008). EBNA-1 was shown to induce CCL20 secretion in RS cell lines and to thereby promote the migration of regulatory T cells which could be envisaged to down modulate EBV T cell response (Baumforth *et al.,* 2008). The protein tyrosine phosphatase receptor Kappa (PTPRK) suppresses the growth of HL cell lines and is down modulated by EBV (Flavell *et al.,* 2008). These results provide a suggestion of how EBV LMP-1, LMP-2A and EBNA-1 may contribute to the development of RS cells.

2.10.2 Kaposi Sarcoma Herpesvirus and AIDS lymphoma

KS associated herpes virus (KSHV) is the AIDS human herpes virus, its former name according to the international committee on taxonomy of viruses is HHV8. This virus causes Kaposi sarcoma, a cancer commonly occurring in AIDS patients as well as primary effusion lymphoma and some types of multicentric Castleman's disease (MCD).

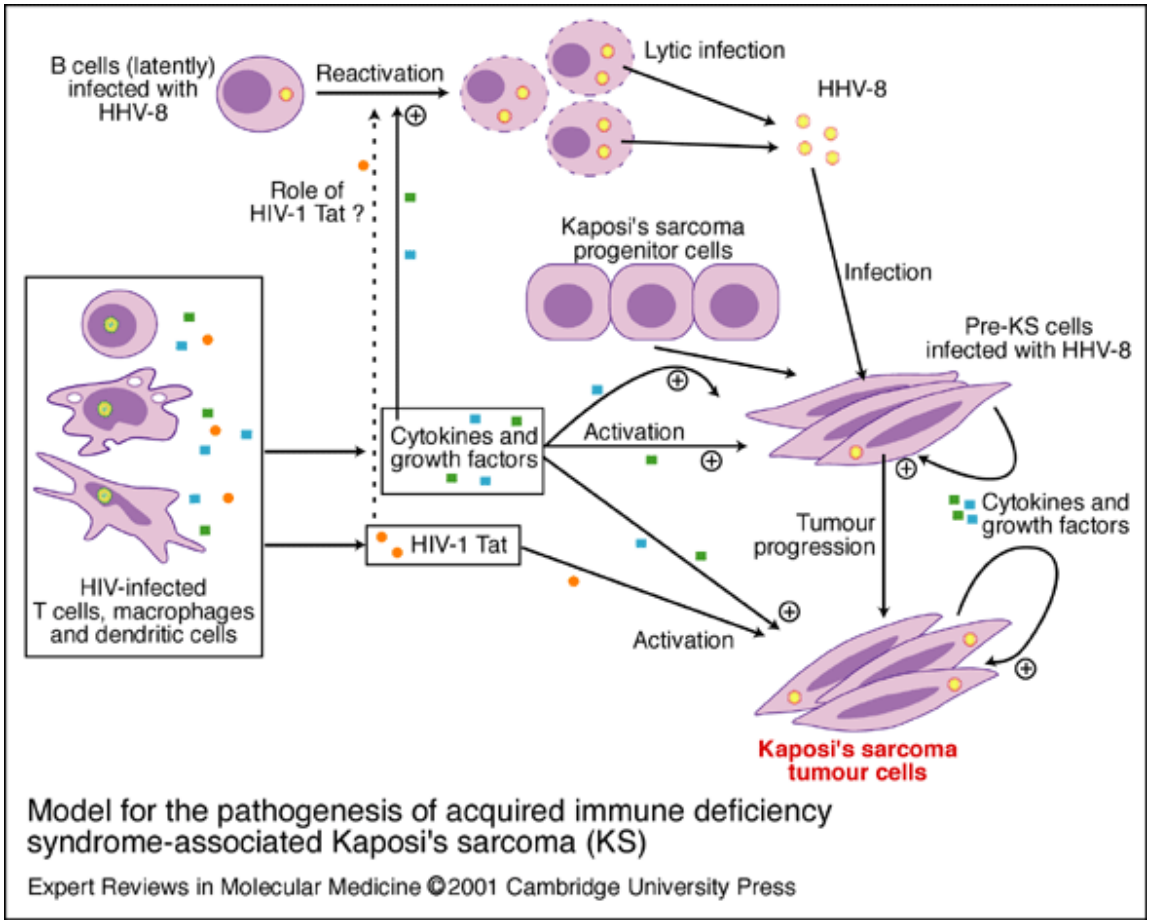

Figure 10. Model for the pathogenesis of acquired immune deficiency syndrome-associated KS

2.11 Virology

KSHV is a herpes virus and is a large double stranded DNA virus, with a protein covering that packages its nucleic acid called the capsid, which is then surrounded by an amorphous protein layer called the tegument. It is finally enclosed in a lipid envelope, derived in part from the cell membrane. The HHV-8 genome is approximately 165 kb long and has a central segment of low-GC DNA (L DNA, 140 kb), containing about 80 open reading frames and flanked by multirepetitive

high-GC DNA (H DNA). The HHV-8 genome, like other rhinoviruses, contains several genes that seem to be derived from the host cell. These genes code for enzymes involved in nucleotide metabolism, proteins interfering with the immune system (IL-6, IRF, chemokines) and regulators of cell growth *(cyclin D2, bcl-2)* (IAS, 2008).

After infection, the virus enters lymphocytes where it remains in a latent state. The virus exists as a naked circular piece of DNA called an episome and uses the cellular replication machinery to replicate itself. Various stimuli such as inflammation may provoke the virus to enter into *"lytic replication"*. When this occurs, the virus starts replicating itself in the form of linear DNA molecules that are packaged into virus particles which are expelled from the cell, to infect new cells or to be transmitted to a new host. When the virus enters into lytic replication, thousands of virus particles can be made from a single cell, which usually results in cell death (Wikipedia).

