HAEMATOLOGICAL CHARACTERISTICS OF HIV POSITIVE PATIENTS SEEN AT MUHIMBILI NATIONAL HOSPITAL AND THEIR RELATION TO THE CLINICAL STAGE

A Cross-sectional analytical study carried out at Muhimbili National Hospital

By

Magdalena Amani Lyimo, MD (DAR)

A dissertation submitted in partial fulfilment of the requirements for the degree of Master of Medicine (Internal Medicine) of the University of Dar es Salaam.

University of Dar es Salaam

March 2003

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the University of Dar es Salaam a dissertation entitled: *Haematological characteristics of HIV positive patients seen at Muhimbili National Hospital and their relation to the clinical stage,* in partial fulfilment of the requirements for the degree of Master of Medicine (Internal Medicine).

.....

Dr Ferdinand Mugusi

(Supervisor)

.....

Dr PM Magesa

(Second Supervisor)

.....

Prof Øystein Bruserud

(Third supervisor)

Date.....

DECLARATION AND COPYRIGHT

I, Magdalena Amani Lyimo, hereby declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature.....

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to all those who in one way or another made the production of this dissertation possible.

I would like to thank my supervisors Dr Mugusi, Dr Magesa and Prof. Øystein Bruserud for their guidance, contributions and advice. I extend my sincere gratitude to the members of the Department of Internal Medicine. To those who believed in me and for giving me the opportunity and providing me with invaluable knowledge in Internal Medicine, both at Muhimbili and Haukeland teaching hospital in Bergen. Gratitude goes to all those who helped with the laboratory work and especially Dr Magesa and Dr Nkya who helped report on the bone marrow aspirations. I would like to thank Candida Moshiro for her great assistance with the statistical analysis.

A special thanks goes to my father and late mother for giving me the most valuable gift of all, a solid educational foundation. I thank my sister Dr Elizabeth Lyimo and all the good friends who had faith in me and encouraged me constantly. Special mention goes to my husband, Dr Faustine Ndugulile for always encouraging me and supporting me in my career. Lastly I thank my daughter Martha for enduring being away from her mother most of the time in order to make this work possible.

ABSTRACT

Background

HIV infection in addition to having significant immunologic, infectious and neoplastic manifestations, has been reported to be associated with a number of haematological complications which result in significant clinical sequelae. The extent of haematological abnormalities in HIV infected patients in Tanzania had not been established.

Methods

In order to determine the haematological characteristics of HIV positive patients a cross-sectional analytical study was carried out. 196 patients admitted to the medical wards of Muhimbili National Hospital were recruited into the study between 15th of November 2001 to 28th December 2001. The total red and white blood cell counts, red blood cell indices, platelets and ESR were determined in each patient. HIV serostatus was determined in 183 patients. Bone marrow aspiration and examination was carried out in some patients. The differences in haematological characteristics were compared by HIV serostatus, and in those who were HIV positive, by CDC clinical stage. Statistical analysis was carried out using appropriate statistical methods and packages.

Findings

Patients did not differ much in terms of demographic and physical characteristics. Pulmonary tuberculosis and mucocutaneous fungal infections were found to be significantly higher in those who were HIV positive. The rest of the admitting diagnosis did not differ significantly between HIV positive and HIV negative patients. HIV positive patients were found to have a higher prevalence of blood cytopenias as compared to HIV negative patients. Anaemia, with a prevalence of 71.7% was the most frequent abnormality. The prevalence of anaemia was shown to increase with advancing clinical stage of HIV infection, but this difference was not statistically significant. The other cytopenias observed were, thrombocytopenia (37.4%), leucopenia (28.6%), and lymphopenia (12.2%). Normochromic-normocytic anaemia was common in HIV positive patients, as compared to hypochromic-microcytic anaemia in HIV negative patients, though this difference was not statistically significant.

HIV positive patients had lower mean red cell and white cell indices. Logistic regression analysis was done on haematological parameters which differed significantly in those who were HIV positive and HIV negative. Anaemia, lymphopenia and a raised ESR were found to be predictive of HIV positive serostatus.

Conclusion

Haematological abnormalities were more prevalent in HIV positive patients, with anaemia being the most prevalent. Anaemia, lymphopenia and a raised ESR were found to be predictive of HIV positive serostatus. These results highlight the existence of these abnormalities in HIV positive patients since they have important clinical implications.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
CDC	Centres for Disease Control
CFU	Colony Forming Units
ELISA	Enzyme Linked Immunosorbent Assay
EPO	Erythropoietin
ESR	Erythrocyte Sedimentation Rate
G-CSF	Granulocyte Colony Stimulating Factor
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
HCT	Haematocrit
HGB	Haemoglobin
HIV	Human Immunodeficiency Virus
IL-1	Interleukin 1
MAC	Mycobacterium Avium Complex
MCHC	Mean Coropuscular Haemoglobin Concentration
MCH	Mean Corpuscular Haemoglobin
MCV	Mean Corpuscular Volume
NACP	National Aids Control Programme (Tanzania)
TGF-β-1	Transforming Growth Factor-β -1
TNF	Tumour Necrosis Factor
UNAIDS	Joint United Nations Programme on HIV and AIDS

WHO	World Health Organization

1. INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

HIV/AIDS in addition to having significant immunologic, infectious, and neoplastic manifestations, is associated with haematological abnormalities. Infection with HIV has been reported to be associated with a number of haematological complications some of which may result in significant clinical sequelae.[1]

The mechanisms that lead to these changes are multiple. Qualitative and quantitative marrow defects and immune cytopaenias are a direct effect of HIV infection. The effect of opportunistic infections, lymphoma and a myriad of drugs against infections, malignancies and HIV infection itself also play an important role.[2, 3]

1.1.1 HIV/AIDS

AIDS was first described in 1981 among homosexual men in the United States of America. They were diagnosed as having by then a rare disease Pneumocystis Carinii Pneumonia (PCP) and other unusual infections. A similar picture was seen in intravenous drug users.[4] Over the past 22 years numbers have continued to rise and AIDS is now a global pandemic. At the end of the year 2002 there were 42 million adults and

children living with HIV/AIDS. 29.4 million were from Sub-Saharan Africa.[5]

In Tanzania the first cases of AIDS were reported in Kagera in 1983.[6] During the year 2001 a total of 14,112 AIDS cases were reported to the National AIDS Control Programme (NACP) from 20 regions. This resulted in a cumulative total of 144,498 cases of AIDS reported since 1983. Estimating that only 1 in 5 cases is actually reported, the actual cumulative total of AIDS cases since the beginning of the epidemic is estimated to be about 722,490. Of all the cases reported 79.2% fall within the age group 20- 49 with the highest number of cases in the age group 25 – 34 and 30 – 39 for females and males respectively.[7]

1.1.2 ANAEMIA

Anaemia is a worldwide problem. Globaly it is estimated that about 2 billion people suffer from anaemia, 9 out of 10 of these are from developing countries.[8] Anaemia has been recorded in African countries for a long time.[9, 10] Few studies have shown extremely high prevalence levels, 80% in some areas. Most studies however have shown prevalences between 30 - 50 % and 50% in child bearing age and in children under five respectively.[9, 10] In Tanzania anaemia is estimated to affect nearly 32% of the population. All age groups and both sexes are

affected suggesting that several factors may be involved as causes of anaemia.[9, 10] Anaemia features prominently among the top ten diseases in many hospitals all over the country.[9, 10] In Tanzania like in many other sub Saharan African countries there is a high prevalence of many tropical diseases that are associated with anaemia. Hookworm and Malaria are the two most important examples that are associated with anaemia. According to Nhonoli (1974) hookworm anaemia was common in Tanzania and accounted for 10% of all patients admitted with severe anaemia to the medical wards at Muhimbili Medical Center.[9, 10] A variety of haemoglobinopathies common in the tropics occurs in Tanzania and sickle cell anaemia appears to be the most common. In Dar es Salaam, approximately 17% of children entering hospital for any reason are carriers of the sickle cell gene.[11]

1.1.3 HIV AND ANAEMIA

Despite important advances in anti retroviral therapy, anaemia remains a problem in many HIV-infected patients. Although the incidence of anaemia in these patients has decreased, its prevalence appears to have stabilized or decreased only slightly.[12] In patients with HIV infection, anaemia occurs in 17% of asymptomatic patients .[13] The prevalence of anaemia in patients with AIDS has been estimated at 63% to 95%,

making it more common than thrombocytopaenia or leukopaenia.[14] The incidence of anaemia is associated with progression of HIV disease, use of certain therapeutics, being of African American descent, and female sex. [14] Haemoglobin level was found to be predictive of survival independent of the CD4 cell count, and the treatment and management of HIV- infected patients was associated with decreased risk of death.[14] Anaemia was also associated with an increased risk of death for HIV infected individuals. The incidence of anaemia was strongly and consistently associated with the progression of HIV disease as measured by diagnosis of an AIDS-defining illness and CD4 cell count of < than 200 cells/ul. Recovery from anaemia was shown to directly increase survival.[14]

Similar results have been found in other studies. In the EuroSIDA study, severe anaemia was found to occur infrequently among HIV infected patients. 58.2% had mild anaemia and 1.4% had severe anaemia.[15] Anaemia was associated with a much faster rate of disease progression. Among patients with similar CD4 cell counts and viral loads, the latest value of haemoglobin was a strong independent prognostic marker for death.[15]

In Zimbabwe, adults with HIV infecion were shown to have severe haematological derangements. Blood cytopaenias was found in 47.5% The most frequent abnormalities were lymphopaenia 31.5%, anaemia 30.8%, neutropaenia 29.6%, thrombocytopaenia 24.7%, eosinophilia 23.5% and leucopaenia 11.7%.[16,17] In Cote d'Ivoire the prevalence of anaemia was found to be significantly higher among HIV positive pregnant women as compared to HIV negative pregnant women.[18] In Kenya mortality attributable to anaemia was found to be 31% and that due to HIV infection 75%.[19] This shows the importance of both HIV infection and anaemia, and the need of establishing appropriate intervention measures.