2.11.1 Primary Effusion Lymphoma

PEL cells contain multiple copies (50-150 copies/ cell) of episomal KSHV genomes (Lallemand *et al.,* 2000). In most cells, latent viral gene expression patterns involve the expression of the LANA, a viral D-type cyclin homologue (VCYC) of FLICE inhibitor protein (VFLIP) a premiRNA transcript encoding 11 viral miRNAs, as well as vIRF3/ K105/LANA-2 (Chang *et al.,* 1996; Schulz, 2006). In addition, a homologue of IL-6 is also expressed in some PEL cells (Parravicini *et al.,* 2000;

Schulz, 2001; Gaidano *et al.,* 2001). The viral IL-6 homologue VIL-6 is expressed in a subpopulation of PEL cells in vivo and in many KSHV B cells in MCD lymphoid follicles (Parravicini *et al.,* 2000; Katano *et al.,* 2000; Moore *et al.,* 1996). It induces proliferation, angiogenesis and haematopoiesis in IL-6 dependant cell lineages (Moore *et al.,* 1996; Burger *et al.,* 1998) and serves as an autocrine factor in the PEL cell lineage (Jones *et al*., 1999). It also induces vascular endothelial growth factor (VEGF) which has been implicated in the pathogenesis of PEL and KS (Aoki *et al.,* 1999). Therefore, VIL-6 may contribute to PEL cell proliferation and to the angiogenesis noted in patients with this lymphoma.

2.11.2 Multicentric Castleman's disease

Multicentric Castleman's disease (MCD), also called multicentric angiofollicular lymphoid hyperplasia, is an atypical, polyclonal lymphoproliferative disorder frequently associated with severe systemic symptoms. Whereas AIDS-related MCD is closely linked with KS (75% of cases), non-AIDS-related MCD is only associated with KS in 13% of cases. In patients affected by both KS and MCD, KS may be already present at diagnosis, or develop during the course of the disease. HHV-8 infection has been reported in 100% of AIDS-related MCD patients both with and without KS. Among MCD of the immunocompetent host, HHV-8 infection is restricted to approximately 40% of the cases. The biologic significance of HHV- 8 infection in MCD, and its relationship with KS development, is presently unclear. It is suggestive that MCD and KS share several features, including the fact that both proliferations display vascular hyperplasia, associated with immune dysregulation and are sustained by the growth factor activity of IL-6,

which is present at high levels in the involved tissues (IAS, 2008; Swerdlow *et al.,* 2008).

Among the KSHV associated pathology entities, MCD appears to be one with the highest number of productively infected cells (Judde *et al.,* 2000; Katano *et al.,* 2000). MCD B cells express VIL-6 and it is thought that its downstream effect on the B cell proliferation and VEGF secretion play a role in the proliferation and in the strong angiogenic component characteristic for MCD lesions. In patients with MCD, exacerbation of the disease was reported to correlate with increased viral load and increased IL-6 and IL-10 levels underlying the importance of productive viral replication and cellular cytokines in the pathogenesis of this disorder (Oksenhendler *et al.,* 2000).

2.11.3 Epstein-Barr virus associated lymphomas

Epstein-Barr virus (EBV) has been implicated in a wide range of lymphoproliferative disorders, including BL, classic Hodgkin's lymphoma (HL) and lymphomas arising in immunocompromised individuals (post-transplantation and HIV associated proliferative disorders) (Young & Rickinson, 2004; WHO, 1996).

EBV is also associated with B cell lymphoma in conjunction with congenital immunodeficiencies such as X-linked lymphoproliferative syndrome (XLP). T cell lyphoproliferative disorders that have been reported to be EBV associated include a subset of peripheral T cell lymphoma, angioimmunoblastic T cell lymphoma,

extranodular nasal type, natural killer T cell lymphoma (Young & Rickinson, 2004; WHO, 1996).

EBV associated lymphoma in AIDS include BL, diffuse large B cell lymphomas (DLBCL) with immunoblastic (IB) morphology primary central nervous system lymphoma (PCNSL), KSHV+, PEL and its solid variants and PBL of the oral cavity type (Raphael et al., 2001; Carbone *et al.,* 1999; Swerdlow *et al.,* 2008).

A defining property of PEL is its consistent association with KSHV infection. At least KSHV viral genes are expressed which provide proliferative and antiapoptotic signals. In cytospin preparations, the cells can have a range of appearances, from cells with an anaplastic morphology to large immunoblastic or plasmoblastic cells. Binucleated and multinucleated cells resembling Reed-Sternberg (RS) cells can be found. Nuclei are large, with prominent nucleoli, cytoplasm is usually abundant and deeply basophilic; some cells have cytoplasmic vacuoles. There is high proliferation rate with numerous mitotic figures. The cells often appear more uniform in histologic sections than in cytospin preparations. (Ansari *et al.,* 1996; Nador *et al.,* 1996).

Extracavitary PELs are usually immunoblastic in appearance and have a high mitotic rate and variable amount of apoptotic debris; some cases have a permanent *"starry-sky"* appearance. PELs are of B cell origin which can be demonstrated by the presence of clonal immunoglobulin gene rearrangement. Evidence points towards a post-germinal centre B cell derivation as most PELs contain somatic

hypermutation of Ig genes as well as frequent somatic hypermutation of the noncoding region of the *BCL6* gene and consistent expression of plasma cell markers such as CD138 and CD38 (Gaidano *et al.,* 1999; Maltocsy *et al.,* 1998).

Recently gene expression analysis of PEL showed features most similar to AIDS immunoblastic lymphoma and multiple myeloma, again indicating a preplasma cell or *"plasmoblastic"* cell. PEL cells commonly express CD45 but lack pan B cell markers including CD19, CD20 and CD79a as well as surface and cytoplasmic immunoglobulins (Knowles *et al.,* 1989; Nador *et al.,* 1996). However, cases of extra-cavitary PEL express immunoglobulin somewhat more often than the classical effusion PEL (Chadburn *et al.,* 2004). Expression of BCL6 gene is generally absent. Activation and plasmas cell markers and miscellaneous non lineage associated antigens such as HLA-DR, CD30, CD38, versus CD38c, CD138 and EMA are often expressed (Raphael et al., 2001; Cesarman *et al.,* 1995; Swerdlow *et al.,* 2008; Carbone et al., 2001; Klein *et al.,* 2003).