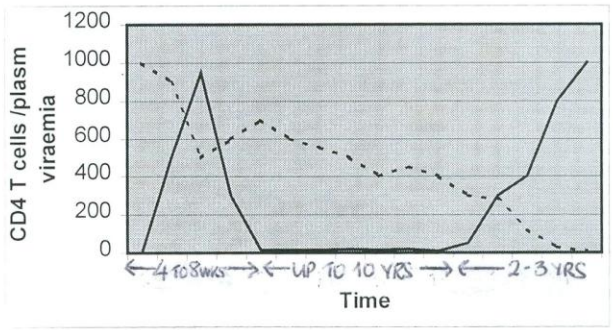
A study done in Tanzania on the immunological markers in HIV infected individuals, found that mean haemoglobin levels were lower in those who were HIV positive (8.3g/dl) compared to controls (11.6g/dl)[20] In a double blind, placebo controlled trial of vitamin A among children infected with HIV, being HIV infected was one of the independent determinants of adverse haematological profiles.[21] In a different study among HIV infected pregnant women in Tanzania, iron deficiency and infectious disease were found to be predominant causes of anaemia. Other factors associated with anaemia were BMI less than 19kg/m^3,

malaria parasites more than 1000/mm^3, geophagia and CD4cell count less than 200/μl.[22]

1.2 PATHOGENESIS AND PATHOLOGY OF HIV

Acquired Immunodeficiency Syndrome (AIDS) is a result of destruction of the CD4+T lymphocytes by the human immunodeficiency virus. The disease is characterised by many opportunistic infections and malignancies.[23, 24] Two types of HIV have been demonstrated, HIV-1 which occurs world-wide and HIV-2 which occurs predominantly in West Africa. Several genetic subtypes of the major (M) group of HIV-1 designated A- K have been described and their distribution varies in different parts of the world. HIV-1 is responsible for a more virulent disease and epidemic compared to HIV-2.[6]

Figure I; Typical course of HIV infection



The typical course of untreated HIV infection spans about a decade. Stages include the primary infection, dissemination of virus to lymphoid organs, clinical latency, elevated HIV expression, clinical disease and death. The duration between primary infection and progression to clinical disease averages about 10 years. Death usually occurs within 2 years after the onset of clinical symptoms.[23, 24] Progression time to the development of AIDS is highly variable and may rarely occur within a year, or take more than 10 years. Based on data as of 1995, it is believed that the median period to the development of severe immune deficiency as measured by a CD4+ cell count of less than 200/mm^3 is 8 to 10 years. In a cohort study done in Uganda, since 1990, 107 HIV prevalent cases, 168 incident cases and 235 HIV seronegative controls were recruited. Median time from seroconversion to death was found to be 9.8 years. When the patients died many were severely immunocompromised and had clinical features of AIDS.[25]

Following primary infection, viral replication occurs and viraemia is detectable for about 8 – 12 weeks. The virus is widely spread throughout the body during this time, and the lymphoid organs become seeded. There is a significant drop in numbers of circulating CD4 T cells at this early time. An immune response to HIV occurs 1 week to 3 months after infection, plasma viraemia falls, and the levels of CD4 T cells rebounds.

However, the immune response is unable to clear the infection completely, and HIV infected cells persist in the lymph nodes. This period of clinical latency may last 10 years. During this time, there is high level of viral replication. It is estimated that 10 billion HIV viral particles are produced and destroyed each day. CD4+ T lymphocytes, major targets responsible for viral production, appear to have similar turnover rates. Higher levels of virus are readily detectable in the plasma during the advanced stages of infection. HIV in patients with late stage disease is usually more virulent and cytopathic than the strains found in early disease.[23, 24]

1.2.1 CD4+ T lymphocytes

The cardinal feature of HIV infection is the depletion of T helper- inducer lymphocytes- the result of the tropism of HIV for this population of lymphocytes, which express the CD4 phenotypic marker on their surface. The CD4 molecule is the major receptor for HIV; it has a high affinity for the viral envelope. Early in infection, primary HIV isolates are M-tropic (monocyte or macrophage). However, all strains of HIV infect primary CD4+ T lymphocytes. As the infection progresses, the dominant M-tropic viruses are replaced by T-tropic (lymphocyte) strains of the virus. Consequences of CD4+ T cell dysfunction caused by HIV are devastating because the CD4+ T lymphocyte plays a critical role in the

human immune response. It is responsible for induction of a wide array of lymphoid and nonlymphoid functions. These effects include activation of macrophages, induction of functions of cytotoxic T cells, natural killer cells, and B cells; and secretion of growth and differentiation of lymphoid cells and affect haematopoietic cells. Macrophages and monocytes play major role in the dissemination and pathogenesis of HIV infection. The macrophage monocyte serves as a reservoir for the HIV virus in the body. Unlike the T lymphocyte it is relatively refractory to the cytopathic effects of HIV, so that the virus can not only survive in this cell but can be transported to other organs in the body such as lungs and brain. [23, 24]

Lymphoid organs play a central role in HIV infection. It is in lymphoid organs that specific immune responses are generated. Throughout the course of HIV infection, even through clinical latency, HIV is actively replicating in lymphoid organs. Cytokines are released, activating a large pool of CD4+ T cells that are highly susceptible to HIV infection. As the disease progresses the lymph node architecture becomes distorted allowing the release of large amounts of virus into the circulation.[23, 24]

1.2.2 Staging of HIV infection

In 1985, WHO developed a clinical case definition of AIDS for public health reporting that relies on specific combinations of major and minor signs/symptoms and diseases for the diagnosis of AIDS. In 1994, an expanded WHO case definition for AIDS surveillance for adults and adolescents was developed; the 1994 WHO surveillance definition incorporates major features of the WHO clinical definition and the 1987 CDC definition. Major features of the 1994 WHO definition include both pulmonary and extrapulmonary manifestations of tuberculosis associated with features of the wasting syndrome, and a positive HIV serologic test.[26]

The HIV disease staging system proposed by the WHO accounts for the independent information provided by clinical and laboratory data, and includes four stages defined by the combination of four clinical groups and three laboratory strata.[26, 27] The clinical groups are characterized by the presence of signs, symptoms, diseases, or a performance score, typically corresponding to asymptomatic infection (group 1), early disease (group 2), intermediate disease (group 3), or late disease (group 4). As to the laboratory strata, these are defined by different ranges of $CD4^+$ T-lymphocyte counts (>500, 200-500, or < 200 cells/mm^3) or total lymphocyte counts (> 2000, 1000-2000, or < 1000 cells/mm^3). The

stages are conceptualised as strictly progressive; that is, once a patient reaches a certain stage, he or she cannot revert to a lower one. In an analysis of a cohort of homosexual men, and another of a cohort of intravenous drug users, the WHO system was found to be prognostically meaningful.[27]

Many similarities may be identified between the WHO staging system and the CDC (1992a) classification of HIV infection. They differ, however, in relation to their purposes: the WHO staging system is concerned with providing prognosis information regarding an individual classified in a given stage; on the other hand, the CDC classification system is mainly descriptive, and intended for surveillance.[27, 28]

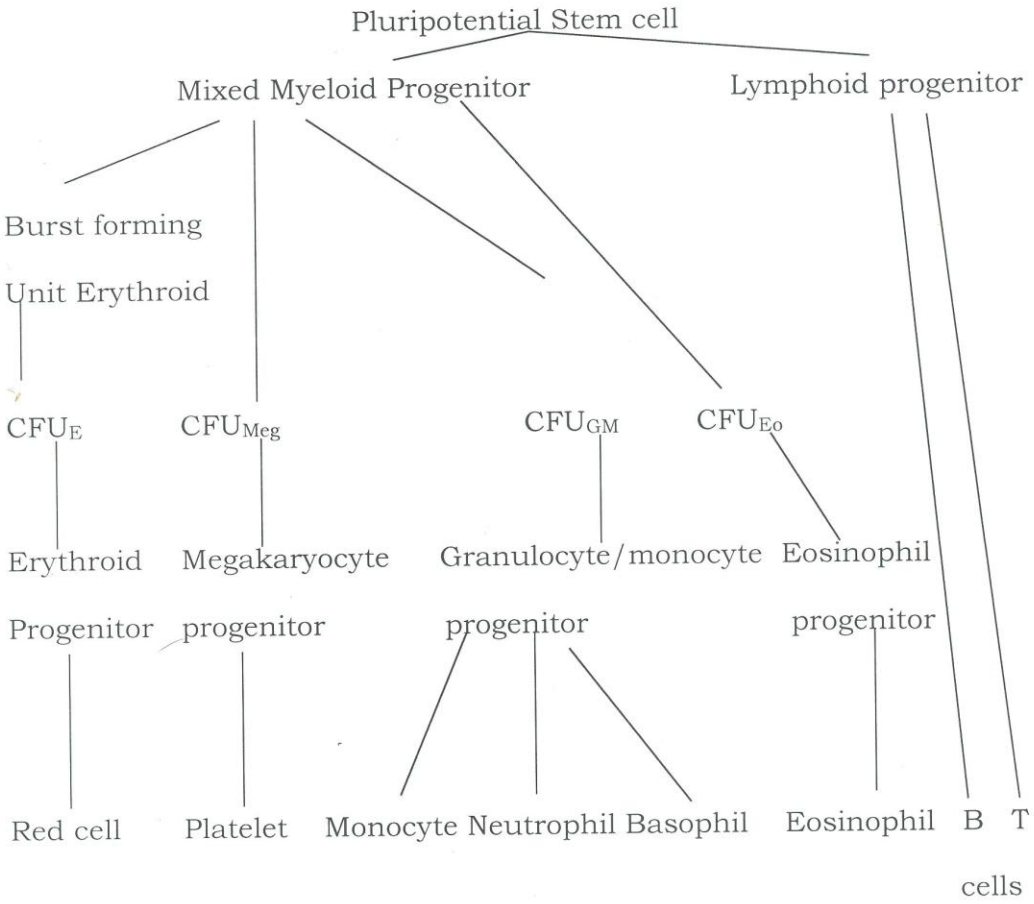
Developing countries often lack adequate laboratory facilities for the histologic or culture diagnosis of the surrogate indicator diseases to meet CDC's 1987 or 1993 AIDS definition.[29]

1.3 NORMAL HAEMAPOIESIS

The haematopoietic system comprises the cells in the blood, the precursors of these cells in the bone marrow, and the haematopoietic cells in the lymph nodes, spleen, and other lymphatic tissue throughout

the body. The system is divided into two major components, myeloid and lymphoid. Both arise from a pluripotential stem cell that is assumed to be of bone marrow origin. The myeloid cells include neutrophils, eosinophils, and basophils (collectively known as granulocytes) and monocytes, erythroid cells and megakaryocytes. Lymphoid cells include both T-cells and B-cells. These arise in the bone marrow and mature at different sites, including lymph nodes, spleen, thymus, extranodal lymphatic tissue, and bone marrow.[30]

Figure 2; Schematic representation of the cell hierarchy in the haemopoietic system.[2, 30]



1.3.1 Marrow morphology

The marrow consists of bone trabeculae and the medullary space. The medullary space includes haematopoietic cells, adipose tissue and stroma. Normal marrow consists of myeloid cells at all stages of

maturation and a relatively small number of well-differentiated lymphocytes, plasma cells and histiocytes. Approximately 60% to 65% of marrow cells are granulocytes and 20% are erythroid precursors. Lymphocytes constitute approximately 10% to 15% of the marrow cells. Megakaryocytes constitute approximately 2% to 5% of marrow cellularity. Myeloid haematopoiesis includes the differentiation and maturation of the granulocytes, monocytes, erythroid cells and megakaryocytes. The differentiation and maturation of myelopoietic cells are regulated by endogenously produced glycoproteins called cytokines. The most recognised are erythropoetin (EPO) granulocyte colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF). Thrombopoietin regulates the development of megakaryocyes and platelets.[2, 30]

1.3.2 Marrow cellularity

Alterations in marrow cellularity result from increases or decreases in the amount of haematopoietic cells with corresponding decreases in the number of fat cells. Hypercellular marrow may occur as a result of proliferation of normal myeloid cells. In disorders such as megaloblastic anaemia there is abnormal maturation of myeloid cells. The hypercellularity in this case is associated with an increase in the rate of intramedullary destruction of the cells. Hypocellular bone marrow may

arise as a result of exposure to drugs, chemicals, ionising radiation, or infectious agents, or it may be idiopathic. This is reflected in the blood by pancytopaenia: anaemia, neutropenia and thrombocytopenia. Isolated hypoplasia of individual myeloid cell lines is uncommon.[2, 30]

1.3.3 Blood cell numbers

Variations in the number of blood cells may result from abnormal processes in the haematopoietic system or from pathological processes in other organ systems. Leucocytosis (increased white cells) is commonly caused by infectious agents. Neutrophilic and monocytic leucocytosis is associated with bacterial infections while lymphocytic leucocytosis is seen with viral infections. Eosinophilic leucocytosis is associated with allergic reactions, parasitic infections and metastatic malignancies. A frequent cause of basophilic leucocytosis is the myeloproliferative disorder especially chronic myeloid leukaemia. Myeloproliferative disorders, iron deficiency, splenectomy and tumours may cause Thrombocytosis. Erythrocytosis may result from decreased oxygen saturation and polycythaemia vera.[2]

Cytopaenia may result from a disorder of the haematopoietic system, or from a disorder that results into excessive destruction of the cells. Pancytopaenia may be transient as in viral infections. Causes of marrow

failure resulting in pancytopaenia include marrow aplasia, myelophthisic process and metabolic deficiencies. Pancytopaenia may also result from immune mediated cell destruction or hypersplenism.[2]

1.4 ANAEMIA

Anaemia is said to be present when the haemoglobin concentration is below the reference range for age and sex of the individual. Normal values are[30, 31]

i. adult males 13.0 – 17.0g/dl

ii. adult non-pregnant female (pre-monopausal) 12.0 – 15.5g/dl

iii. adult pregnant female 11.0 – 14.0g/dl

Anaemia can be classified according to the associated changes in red cell size.

1. Hypochromic-microcytic (reduced MCV, MCH and MCHC)
2. Normochromic- macrocytic (increased MCV)
3. Normochromic- normocytic (normal indices)

Anaemia can also be classified based on the aetiology of anaemia.

1.5 HAEMATOLOGICAL DERANGEMENTS IN HIV

The HIV produces its dominant effects on CD4+ cells, which are lysed or form syncitia with adjacent CD4+ cells. The CD4 antigen appears to be the main receptor for HIV and CD4 antibodies. However CD4 alone is not sufficient for HIV infection (e.g. mouse cells infected with human CD4 will not necessarily become infected) and second receptors are required. CD negative cells including brain cells and haematopoietic cells may also be infected using such alternative receptors.[2, 30]

1.5.1 Haematological changes

Infection with HIV is associated with a range of haematological abnormalities. The mechanisms for these changes are multiple: qualitative and quantitative marrow defects and immune cytopaenias are a direct result of HIV infection, while the effect of opportunistic infections, lymphomas and a myriad of drugs against infection, malignancy or HIV itself play an important role. Peripheral blood and bone marrow abnormalities are common in HIV and increase in frequency with advancing disease.[2, 30]

1.5.2 Haemopoiesis

Cytopaenia is common in HIV disease and is often associated with morphological abnormalities in peripheral blood and bone marrow cells

suggestive of myelodysplasia. It is likely that HIV directly affects marrow production. This may be by direct infection of precursor cells or marrow stromal cells or by inducing altered production of regulatory cytokines. While susceptibility of progenitor cells to infection by HIV in vitro has been demonstrated, the extent to which progenitor cell infection in vivo contributes to abnormal haemopoiesis is uncertain. It is possible that direct infection of progenitor cells induces phenotypic abnormalities resulting in ineffective haemapoiesis and cytopaenia. [2, 30]

Infection of marrow accessory cells, T lymphocytes, macrophages, fibroblasts and dendritic cells with HIV leads to decreased production of stimulatory cytokines or increased production of inhibitory cytokines. The ability of the HIV-1 *tat* protein to induce bone marrow macrophages to increase their expression of TGF-β-1, a potent inhibitor of stem/progenitor cells has been reported. These cytokines such as tumour nccrosis factor (TNF), interleukin-1 (IL-1) and interferon have an inhibitory effect on marrow progenitor cells. A study was done to determine the correlation of serum cytokine levels with haematological abnormalities in HIV infection. Results showed that the production of mononuclear phagocyte-derived cytokines was enhanced in acquired immunodeficiency syndrome, and that the levels of the factors were correlated to the presence of certain haematological abnormalities.[32, 33]

1.5.4 Bone Marrow Changes

Myelodysplasia

Abnormalities are very common at all stages of HIV infection. These increase in frequency as the disease progresses. The most common abnormal finding is of dysplasia affecting one or more of the cell lines. In general, the more advanced the disease, the more marked the dysplasia. Although erythroid dysplasia is the most common finding, being recognized in over 50% of HIV-infected patients, abnormal granulocytic and megakaryocytic development is encountered in approximately one-third of patients. [2, 30]