2.11.4 PEL and KSHV unrelated effusion lymphomas

PEL needs to be differentiated from those lymphomas occurring in patients in whom effusion complicates a tissue based lymphoma, the so-called secondary lymphomatous effusion.

A case diagnosis requires differentiation of PEL from other types of lymphoma primarily involving the serous body cavities that can present with a primary neoplastic effusion (Carbone & Gloghini, 1996; Carbone & Gloghini, 2006;

Kobayashi *et al.,* 2007; Matsumoto *et al.,* 2005; Simonelli *et al.,* 2003). Many of the cases are KSHV unrelated large B cell lymphomas, but KSHV unrelated PEL like lymphomas are also found (Kobayashi *et al.,* 2007). KSHV unrelated PEL like lymphoma cases are associated with hepatitis C virus (HCV) (30-40%), the most involved sites are peritoneum and pleura. Lymphoma cells most commonly show large cell type morphology and B cell immunophenotype. In contrast, PEL cases are universally associated with KSHV and mostly occur in immunodeficiency states. They demonstrate distinctive morphology and lack c-MYC gene rearrangement and B cell associated antigen expression that expresses PEL and KHSV unrelated PEL. Unrelated PEL-like lymphomas are different in terms of pathogenesis, morphology, immunophenotypic features, clinical behaviour and prognosis (Carbone *et al.,* 2008).

Lymphomas primarily involving the serous body cavity include a certain number of BL mainly occurring in the context of AIDS, which present as primary lymphomatous effusion without mass formation. The most specific biologic markers discriminating PEL from BL presenting as a primary lymphomatous effusion are represented by KSHV infection, which clusters with PEL and by translocation of the c-MYC protooncogene, which segregates with BL. KSHV-unrelated large B cell lymphoma, also termed as KSHV-unrelated PEL like lymphoma, shows evidence of KSHV infection, but display features related to large B cell lymphoma.

Large B cell lymphoma arising in KSHV associated MCD (Multicentric Castleman's Disease). Castleman's disease actually represents several different clinical pathologic entities. Prior to the discovery of KSHV, two histologic types of CD had been described; in the hyaline vascular (HV) variant, that is the most common form affecting 90% of patients usually involves a single lymph node in mediasternum; the plasma cell (PC) variant which is characterized by hyperplastic germinal centres, abundant plasma cells in the interfollicular areas, persistence of sinuses and associated clinical and laboratory abnormalities (Ballon & Cesarman, 2006). Two clinical entities were also described; (1) localized forms, which usually present as single lymph node hyperplasia in a single lymph node bearing region (mediasternum); (2) MCD, which manifests as generalised lymphadenopathy with systemic symptoms and characterised by a more aggressive clinical course and the potential for malignant transformation. MCD resembles the PC variant histopathologically. The PC variant can also be localised and the histologic appearance of MCD is somewhat different, so MCD should be classified separately. Besides primary MCD cases associated with other diseases, secondary MCD is common. Secondary MCD is a large and heterogenous group of clinical entities and is often referred to as interleukin-6 (IL-6) syndrome because of evidence that an overproduction of IL-6 probably in association with other cytokines occurs in MCD associated disease as well as in MCD itself suggesting a common underlying pathogenic mechanism.

Approximately half of the cases of MCD occurring in immunocompetent patients and in almost all those infected with HIV are associated with KSHV positivity

suggesting a pathogenic role of the virus in this disease (Soulier *et al.,* 1995). A plasmablastic variant of MCD is characterised by the presence of medium-sized to large lymphoblastic cells scattered in the mantle zone of the follicles, most frequently in the HIV infected individuals. Secondary MCD can be found in association with a variety of pathologic conditions, including HIV infection, plasma cell dyscrasias *(eg. POEMS syndrome)*, KS, B cell lymphoma, and HL. In KSHV positive cases, a common association is KS and a specific variant of NHL referred to as plasmablastic lymphoma, the so called large B cell lymphoma arising in KSHV associated MCD (Dupin *et al.,* 2000; Souller *et al.,* 1995). This lymphoma is specifically associated with KSHV and is considered a KSHV linked disease entity.

2.11.5 Plasmablastic lymphomas (PBLs)

Plasmablastic lymphomas (PBLs) of the oral cavity type were first described as lymphoma occurring mostly in HIV positive individuals having an unusual immunophenotype (low or no CD45 and CD20) and frequent presence of EBV (Delecluse *et al.,* 1997). This rare entity typically involves the jaw and oral cavity of HIV patients even if it has been documented in sites other than oral cavity such as the anorectum, nasal and paranasal region, skin, testes, bone and lymph nodes. PBLs of the oral cavity type are composed of large neoplastic cells with a very high proliferation rate, displaying a marked degree of plasma cell differentiation and features of CD are absent (Delecluse *et al.,* 1997; Carbone *et al.,* 1999; Dong *et al.,* 2005). The neoplastic cells have round nuclei, moderately clumped chromatin, a single prominent nucleolus and moderate to abundant basophilic cytoplasm with an

eccentric nucleus (Dong *et al.,* 2005). The mitotic rate is very high and there are frequent apoptotic cells and single cell necrosis. The cytoplasm is usually deeply basophilic with a paranuclear hof while binucleation and multinucleation are common. Cells with features of maturing plasma cells can be seen and there is usually a spectrum of differentiation that can be appreciated morphologically. Phenotypically, PBLs of the oral cavity type display an unusual profile characterised by a weak or absent expression of B cell antigen (for example CD20 and PAX5) coupled to strong immunostaining with the plasma cell markers CD138/ syndicam-1, MUM1/ IRF4 and VS38c. CD45 is expressed in most cases but may be weak or negative. A recent study reported that only five of 11 cases express cytoplasmic Ig, which were IgGκ or IgGλ (Dong *et al.,* 2005). EBV can be detected by EBEr or ISH, but LMP-1 and LMP-2 are not expressed. This is consistent with a restricted latency, which is in contrast to AIDS related IB (immunoblastic lymphoma) that usually expresses LMP-1 (WHO, 1996).