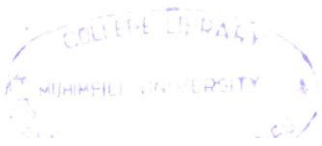
Dyserythropoiesis may be manifest by florid megaloblastic change. This is unrelated to serum cobalamin and folate levels, or to drug therapy with Zidovudine or folate antagonists. Erythroblasts are often bi- or multinucleated with an irregular nuclear outline and basophilic stippling. Abnormal sideroblasts including ringed forms may be present. The frequently observed reticulo-endothelial iron block is likely to be secondary to concurrent infection or chronic disease.[30, 33]

Dysplastic features in megakaryocytes are common, occurring in one-third of marrow and including nuclear hypolobulation and micromegakaryocytes. Granulocytic dysplasia may be apparent at all

stages of maturation with megaloblastic change, nuclear abnormalities and Pelger cells reflecting dysfunctional nuclear maturation. The rarity of acute leukaemia in HIV disease and the failure to detect chromosomal abnormalities in patients with marked dysplastic changes suggest that myelodysplasia in the setting of HIV disease is not generally a pre-leukaemic state, thus differing from the classic myelodysplastic syndrome. The term 'HIV-related myelodysplasia' has been coined to describe the changes in HIV disease. [2, 30]

Cellularity

Marrow from HIV-infected patients is sometimes difficult to aspirate and the trails are of decreased cellularity. The true marrow cellularity is better appreciated on trephine biopsy, which is hypercellular in a majority of patients. The difficulty in aspiration may be in part due to the increase in reticulin fibrosis seen especially in hypercellular marrows. Hypercellularity in the face of peripheral cytopaenia is a common finding. It is likely to reflect myeloid dysplasia and ineffective haemopoiesis. Indeed, there is a correlation between observed marrow dysplastic changes and the peripheral blood findings of anaemia and leucopenia. Red cell hypoplasia has been described in patients with HIV disease and may be associated with infection with B19 parvovirus or disseminated *Mycobacterium avium-intracellulare*. Severe erythroid



hypoplasia has also been described in patients receiving therapy with Zidovudine. A recent study of the ultrastucture of bone marrow cells in patients infected with HIV showed abnormalities in erythroid cells, marrow granulocytes, plasma cells and stromal cells, which were attributed to a direct effect of HIV infection. [2, 30]

Increased numbers of histiocytes are often seen in the bone marrow and in many patients haemophagocytosis is striking. In many patient with HIV infection who show increased numbers of marrow histiocytes, there is no obvious infective cause and it is likely that HIV itself is the trigger to histiocyte proliferation and phagocytosis. It is likely that, as a result of HIV infection, the marrow produces a histiocytic reaction, which arise from increased numbers of histiocytes to a full-blown haemophagocytic syndrome with severe pancytopaenia. [2, 30]

Plasma cells are often strikingly increased in the marrow of HIV-infected patients. They may represent a physiological response to antigenic stimulation by viruses or other infective agents, or may be secondary to dysregulated B-cell proliferation due to HIV. The marrow plasmocytosis is not confined to those patient with advanced disease in whom opportunistic infections could be implicated but is seen also in patients

at an early stage who have no concurrent infections. Plasma cells are often morphologically abnormal and appear in clusters.

Paraproteinaemia occurs in 9% of homosexual HIV positive men. This may result either from changes in T-cell regulation or from the activation of B-lymphocytes directly infected by HIV. The development of multiple myeloma is very uncommon, although it has been described. [2, 30]

The clinical value of bone marrow examination in HIV infection is;

1. Microbiological culture in patients with fever of unknown origin
2. Exclusion or staging of lymphoma
3. Diagnosis of immune thrombocytopaenic pupura
4. Elucidation of cytopaenias

2.0 STATEMENT OF THE PROBLEM

Studies have shown that HIV positive patients experience a wide range of haematological complications which include anaemia, neutropenia, lymphopenia, and thrombocytopenia, which all result in significant clinical sequelae.[1] Recovery from anaemia is shown to directly increase survival, therefore screening for anaemia should be aggressive and patients with anaemia should be treated.[14] Surveys have shown an improvement in HIV-infected patients energy level and health perception when haemoglobin levels increased after treatment with recombinant erythropoietin. [34] There was an increase in the quality of life in AIDS patients when the haemoglobin was raised from 11g/dl to 12g/dl as demonstrated by evaluation of fatigue, shortness of breath and Kaarnofsky performance scale after 16 weeks.[34] As HIV infected patients live longer, maintaining their quality of life becomes an increasingly important goal of treatment. Clinicians should consider the fall in the quality of life caused by anaemia when treating patients with HIV infection.[12] Information on the extent of haematological abnormalities on Tanzanian patients who are HIV-infected is not known. It has not been well documented. There is also no information on the prevalence of anemia in Tanzanian adults in the HIV era. Studies which have been done have been confined to children and pregnant women.[21, 22] It is therefore important to describe and establish the extent of

haematological abnormalities bearing in mind that HIV/AIDS is the leading cause of morbidity and mortality in adults in Tanzania today.[35]

3.0 STUDY OBJECTIVES

3.1 Broad Objective

To determine the haematological characteristics of HIV positive patients and compare them to those of HIV negative patients seen at Muhimbili National Hospital and the relation to their clinical stage.

3.2 Specific objectives

1. To determine the prevalence of anaemia in HIV positive compared to HIV negative patients and the relation to HIV clinical stage.

2. To describe the morphological types of anaemia and red blood cell indices seen in HIV positive compared HIV negative patients in relation to HIV clinical stage.

3. To determine the white blood cell counts of HIV positive patients compared to HIV negative patients and the relation to HIV clinical stage.

4. To determine the platelet counts in HIV positive patients compared to HIV negative patients in relation to HIV clinical stage.

5. To determine the erythrocyte sedimentation in HIV positive patients compared to HIV negative patients and the relation to HIV clinical stage.

6. To describe the bone marrow characteristics in HIV positive patients.

7. To determine the haematological indices that can be predictive of HIV positive serostatus.

4.0 METHODOLOGY

4.1 Study design and setting

This was a cross-sectional analytical study. The study was conducted at Muhimbili National Hospital. This is a tertiary health institution. It receives patients who are referred from the three district hospitals in Dar es Salaam, mainly Temeke, Amana and Mwananyamala Hospitals. Patients are also referred from other health facilities and from the rest of the country. Patients were recruited from the medical wards between November 15th 2001 and December 28th 2001.

4.2 Study population

Patients admitted to the medical wards of Muhimbili National Hospital were eligible for participation in the study. Those who were included were;

-males and females between the age of 18 and 65 years of age

-those who provided written or oral witnessed informed consent

Those who were excluded from the study were;

-pregnant women

-patients who were on antiretroviral treatment

4.3 Study procedure

Every third admitted patient was selected and invited to participate in the study. All the information regarding the study objectives and procedures were explained to the patients. They were provided with an information sheet. Patients were required to provide written or oral witnessed informed consent before proceeding with the study. No names were used for purposes of confidentiality. A coded questionnaire was used, in which demographic details were recorded. A physical examination was done which included determination of weight, height, body temperature, blood pressure and respiratory rate. The working diagnosis for the patient in the ward was also recorded. Pre test counselling was done before blood for HIV testing was done. Each HIV positive patient was then assigned to the CDC clinical stage of HIV infection to which they best fitted, based on the working diagnosis made during the period of admission in the ward.

Specimens for laboratory examinations were then taken. Venous blood was drawn and put into two separate vacutainers. One contained an anticoagulant, EDTA. The other had no anticoagulant. Every third patient who agreed to participate in the study was requested to have a bone marrow done on him or her. Those patients who gave consent had a bone marrow aspiration done. A sample of bone marrow was obtained

Behring). A positive result was confirmed by using a second ELISA test. Discordant results were confirmed using Western Blot Assay (Diagnostic Bio-Technology Immunoblot).

Bone marrow aspiration slides were air dried. Smears were stained using May-Grunwald Giemsa stain and potassium ferrycyanide. Slides were examined using a light microscope and cellularity determined. Trephine biopsy was not done.

All the results were taken back to the respective wards from which the patients were obtained. These were used by the admitting doctor in the care and management of the patients.

4.5 Statistical analysis

A sample size of 110 was calculated using Epiinfo version 6.0 statistical package. Data was analysed using Epiinfo version 6.0 and SPSS 10. Patients were divided into two groups according to their HIV serostatus. Those who were HIV positive were grouped into their respective CDC clinical stage of HIV infection. Mean values obtained were compared using χ^2Kruskall-Wallis and ANOVA tests. A p-value of 0.05 was considered to be statistically significant. Logistic regression analysis was

also done on haematological indices to determine those which could predict HIV positive serostatus.

4.6 Limitations of the study

Due to financial constriants it was not possible to do CD4 cell counts for the staging of those who were HIV seropositive. Patients were assigned to the stage of HIV infection based on clinical features and other investigations which were done during their admission.

Very few patients gave consent to have a bone marrow aspiration done. It was not possible therefore to do any tests for statistical significance on the results obtained from the bone marrow examinations.

5.0 RESULTS

224 patients admitted to the medical wards were invited to participate in the study. A total of 196 patients, gave consent to participate in the study. Results of HIV testing and full blood counts were available in 183 patients. 92 (50%) were found to be HIV positive and 91 HIV negative. The mean age (sd) of study subjects was 36.80 (13.04) years. HIV positive patients were younger, 35.74 (9.96) compared to HIV negative 38.48 (14.94) (p-value 0.336). The age distribution of HIV positive patients was significantly different from HIV negative patients as shown in table 1, 77.2% of those who tested HIV positive were in the age group of 26 – 49 years (p-value 0.001). HIV positive patients and HIV negative patients did not differ significantly according to other demographic characteristics like sex, level of education, occupation marital status or income. (Table 1)

TABLE 1
DEMOGRAPHIC CHARACTERISTICS OF THE STUDY PATIENTS ACCORDING TO THEIR HIV SEROSTATUS

Demographic characteristic	HIV positive No. (%)	HIV negativeNo (%)	p-value
SEX			
Male	41 (44.6)	49 (53.8)	
Female	51 (55.4)	42 (46.2)	0.21
AGE GROUP			
18 – 25	12 (13)	27 (29.7)	
26 – 49	71 (77.2)	34 (37.4)	0.00
50 – 65	9 (9.8)	30 (33)	
OCCUPATION			
Petty trade	14 (15.4)	8 (8.9)	
Business	10 (11)	3 (3.3)	
Civil service	13 (14.3)	13 (14.3)	0.132
Casual labour	33 (36.3)	38 (42.2)	
Housewife	13 (14.3)	12 (13.3)	
Unemployed	8 (8.8)	16 (17.8)	
MARITAL STATUS			
Single	21 (22.8)	27 (29.7)	
Married	50 (54.3	48 (52.7)	
Cohabiting	3 (3.3)	1 (1.1)	0.24
Divorced	14 (15.2)	7 (7.7)	
Widowed	4 (4.3)	8 (8.8)	
INCOME (per month in Tshs)			
0 – 30,000	40 (48.2)	40 (51.9)	
30,000 – 100,000	35 (42.2)	30 (39)	0.89
>100,000	8 (9.6)	7 (9.1)	
EDUCATION			
None	8 (8.7)	15 (16.5)	
Primary	65 (70.7)	55 (60.4)	0.24
Secondary	15 (16.4)	15 (16.4)	
Post secondary	3 (3.2)	6 (6.5)	

HIV positive patients had significantly lower BMI compared to HIV negative patients.(p-value 0.004). The mean temperature was significantly higher in HIV positive patients compared to HIV negative patients (p-value 0.006). There was no significant difference in the mean systolic blood pressure, respiratory rate or pulse rate in HIV positive patients compared to HIV negative patients. (Table 2)

TABLE 2
PHYSICAL CHARACTERISTICS OF PATIENTS BY HIV SEROSTATUS

Physical characteristic	HIV positive No.(%)	HIV negative No. (%)	p-value
BODY MASS INDEX			
<18.4	25 (69.4)	11 (30.6)	
18.5 – 24.9	54 (47.4)	60 (52.6)	0.004
>25	4 (22.2)	14 (77.8)	
Mean Temp (C)	37.35 ±0.62	37.13 ±0.48	0.006
Mean Blood Pressure			
Systolic	114.03 ±13.06	117.82 ±13.76	0.115
Diastolic	72.16 ±11.79	85.72 ±18.57	0.001
Mean Resp Rate/min	19.90 ± 3.53	19.39 ±2.55	0.392
Mean Pulse Rate/min	98.78 ±11.41	80.90 ±10.38	0.722

Patients who were HIV positive had a higher frequency of pulmonary tuberculosis and candidiasis and mucocutaneous fungal infections as compared to those who were HIV negative,p values 0.03 and 0.003 respectively. (Table 3) There was no difference between HIV positive

patients and HIV negative patients in terms of the other addmission diagnoses.

TABLE 3
FREQUENCY OF DIAGNOSES AMONG THE STUDY PATIENTS ACCORDING TO HIV SEROSTATUS

DIAGNOSIS	HIV positive No.(%)	HIV negative No. (%)	Total	P value
Malaria	19 (20.65)	23 (25.27)	42 (22.95)	0.45
Pulmonary Tuberculosis	26 (28.26)	14 (15.38)	40 (21.86)	0.03
Extra Pulmonary Tuberculosis	12 (13.04)	7 (7.70)	25 (13.66)	0.23
Heart Failure	5 (5.43)	17 (18.68)	22 (12.02)	0.005
Central Nervous system disorder	12 (13.04)	7(7.70)	19 (10.38)	0.23
Candidiasis/Mucocutaneous fungal infection	12 (13.04)	0 (0)	12 (6.56)	0.003
Gastroenteritis	7 (7.61)	5 (5.49)	12 (6.56)	0.56
Pneomonia other than PTB	5 (5.43)	4 (4.4)	9 (4.92)	0.74
Peptic Ulcer Disease	4 (4.34)	4 (4.4)	8 (4.37)	0.98
Urinary Tract Infection	0 (0)	6 (6.6)	6 (3.28)	0.01
Liver Disease	3 (3.26)	3 (3.3)	6 (3.28)	0.98
Diabetes Mellitus	0 (0)	4 (4.4)	4 (2.19)	0.04
Kaposi's Sarcoma	2 (2.17)	0(0)	2 (1.09)	0.15
Hypertension	0 (0)	2 (2.2)	2 (1.09)	0.15
Others	19 (20.65)	16(17.58)	35 (19.13)	0.59

The prevalence of anaemia was significantly higher in those who were HIV positive (71.7%) compared to those who were HIV negative (44%) (p-value 0.001). Anaemia also increased with advancing clinical stage among those who were HIV positive. However this difference was not statisticaly significant among the different clinical stages. Peripheral Blood cytopaenias were more common in HIV positive than in HIV negative patients. HIV positive patients had significantly higher

frequency of anaemia (p-value 0.001), leucopaenia (p-value 0.008), lymphopaenia (p-value 0.01), thrombocytopaenia (p-value 0.01).(Table 4)

TABLE 4
FREQUENCY OFANAEMIA AND PERIPHERAL BLOOD CYTOPAENIA ACCORDING TO HIV SEROSTATUS

Cytopaenia	HIV positive No. (%)			HIV negative No.(%)	p-value
Anaemia	66 (71.7)			40 (44)	0.001
	Stage A 16 (61.5)	Stage B 26 (68.4)	Stage C 24 (85.7)		0.120
Leucopenia	26 (28.6)			10 (11.1)	0.008
Lymphopenia	11 (12.2)			2 (2.2)	0.01
Thrombocytopenia	34 (37.4)			16 (17.8)	0.01

Among all the patients, normochromic-normocytic red blood cells were the most common. Among those who were HIV positive, normochromic-normocytic red blood cells were the most common (51.1%), followed by hypochromic-microcytic red blood cells (35.9%), and then macrocytic red blood cells (13%). The same pattern was observed in those who were HIV negative. The red blood cell morphology did not differ significantly among the HIV positive and HIV negative patients (p-value 0.57)(Table 5)

TABLE 5
RED BLOOD CELL MORPHOLOGY IN ALL PATIENTS BY HIV SEROSTATUS

RBC Morphology	HIV positive No. (%)	HIV negative No. (%)	p-value
Normochromic normocytic	47 (51.1)	43 (47.3)	
Hypochromic microcytic	33 (35.9)	39 (42.9)	0.57
Macrocytic	12 (13.0)	9 (9.9)	

Among HIV positive patients with anaemia normochromic normocytic anaemia was the most common (43.9%). This was followed by hypochromic-microcytic anaemia and the macrocytic anaemia, 42.2 and 13.6% respectively. In the HIV negative patients with anaemia, hypochromic-microcytic anaemia (55%) was more common than normochromic-normocytic and macrocytic anaemia, (32.5% and 12.5%). This difference was however not statisticaly significant.(p-value 0.43) (Table 6)

TABLE 6
RED BLOOD CELL MORPHOLOGY IN THOSE WITH ANAEMIA (HGB<10g/DL) BY HIV SEROSTATUS

RBC Morphology	HIV positive No. (%)	HIV negative No. (%)	p-value
Normochromic normocytic	29 (43.9)	13 (32.5)	
Hypochromic microcytic	28 (42.4)	22 (55.0)	0.43
Macrocytic	9 (13.6)	5 (12.5)	

In HIV positive patients with stage A disease, normochromic-normocytic anaemia was the most common (56.3%). In those patients with stage B

and C disease hypochromic-microcytic anaemia was the most common. In all the stages macrocytic anaemia was the least common type of anaemia.(Table 7)