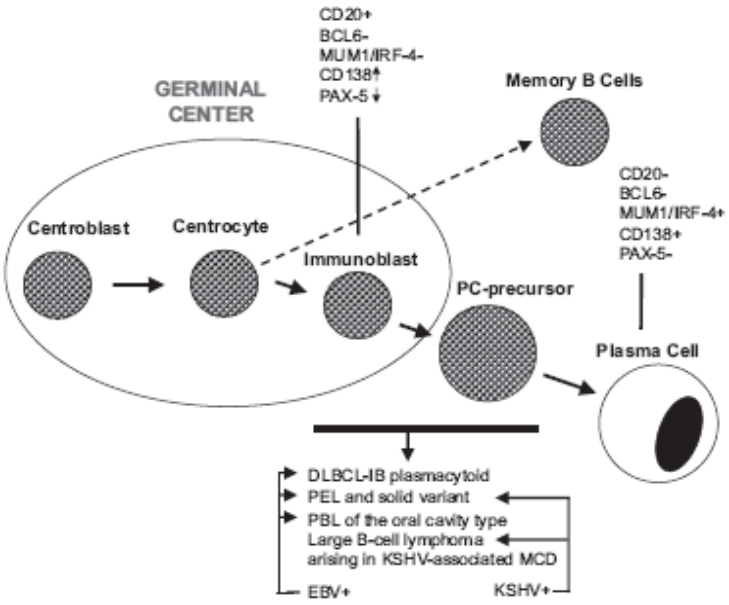

Figure 11. Diffuse Large B cell Lymphoma pathogenesis

2.11.6 Diffuse large B cell lymphoma

DLBCL, the transforming EBV protein LMP-1 is frequently expressed (Hamilton-du Toit *et al.,* 1993; Carbone *et al.,* 1993). LMP-1 plays a crucial role in the transformation of B lymphocytes by EBV. LMP-1 activates the NFKD as well as the GNK and p 38 pathway (Mosialos *et al.,* 1995; Eliopoulos *et al.,* 1999) by recruiting cellular TRAF1-3 and TRADD molecules to short sequence motifs, CTAR-1 and CTAR-2 respectively in the cytoplasm domain of the LMP-1 molecule (Huen *et al.,* 1995; Izumi *et al.,* 1997).

In B cells, LMP-1 increases the expression of the antiapoptotic proteins A_{20} and bcl-2, the adherin molecule CD54/ICAM-1 and the cell cycle regulator $P27^{kit}$

(Gloghini *et al.,* 2002). In DLBCL, expression of LMP-1 correlates inversely with the expression of BCL6, a marker for germinal centre B cell suggesting that among DLBCLs the impact of EBV LMP-1 is likely to be strongest in tumours representing a post germinal plasmacytic differentiation profile (Gaidano *et al.,* 1998).

The role of EBV in DLBCLs has been considered as driving lymphoproliferation in defective T cell immunity against EBV (Rowe *et al*., 1991). DLBCL is always monoclonal. This suggests that in addition to the effects contributed by EBV LMP-1, additional factors such as genetic damage are likely to contribute to the pathogenesis of AIDS-associated DLBCL (Gaidano *et al.,* 1998).

2.12 Burkitt's Lymphoma

Burkitt's lymphoma was first described by Dr Denis Burkitt in Uganda although Sir Albert Cook, a missionary doctor had reported similar manifestations in children in 1887. The advent of HIV has changed the epidemiology of BL especially in the high burdened countries. BL is a B-cell lymphoma genetically characterized by a chromosomal translocation that results in deregulation of the c-MYC oncogene. There are three main forms of BL according to its geographic distribution, incidence magnitude and risk factors. There is the endemic BL (eBL) mainly found in Africa, which affects children between two to nine years of age; Sporadic Burkitt's lymphoma (sBL) the form subsequently described outside the African region, but similar to eBL and affecting mainly abdominal viscera and is not age specific; A third subtype of BL, which is based on its association with HIV

infection mainly affecting adults. HIV associated BL can be identified in any geographical area and in all age groups and is of great importance especially in Sub-Saharan Africa (Orem *et al.,* 2007).

Approximately 30 to 60% of AIDS BLs are EBV positive and the transforming EBV protein LMP-1 is not expressed in BL (Young & Rickinson, 1985). EBNA-1, a viral protein required for the replication and maintenance of the latent viral episomal DNA, is found consistently in BL cells (Young & Rickinson, 1985). The presence of latent EBV in BL cells has been shown to promote genetic instability (Kamranvar *et al.,* 2007), suggesting a mechanism by which latent EBV could contribute to genetic alteration required for the development of BL. Some latent EBV transcription patterns found in BL produce viral proteins that are likely to protect BL cells from apoptosis induced by the regulated c-myc expression (Kelly *et al.,* 2006). The importance of apoptosis protection during BCL in immortalisation has recently been highlighted by the failure of EBV deletion mutants lacking both viral bcl-2 homologues (BALF-1, BHRF-1) to efficiently immortalise human B cells (Altmann & Hammerschmidt, 2005). Given the strong apoptotic effect of overexpressed c-myc, the role of EBV in some cases of BL could therefore consist of protecting BL cells against this side effect of c-myc translocation.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design and sample population

The data were extracted from the DISA Laboratory Information System at the DGMH, Tertiary Laboratory from 2003 to 2008. The study reviewed the medical and laboratory records of HIV positive patients who underwent bone marrow examination for investigation of fever and/or cytopenia. Eighty (80) patients with HIV/AIDS were selected. The ages varied between 20 to 50 years. There was no gender preference and no pregnant women were included in this study.

3.2 Data Collection

The following data were collected from archives and stores:

a) Peripheral blood result

b) Reticulocyte count result

c) CD4 count result

d) Bone marrow result

e) Trephine biopsy result

3.3 Statistical Analysis

The statistical analysis was of a descriptive nature. Numerical variables were summarized by sample size, mean, minimum and maximum values. Categorical

variables were summarized by frequency counts and percentages. Where feasible, 95% confidence intervals were calculated for percentage values.

All statistical analyses were performed on SAS® Release 9.1.3, run under Microsoft® Windows® XP for a personal computer.

CHAPTER FOUR

RESULTS

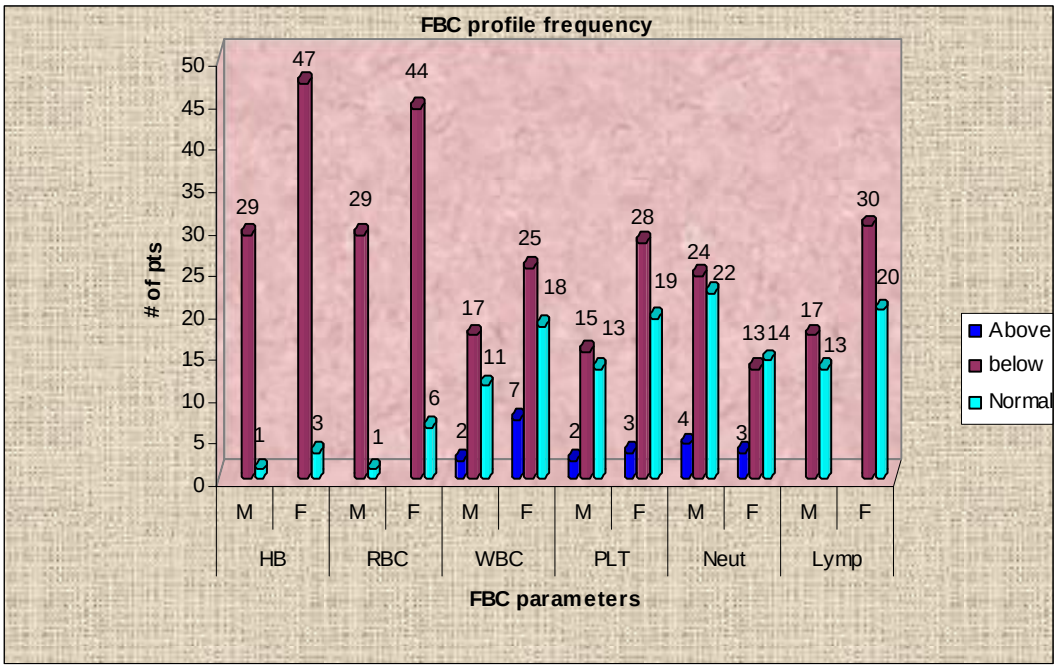

Figure 12. The distribution of Full Blood Count (FBC) parameters in the study Population

Twenty-five patients out of a total of 80 (31.25%), had pancytopenia. Of the 25, eight (32%) were males and 17 (68%) were females. In this study, pancytopenia was described as a haemoglobin concentration, granulocyte count and platelet count below normal ranges.

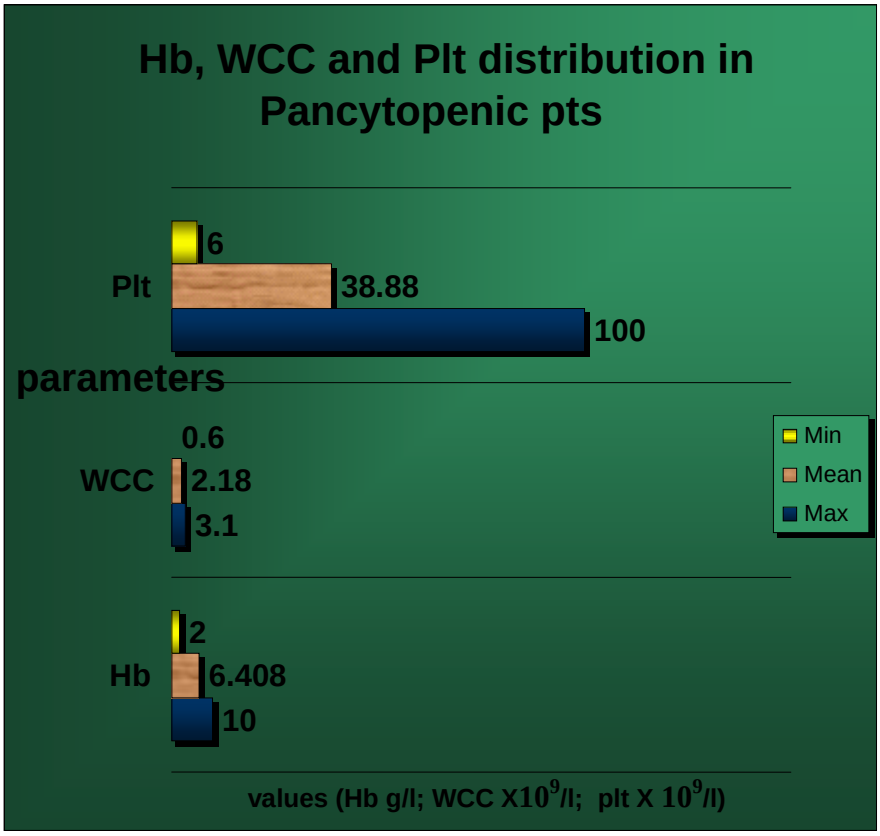

Figure 13. The WCC, Plt count and Hb distribution in pancytopenic patients

The white cell count (WCC) ranged from 0.6 to 3.1 x 10^9 /l with an average of 2.18 x 10^9/l. The haemoglobin concentration ranged from 2 to 10 g/l (average, 6.41 g/l). The platelet count in the study population had a range of 6 to 100 x 10^9/l.