TABLE 7

RED BLOOD CELL MORPHOLOGY IN HIV POSITIVE PATIENTS WITH ANAEMIA (HGB<10g/dl) BY CDC CLINICAL STAGE

RBC Morphology	Stage A No. (%)	Stage B No. (%)	Stage C No. (%)	p-value
Normochromic normocytic	9 (56.3)	10 (38.5)	10 (41.7)	
Hypochromic microcytic	6 (37.5)	11 (42.3)	11 (45.8)	0.703
Macrocytic	1 (6.3)	5 (19.9)	3 (12.5)	

The mean RBC count was significantly lower in HIV positive patients compared to those who were HIV negative. (p-value 0.0001). Haemoglobin and haematocrit were also significnlty lower in those who were HIV positive (p-value 0.0024 and 0.0026 respectively). MCV, MCH and MCHC were higher in those who were HIV positive but these differences were not statisticaly significant. (Table 8)

TABLE 8
MEAN RED BLOOD CELL INDICES BY HIV SEROSTATUS

RBC INDEX (Normal Range)	HIV positive	HIV negative	Test statistic	p-value
RBC counts 3.8 – 6.5 M /uL	3.13 ±1.01	3.80 ±1.39	Kw 15.23	0.0001
HGB g/dl	8.42 ±2.77	9.84 ±3.70	Kw 9.25	0.0024
MCV 80 – 95 fL	83.18 ±11.80	80.59 ±14.40	F=1.77	0.1843
MCH 27- 32 pg	27.10 ±3.37	26.36 ±5.13	Kw 9.19	0.276
MCHC 31 –36 g/dl	32.17 ±2.41	31.93 ±2.29	F=0.51	0.4785
HCT 37 - 54 %	26.07 ±8.99	30.72 ±11.31	Kw 9.09	0.0026

Patients who were in stage C had lower RBC counts, haemoglobin, MCH, MCHC and HCT than those who were in stage A or B disease. (Table 9)

TABLE 9
MEAN RED BLOOD CELL INDICES IN HIV POSITIVE PATIENTS BY CLINICAL STAGE

RBC INDEX (Normal Range)	Stage A	Stage B	Stage C	Test statistic	p-value
RBC COUNTS 3.8 – 6.5M/uL	3.14 ±1.07	3.21 ±0.97	3.06 ±1.03	F=0.345	0.7027
HGB g/dl	8.61 ±2.90	8.85 ±2.61	7.66 ±2.79	F=1.574	0.2128
MCV 80 – 95 fl	80.94 ±16.17	85.41 ±9.15	82.05 ±9.98	Kw 1.79	0.4085
MCH 27 – 32 pg	27.67 ±3.14	27.76 ±3.26	25.69 ±3.41	F= 3.779	0.0266
MCHC 31 – 36 g/dl	32.92 ±2.48	32.40 ±2.59	31.17 ±1.73	F= 4.129	0.0193
HCT 37 – 54 %	25.55 ±10.01	27.43 ±8.29	24.73 ±8.99	F= 0.782	0.4604

Compared to HIV negative patients, patients who tested positive for HIV had significantly lower white cell counts (p-value 0.034) , lymphocyte counts (p-value 0.002), granulocyte count (p-value 0.063), and platelet counts (p-value 0.014). They had a significanlty higher mean ESR (p-value 0.001). (Table 10)

TABLE 10

MEAN WHITE BLOOD CELL INDICES, PLATELET COUNTS AND ESR BY HIV SEROSTATUS

WBC INDEX (Normal Range)	HIV Positive	HIV Negative	Test statistic	P value
WBC count (4 – 11 K/uL)	6.52 ±4.05	7.75 ±3.76	F=4.525	0.0348
Lymphocyte count (0.6-5.2K/uL)	1.72 ±1.15	2.25 ±1.12	F=9.806	0.002
Granulocyte count (2 – 8.7 K/uL)	4.11 ±3.32	4.85 ±3.55	Kw 3.46	0.063
Platelets (150 – 400 K/uL)	215.26 ±126.88	272.69 ±160.76	Kw 6.053	0.014
ESR mm/1st hr	103.68 ±45.08	66.20 ±48.64	F=28.91	0.000

Patients with stage C disease had a higher total white cell count, granulocyte count and platelet count as compared to those with stage A and stage B disease. However those with stage C disease had a lower total lymphocyte count than those with stage A disease. The ESR increased with advancing stage of HIV disease.(Table 11).

TABLE 11
MEAN WHITE BLOOD CELL INDICES, PLATELETS AND ESR IN HIV POSITIVE PATIENTS BY CLINICAL STAGE

WBC INDEX(Normal Range)	Stage A	Stage B	Stage C	Test statistic	P value
WBC Count(4- 11 K/uL)	6.91±3.26	5.46±3.48	7.61±5.10	Kw 7.370	0.025
Lymphocyte count (0.6 - 5.2K/uL)	1.90±1.13	1.54±1.18	1.77±1.16	F=0.812	0.4475
Granulocyte count(2 – 8.7K/uL)	4.30± 2.57	3.35± 2.48	4.95± 4.59	Kw 3.73	0.154
Platelet count(150-400 K/uL)	220.12±103	187.47±138.58	248.46±125.97	F=1.928	0.1515
ESR mm/1st hr	93.58±45.59	97.22±47.07	121.61±37.52	F=3.421	0.0371

Of the 196 patients who agreed to participate in the study only 46 (23.5%) agreed to have a bone marrow aspiration done.Examination of the slides was posssible for 33 patients. The commonest type of bone marrow morphology seen among those who were HIV positive was reactive marrow. There were no normal marrows. Among those who were HIV negative, megaloblastic anaemia reactive marrow, and depleted iron stores were equaly common. (Table 12)

TABLE 12
BONE MARROW CHARACTERISTICS OF SOME OF THE PATIENTS BY HIV SEROSTATUS

Characteristic	HIV Positive No.(%)	HIV Negative No.(%)
Megaloblastic Anaemia	5 (10.47)	3 (23.07)
Reactive Marrow	6 (33.33)	3 (23.07)
Depleted Iron Stores	2 (11.11)	3 (23.07)
Iron Deficiency Anaemia	0	2 (15.38)
Leucoerythroblatic	1 (5.5)	0
Combined Deficiency	2 (11.11)	0
Normal Marrow	0	2 (15.38)
Non-diagnostic Marrow	2 (11.11)	0
TOTAL	18	13

Haematocrit and RBC counts were removed from the logistic regression model because they were highly correlated to HGB. Anaemia, lymphopenia, and raised ESR were found to be predictive of HIV positive serostatus. (Table 13)

TABLE 13

LOGISTIC REGRESSION OF HAEMATOLOGICAL CHARACTERISTICS PREDICTIVE OF HIV POSITIVE SEROSTATUS

VARIABLE	Univariate OR (95% CI)	Multivariate OR (95% CI)
Haemoglobin >10g/dl	1.0	
<10g/dl	3.23 (1.75 – 5.98)	2.90 (1.36 – 6.18)
WBC count 4 -11K/uL	1.0	
< normal	1.58 (0.83 – 2.89)	1.67 (0.76 – 3.58)
Lymphocyte count 0.6-5.2K/uL	1.0	
<normal	3.99 (1.07 - 14.83)	5.28 (1.18 – 23.63)
Platelet count 150-400	1.0	
< normal	1.97 (1.08 – 3.62)	1.81 (0.86 – 3.83)
ESR upto 35mm/hr	1.0	
>35mm/hr	4.56 (2.02 – 10.30)	3.31 (1.29 – 8.48)

6.0 DISCUSSION

50% of the patients recruited into the study were HIV infected, among them 54.84% were females and 77.2% were between the age group 24-49 years. These results concur with the prevalence of 79.2% reported in the Tanzanian national surveillance report, in the age group 20-49 years. In the survellance report it was found that 55.35% were females. A 40% to 50% hospital-bed occupancy by HIV/AIDS has also been reported by the NACP.[7]

Studies have shown that HIV positive patients experience a wide range of haematological complications which include anaemia, neutropenia, lymphopenia, and thrombocytopenia. [37, 38] This finding was also observed in the current study in which peripheral blood cytopenias were found to be more prevalent in HIV positive patients than in HIV negative patients. The most frequent abnormality seen was anaemia which occurred in 71.7% of the HIV positive patients. The proportion of patients with anaemia was shown to increase with advancing clinical stage of HIV disease,thought this was not found to be statistically significant. Other haematological abnormalities in the study were thrombocytopenia 37.4%, leucopenia 28.6% and lymphopenia 12.2%. These findings differ only slightly from those obtained from HIV infected

adult Zimbabweans. [16, 17] It was observed that lymphopenia was the most common abnormality (31.5%) followed by anaemia (30.85), thrombocytopenia (24.7%) and leucopenia (11.7%). [16,17]

The prevalence of anaemia in patients with AIDS has been estimated to be 63% to 95% making it more common than thromboocytopenia or leucopenia in patients with AIDS.[14] In Abidjan, among HIV infected pregnant women, the prevalence of anaemia was higher when compared to those who were HIV negative.[18] Anaemia, apart from being the most frequent haematological abnormality, is important in terms of the clinical implications to the patient. Anaemia has been found to be a prognostic marker of future disease progression or death, independent of CD4 counts and viral load. [32,34] In the Multistate Adult and Adolescent Spectrum of HIV disease Surveillance Project the presence of anaemia was strongly and consistently associated with the progression of HIV disease as measured by an AIDS defining illness and measurement of a CD4 count of less than 200 cells/μl.[14]