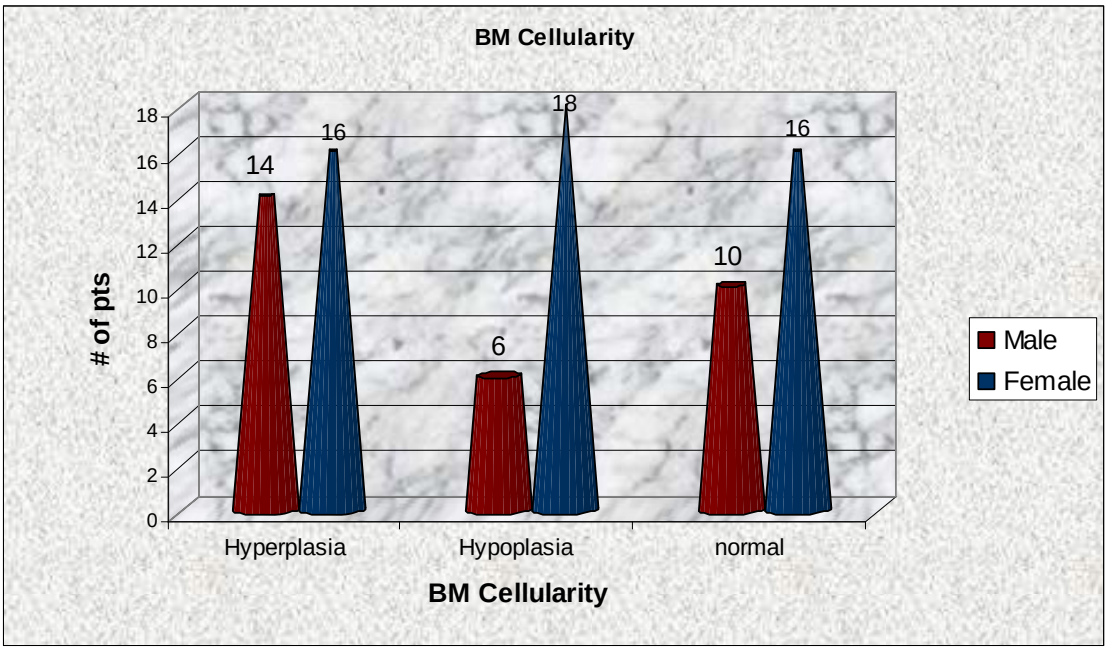

Figure 14: Bone marrow cellularity in the study population

The majority of the patients in the study were females representing 63% (50) of the total patients in the study population. Twenty-four patients showed hypoplasia in the BM with 75% (18) of them females.

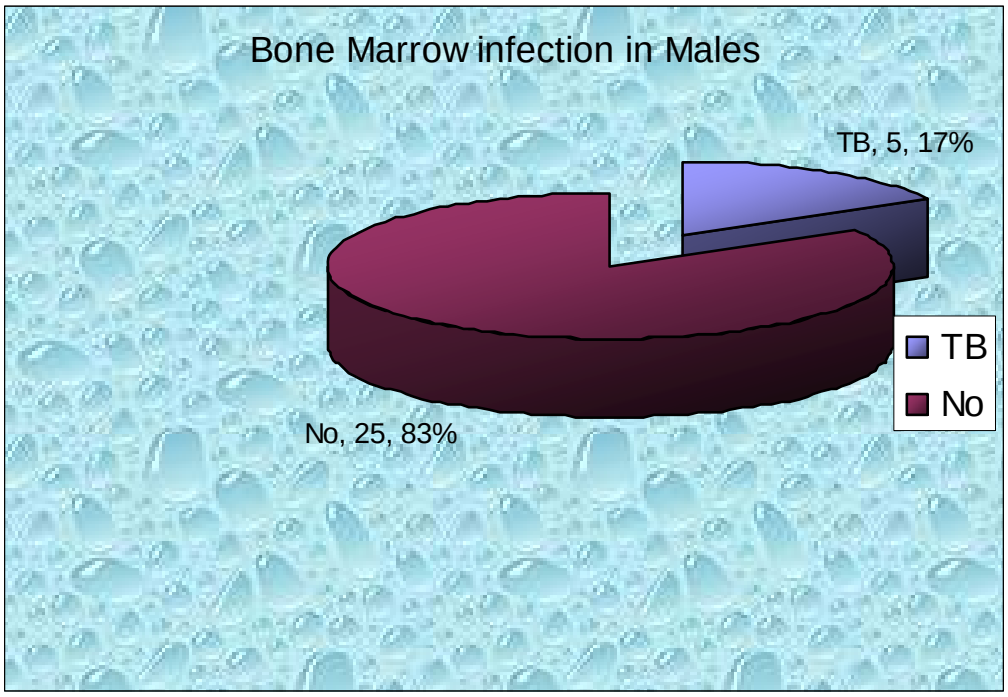

Figure 15. Bone Marrow infections in males

Among male patients in this study five (17%) patients had TB out of the 30.

Among female patients, five (10%) out of the 50 patients had TB.

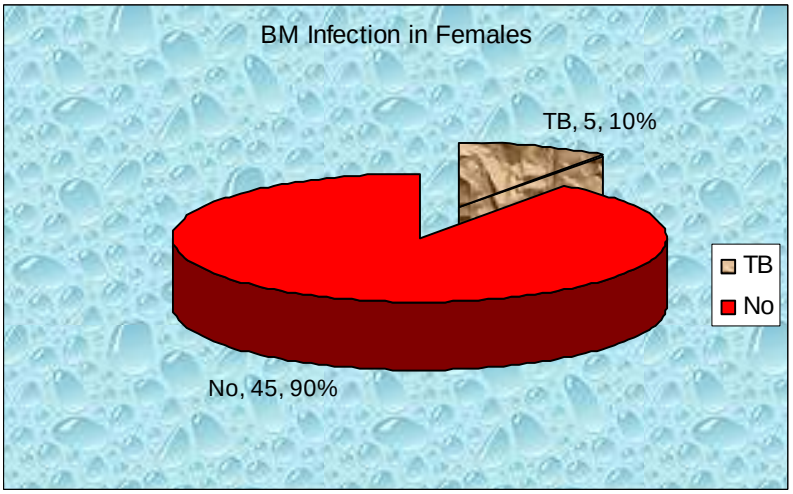

Figure 16. BM infection in females

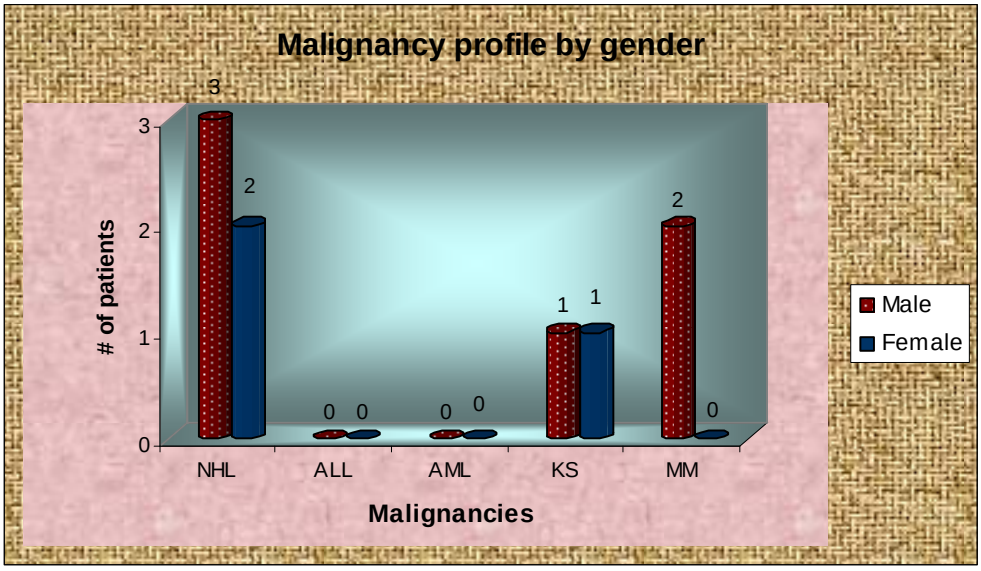

Figure 17. Malignancy profile by gender in the study population.

The majority of patients with malignancies were males, six out of nine (67%). Three of the five patients with NHL and both of the patients with multiple myeloma were males.

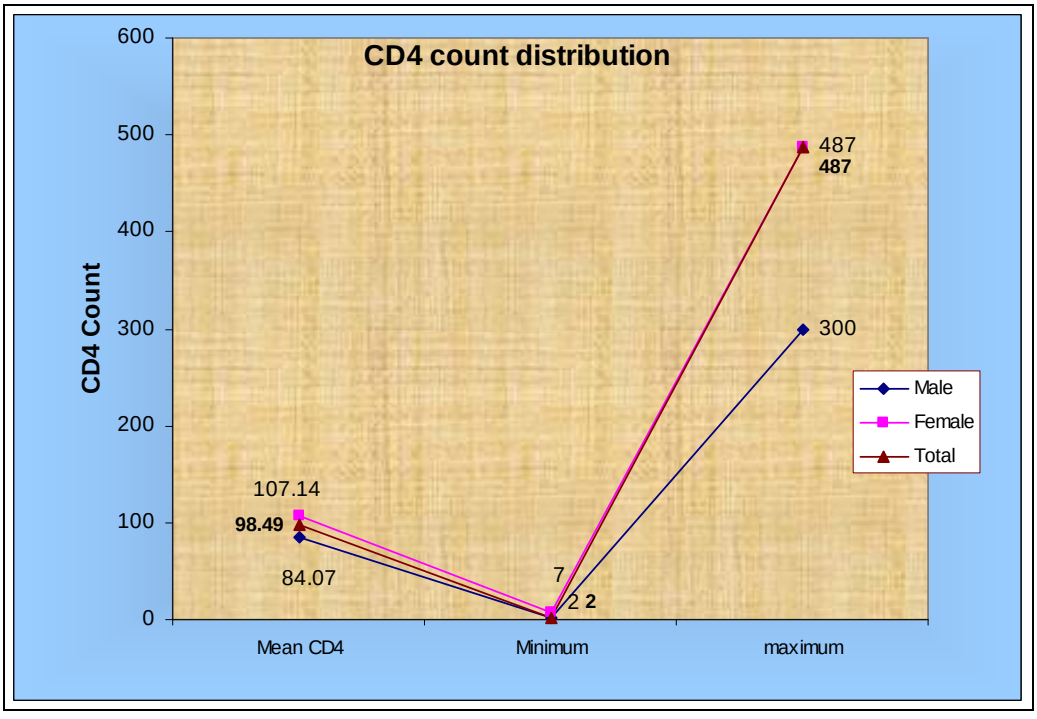

Figure 18. CD4 count distribution among the study population

The CD4 count in the study population ranged from 7 to 487 x 10^6/l.