Thrombocytopenia was th second most common abnormality observed in this study. Its importance is supported by the Multistate Adult and Adolescent Spectrum of HIV Disease study which showed that thrombocytopenia was associated with clinical AIDS and significantly

decreased survival in patients who have HIV infection.[39] Thrombotic thrombocytopenic pupura, although rare has also been well described as a complication occuring in HIV infected patients at all stages of HIV infection.[40, 41] In trying to elucidate the causes of thrombocytopenia in HIV infected patients, several studies have been done. An autoimmune mechanism has been postulated for the destruction associated with some forms of thrombocytopenia. Recent studies have shown that megakaryocytes are susceptible to HIV infection and suggest the possibility that HIV can directly impair the platelet production from megakaryocytes.[42, 43]

Leucopenia and lymphopenia were also observed with a higher frequency in HIV infected patients when compared with HIV negative patients in this study. This finding is of clinical importance. Neutropenia frequently complicates infection with HIV and is a significant risk factor for bacterial infection. [44] Findings from a case-control study by Hermans et al showed that severe bacterial and fungal infections and bacteremia were significantly higher in neutropenic patients than in non-neutropenic patients. Neutropenic HIV infected patients were at increased risk of developing severe infections at the end stage of HIV disease.[45] Neutropenia has been identified as an important independent

risk factor in the development of infectious complications in patients with HIV and AIDS. [46]

In this study red blood cell morphology in HIV positive and HIV negative patients was commonly normochromic-normocytic. This picture changes when the red blood cell morphology is examined in patients with anaemia. Among those who were HIV positive the commonest picture was that of normochromic-normocytic anaemia while in HIV negative patients it was hypochromic-microcytic anaemia. The anaemia of chronic infections is normochromic-normocytic. It may also be hypochromic-microcytic. Early on the red blood cells are normochromic and during the course of the disease they become more microcytic.[47] Patients withHIV infection usualy have long standing chronic infections and this could be the reason why their anaemia is normochromic – normocytic.[47] It was found that the study patients had significantly higher prevalence of pulmonary tuberculosis and mucocutaneous fungal infections than the HIV negative patients, and this may have contributed to the higher prevalence of anaemia as well as to the type of anaemia that was seen in these patients. In those who are HIV negative other factors like nutritional deficiencies might be more important as the cause of anaemia. In Tanzania iron deficiency in the most important cause of anaemia in the general population.[9,10] With HIV disease

progression the red blood cell morphology becomes hypochromic microcytic which could again result from increasing frequency of opportunistic infections.[1]

The mean red blood cell counts, haemoglobin level and haematocrit were found to be significantly lower in HIV positive patients than in HIV negative patients. They also had significanlty lower white cell counts, lymphocyte counts and platelet counts. Tanzanian blood donors were examined by Urassa et al and those who were HIV infected found to have lower mean haemoglobin 8.3g/dl than those who were not infected 11.6g/dl. They were found to have lower total white cell counts and total lymphocyte counts. The total white cell counts, total lymphocyte counts and total T-lymphocytes were not significantly different in the 4 clinical stages (WHO clinical stages) of HIV infection. [20] A progressive decline in number of leucocytes and erythrocytes, haemoglobin level, transferrin and serum iron with disease progression have been reported. These changes have been attributed to the effects of the HIV virus.[47] Although patients in stage C had lower mean red cell and white cell indices than those in Stage A, the differences among the 3 clinical stages were not statistically significant. This finding may have been influenced by the fact that CDC clinical staging was used to indicate disease progression. Studies have been done to assess the accuracy of clinical case

definitions in HIV infected patients. In one study case definitions were found to be specific but only moderately sensitive for advanced HIV disease. It has been suggested that other surrogate markers like CD4 cell counts, plasma viraemia etc, be used instead as better indicators of clinical disease progression.[49]

Among the study patients ESR was found to be significantly higher in those with HIV infection. ESR was found to increase progressively with advancing HIV disease. Laboratory findings from a previous study have been associated with progresssion to AIDS included an elevated ESR.[50, 51] Others were a decrease in the number of CD4 lymphocytes, decrease in CD4/CD8 ratio, HIVp24 antigenaemia, lack of anti HIVp24, anaemia and elevated serum beta-2 microglobulin.[50, 51] This is reflected in the results obtained from logistic regression analysis of the haematological parameters. Anaemia, lymphocyte count and ESR were found to be predictive of HIV seropositive status. Anaemia, lymphopenia, thrombocytopenia and a raised ESR were independent predictors of HIV positive serostatus.

Bone marrow examinations in HIV positive patients have also been carried out in a number of studies. In one study, case records of 40 patients who had bone marrow examinations done, were retrospectively

examined.[52] The bone marrows were found to be non-specific. Dysplasia and hypercellularity were the most common finding. In patients with HIV infection bone marrow examination had a low specific yield (28%) for those with anaemia as compared to a higher yield (63%) in patients with MAC infection. [53, 54] In South Africa a study was done to assess the diagnostic usefulness of bone marrow examination. Consecutive case series of 257 adults with HIV infection who had a bone marrow examination were reviewed. The bone-marrow examination was positive in 38% of the patients and gave a unique diagnosis in 24% of these patients.[55] In this study, among HIV positive patients who agreed to have bone marrow aspirations done, reactive marrows were the most common. In these patients the peripheral blood counts showed some degree of cytopaenia which was not reflected in the bone marrow. In these patients the bone marrows were hypercellular or of normal cellularity. Megaloblastic changes were the next common finding indicating abnormal haemopoieses. This is in keeping with the common finding of hypercellularity in the face of peripheral cytopaenia in the bone marrows of patients with HIV infection. The bone marrow in HIV infected patients has increased haemopoietic activity but is unable to keep up with increased peripheral destruction. It has also been shown that dyserythropoiesis may be manifest by florid megaloblasitc change. [2, 30, 33] There were no normal bone marrows among the HIV positive

patients. This goes to show that the bone marrow picture may not be specific for HIV infection. The multiple causes of marrow failure in patients with HIV infection acccount for the mixed histological findings on bone marrow examinations of these patinents. [38] The bone marrow results that are reactive indicate the presence of infection. The bone marrow may be used together with other tests to establish the cause of the infection. However the number of patients who had bone marrow examination done was small and it was not possible to establish if these findings were statistically significant.

7.0 CONCLUSION

Haematological abnormalities are more prevalent in HIV positive patients compared to HIV negative patients. Anaemia is the most common cytopaenia with a prevalence of 71.7%. Anaemia occurs with increasing prevalence with advancing clinical stage of HIV infection. Thrombocytopenia, leucopenia and lymphopenia are also more common in patients who are HIV infected. They have lower mean red cell and white cell indices, and higher ESR than HIV negative patients. These findings may be as a result of the HIV infection itself or due to opportunistic infections, mainly pulmonary tuberculosis and mucocutaneous fungal infections. Bone marrow findings may be non specific in HIV infection and may not be reflected in the peripheral blood counts. Anaemia, lymphopenia, and a raised ESR may be predictive of HIV positive serostatus. These haematological abnormalities observed have important clinical implications for patients who are HIV positive.

8.0 REFERENCES

1. Martin MD and Rodgers GM. Haematologic and neoplastic aspects of human immunodefiency virus infection. Manual of Clinical Haematology edited by Joseph J Mazza, 3rd edition; Lippincott Williams and Wilkins 2002; pages 389-399.

2. Costello C. The haematological manifestations of HIV disease. Postgraduate Haematology edited by Hoffbrand AV, Mitchell Lewis S, Edward GD, 4th edition; Tuddenham Butterworth Heinmann 1999.; pages 309-323.

3. Fauci AS, Clifford Lane H. Human Immunodeficiency Virus Disease: AIDS and related disorders. Harrison's Principles of Internal Medicine edited by Eugene Brauwald, Stephen L Hauser, Anthony S Fauci, Dan L Longo, Dennis L Kasper, J Lary Jameson, 15th edition; Mc-Graw-Hill 2001; pages 1879-1899.

4. Sepkowitz, Stein-brook and Drazen, Gottlieb et al. AIDS – the first 20 years. The New England Journal of Medicine 2001; 344:1764-1788

5. Global estimates of the HIV/AIDS epidemic, end of 2002. UNAIDS 5th December 2002.

6. Kwesigabo G. Trends of HIV infection in the Kagera region of Tanzania 1987-2000. Umeå University Medical Dissertation. New Series No.710 ISBN 91-7191-966-x ISSN 0346 6612.

7. Tanzanian National AIDS Control Programme, HIV/AIDS/STI Surveiillance Report, January – December 2001, report no 16:1-7.

8. WHO report 2000. Turning the tide of malnutrition, responding to the challenges of the 21st century.

9. Kavishe FP. The public health significance of nutritional anaemia. Tanzania Food and Nutrition Centre report no 1290, 1990.