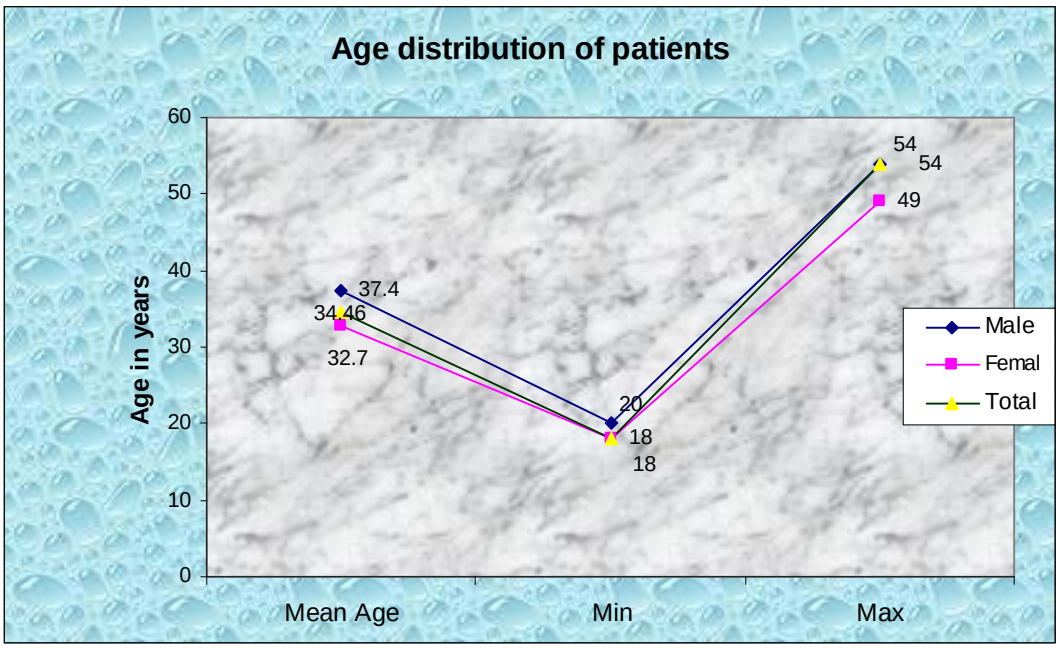

Figure 19. Age distribution among study population

In this study the age ranged from 18 years to 54 years of age with a mean of 34 for females and 37 years for males.

CHAPTER FIVE

DISCUSSION

The United States of America (USA) were the first to discover Acquired Immunodeficiency Syndrome in 1981. In 1983, the human immunodeficiency virus was first isolated from a patient with lymphadenopathy. By 1984, HIV was clearly demonstrated to be the causative agent of AIDS (Tripathi *et al.,* 2005a). The dissemination of HIV-1 in humans represented a catastrophic example of genomic transmission and expansion. All stages of HIV disease present with bone marrow abnormalities, varying with disease progression (Tripathi *et al.,* 2005b).

In this study, the majority of the patients were females (50 out of 80) (62.5%) and 30 were males (37.5%). A similar study was performed at Baragwanath Hospital in Johannesburg, South Africa a decade earlier and found similar proportions (Karstaedt *et al.,* 2000*).* The Johannesburg group found 54 % females and 45% males. In this study the mean age of the patients was 34 years (range 18 to 54 years) and similar to the earlier study where the mean was also 34 years (18 to 65 years). The similarity in age is likely due to the fact that the most affected persons by HIV are between 15 to 40 years (WHO, 2006).

In this study all 80 patients presented with cytopenia contrary to the study at Baragwanath Hospital where 53 out of 257 patients (21 %) had cytopenias. This may have demonstrated a late stage of presentation.

Ten out of the eighty patients in this study had TB representing 12.5 % of the total. These findings are almost half of that found in the Baragwanath study where 26% of the patients had TB (Karstaedt *et al.,* 2000). This may be due to a larger sample size used in the Baragwanath group compared to this study. TB was the only opportunistic infection found in this study. Other studies have also reported *Mycobacterium avium* complex (MAC) and cryptococcal infection (Karstaedt *et al., 2000*).

A study was done in USA involving 74 patients with HIV and bone marrow abnormalities and 7.2% of the patients had hypocellularity (Tripathi *et al.,* 2005a). However, this is contrary to what was found in this study, where 30% of the patients had hypocellularity. The difference in findings can be attributed to different settings and also sampling methods. In this study all HIV infected patients who were hospitalized and had BM examinations performed, were included.

In this study, 11% (9 out of 80) of the patients had malignancies, 55% of which had non-Hodgkins lymphoma (5 out of 9), 22% with Kaposi Sarcoma (2) and 22% with multiple myeloma (2). The Baragwanath study had three percent of the patients with malignancies, predominantly Hodgkin's lymphoma followed by NHL, MM and AML. This group did not report any KS but also had AML which was not found in our study.

The Johannesburg study also found patients with concomitant TB, one with AML and one with NHL. Similar findings were not found in this study.

In this study population, 85% of the patients had CD4 counts of < 200 x 10^6/l with the lowest being 2 x 10^6/l. Of these, six percent (4 out of 68) were TB positive. One study also illustrated that patients presenting with BM abnormalities have very low CD4 counts, less than 200 x 10^6/l (Tripathi *et al.*, 2005a).

CHAPTER SIX

CONCLUSION AND

RECOMMENDATIONS

This research project studied the haematological abnormalities and bone marrow changes in 80 patients with HIV seen at DGMH from 2003 - 2007. This study found there were more women than men affected but the difference was not statistically significant. Haematological abnormalities were present in all patients. Bone marrow involvement by TB occurred in 12.5% in the study population. Malignancies were seen more frequently in males; three patients with NHL, two MM and one KS. The difference in distribution between the sexes was not statistically significant *(p=0.391002).*

The purpose of BM and peripheral haematological examinations in HIV patients is to establish and evaluate peripheral cytopenias or to confirm the presence of systemic infection or malignancies. A bone marrow examination has been shown to be a valuable and frequently used test in our setting. However, it is not usually performed timeously and it is a relatively expensive investigation. It is recommended that health education and health promotion focus on the control of biological carcinogenic agents such as EBV, HPV and HHV-8 by routinely testing for these agents and also promoting positive reproductive behaviour among people living with HIV/AIDS. The use of non-invasive tests will be helpful in our setting where there is high TB prevalence. TB blood culture facilities need

to be widely available at primary level for sputum smear negative patients with unexplained fever weight loss and cytopenias.

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