10. Mnyika KS. Anaemia surveillance system, a milestone. Tanzania Food and Nutrition Centre report no 1436, 1991.

11. Mitchell R, Fupi F. Sickling in Tanzania. East African Medical Journal 1972;49 (9):638-642.

12. Voldbering P and The Anaemia in HIV Working Group. Consensus statement; anemia in HIV infection – Current trends, treatment options and practice strategies. Clinical Therapeutics 2000; 22(9):1004-1020.

13. Hambleton J. Haematological complications of HIV infection. Oncology 1996;10 (5):671-680.

14. Sullivan PS, Debra L, Hanson et al, and the Adult/Adolescent Spectrum of Disease Group. Epidemiology of anaemia in Human Immunodeficiency (HIV) infected persons; Results from the Multistate Adult and Adolescent Spectrum of HIV Disease Surveillance Project. Blood 1998;91 (1):301-308.

15. Mocroft A, Ole Kirk, Barton SE et al, Anaemia is an independent predictive marker for clinical prognosis in HIV-infected patients from acroos Europe. Journal of AIDS 1991;13 (8):943-950.

16. Adewuyi JO, Coutts AM, Latif AS et al, Haematological features of the Human Immunodeficiency Virus infection in adult Zimbabweans Central African Journal of Medicine 1999; 45(2):26-30.

17. Adewuyu JO, Chitisike I. Haematological features of the Human Immunodeficiency Virus infection in Black children in Harare. Central African Journal of Medicine 1994;40 (12):333-6.

18. Ramon R, Sawadogo D, Koko FS et al, Haematological characteristics and HIV status of pregnant women in Abidjan, Cote d'Ivoire 1995- 1996. Trans R Society of Tropical Medicine and Hygiene 1999;93 (4):419-22.

19. Zucke JR, Lackritz EM, Reuben TK. Anaemia, blood transfusion practices, HIV and mortality among women of reproductive age in wester Kenya. Trans R Society of Tropical Medicine and Hygiene 1994;88 (2):173-6.

20. Urassa WK. Immunolgical markers of disease progression in Human Imuunodeficiency Virus type 1 infection in Dar es Salaam Tanzania. Masters of Medicine in Microbiology and Immunology Dissertation, University of Dar es Salaam; 1995.

21. Villamor E, Mbise R, Spiegelman D et al, Vitamin A supplementation and other predictors of anaemia among children from Dar es Salaam, Tanzania. Americal Journal of Tropical Medicine and Hygiene 2000; 62 (5):590-7.

22. Antelman G, Msamanga GI, Spiegelman D et al, Nutritional factors and infectious disease contribute to anaemia among pregnant women with Human Immunodefifiency Virus infection in Tanzania. Journal of Nutrition 2000;130 (8):1950-7.

23. Brooks GF, Butel JS, Morse SA. Jawetz Melnick and Adelberg's Medical Microbiology. AIDS and Lentiviruses. 21st edition; Appleton and Lange1998; pages 566-583.

24. Peakman M, Vergani D. Human Immunodeficiency Virus and AIDS. Basic and Clinical Immunology 1st edition, Churchill Livingstone 1997; pages 277-287.

25. Morgan D, Mahe C, Mayanja B et al. HIV-1 infection in rural Africa, is there a difference in the median time to AIDS and survival compared with that in industrialised countries. AIDS 2002 ;16(14):597-603.

26. National Guidelines for the clinical management of HIV/AIDS. National AIDS Control Programme(Tanzania), 2002.

27. Montaner JS, Le TN, Le N et al, Application of the WHO system for HIV infection in a cohort of homosexual men in developing a prognosticaly meaningful staging system. AIDS 1992 ;6 (7):719-24.

28. Centers for Disease Control and Prevention (CDC). 1993 Revised classification system for HIV infection and expanded surveillance of definition for AIDS among adolescents and adults MMWR 1992; 41 (RR-17): 1.

29. Lifson AR, Allen S, Wolf A et al, Classificatin of HIV infection and disease in women from Rwanda. Evaluation of the WHO HIV staging system and recommended modifications. Annals of Internal Medicine 1995;122 (4):262-70.

30. Litz CE, McClure JS, Browning RD. Blood and Bone Marrow. Anderson's Pathology edited by Ivan Damjanov and James Linden,10th edition;1996.

31. Weatherall, Ledingham and Warrell. Oxford Textbook Of Medicine. Disorders of Blood. 2nd edition, English Language Book Society/Oxford University Press 1988; pages 19.1-19.98.

32. Maury CP, Lahdevrita J. Correlation of serum cytokine levels with haematological abnormalities in Human Immunodeficiency Virus infection. Journal of Internal Medicine 1990;227 (4):253-7.

33. Giesssler RG, Ottoman OG, Kleiner K et al, Decreased haematopoietic colony growth in long term bone marrow cultures of HIV- positive patients. Respiratory Virology 1993,144; 69-73.

34. Baez J. Trends in HIV- Induced Anaemia. Infectious Diseases Special Edition 1998; 25 (3 Suppl);43-46.

35. Policy Implications of Adult Morbidity and Mortality, end of phase one report. Ministry of Health, Dar es Salaam, Tanzania. Mack Printers 1997

36. Dacie JV and Lewis SM. Practical Haematology. 7th Edition. Churchill Livingstone.

37. Moyle G. Anaemia in persons with HIV- infection, contributor to mortality. AIDS Review 2002; 4 (1):13-20.

38. Moses A, Nelson J and Grover C et al, The influence Of Human Immunodeficiency virus-1 on haematopoiesis. Blood; 1998;91 (5):1479-1495.

39. Sullivan PS, Hanson DL, Chu SY et al, Surveillance for thrombocytopenia in persons infected with HIV; results from the Multistate Adult and Adolescent Spectrum of Disease Project. Journal of Acquired Immune Deficiency Syndrome Human Retrovirology 1997;14 (4):374-9.

40. Sood R, Rakker AS, Carmosino L et al, Thrombotic thrombocytopenic pupura in HIV infection, a report of two cases. AIDS Patient Care STDs 1996;10(6):349-52.

41. Burbano X, Miguez MJ, Lecusay R et al, Thrombocytopenia in HIV-infected drug users in the HAART era. Platelets 2001;12 (8):456-61.

42. Sato T, Sekine H, Kakuda H et al, HIV infection of megakaryocytic cell lines. Leukemia Lymphoma 2000;36 (3-4);397-404.

43. Cole JL, Manzec UM, Gunthel CJ et al, Ineffective platelet production in thrombocytopenic HIV-infected patients. Blood 1998; 91(1):3239-3246.

44. Kuritzkes DR. Neutropenia, neutrophil dysfunction and bacterial infection in patients with HIV disease: The role of Granulocyte Colony Stimulating Factor. Clinical Infectious Diseases 2000;30; 256-260.

45. Hermans P, Sommereijns B, Van Gutsem N et al, Neutropenia in patients with HIV infection; a case control study in a cohort of 1403 patients between 1982 and 1993. Journal Hematother Stem Cell Res 1999;8 suppl;S23-32.

46. Herman P. HIV disease related neutropenia; an independent risk factor for severe infections. AIDS 1999;13 Suppl 2; S11-7.

47. The Merck Manual. Section 11 Haematology and Oncology edited

by Mark H Beers, Robert Berkow. 17th edition; Merck Research Laboratories 1999, pages 849-882, 996-997.

48. Spada C, Treitinger A, Hoshikawa-Fujimura AY. HIV influence on hematopoiesis at the initial stage of infection. European Journal of Haematology 1998;61 (4); 255-60.

49. Gallant JE, Somani J, Chasson RE et al, Diagnostic accuracy of three clinical case definitions for advanced HIV disease. AIDS 1992;6 (3);295-9.

50. Morfeldt-Manson L, Bottiger B, Nilsson B et al, Clinical signs and laboratory markers in predicting progression to AIDS in HIV-1 infected patients. Scandinavian Journal of Infectious Diseases. 1991; 23 (4); 443-9.

51. Zon LI, Arkin C, Groopman JE. Haematologic manifestations of the Human Immunodeficiency Virus. British Journal of Haematology 1987;66 (2) 251-60.

52. Torda A, Jones PD, Beale P. Evaluation of results and clinical

utility of bone marrow examination in patients with HIV infection. Annual Conference Australasian Society HIV Medicine, 1994 3-6; 6; 117.

53. Akpek G, Lee SM, Gagnon DR et al, Bone marrow aspiration biopsy and culture in the evaluation of HIV-infecterd patients for invasive mycobacteria and histoplasma infections. American Journal of Haematology 2001;67 (2):100-6.

54. Ryu T, Ikeda M, Okazaki Y et al, Myelodysplasia associated with aquired immunodeficiency syndrome. Internal Medicine 2001;40 (8); 795-801.

55. Karstaedt AS, Pantanowitz L, Omar T et al, The utility of bone-marrow examination in HIV-infected adults in South Africa. QJM 2001; 94 (2):101-5.