MMED DISSERTATION

ASSOCIATION BETWEEN CLINICAL STAGES (WHO) AND CD4+T-CELL COUNT IN HIV INFECTED ADULTS AT UNIVERSITY TEACHING HOSPITAL IN LUSAKA, ZAMBIA.

By

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MBChB

A Dissertation submitted to the University of Zambia in a partial fulfillment of the requirement for the degree of master of medicine in Internal Medicine.

(School of medicine)

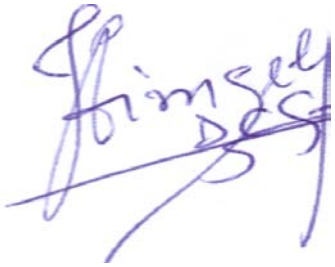
THE UNIVERSITY OF ZAMBIA

LUSAKA

JULY 2010

DECLARATION

I, Dominique Ganywamulume CHIMANUKA, declare that this dissertation is my own work. It is being submitted for the degree of Master of Medicine in Internal Medicine at the University of Zambia. It has not been submitted previously for any qualification in this university or any other.



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This dissertation of **DOMINIQUE GANYWAMULUME CHIMANUKA** has been approved as fulfilling the requirements or partial fulfillment of the requirements for the award of Master of Medicine in **Internal Medicine** by the University of Zambia on the **13th of July 2010**

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ABSTRACT

Infection with HIV ultimately results in profound immunodeficiency, in which patients may present with various AIDS – defining clinical conditions.

The CD4+T-cell count is an important parameter to understand HIV-related opportunistic infections. In poor countries and populations with limited resources, WHO staging system may more useful than CDC classification system of HIV infection. This study was conducted to examine the relationship between WHO clinical stages and CD4+T-cell count in HIV-infected adults at UTH in Lusaka, Zambia.

Selected HIV-infected Zambians adults attending UTH were examined and categorized in WHO clinical stages in a cross-sectional study. Blood was collected for CD4+T-cell count using the FACS method. Different models were developed for the distribution of CD4+T-cell count with other variables. The mean CD4+T-cell counts were estimated and compared between WHO clinical stages. We utilised Epi –Info 6 for descriptive and bi -variable analysis with linear regression for correlation.

Of 216 HIV participants of the study, 107 (49.5%) were males and 109 (50.5%) females. The mean BMI was estimated at 21.2kg/m^2 for the all study population and 44 (23.3%), 16 (8.5%), 83 (43.9%) and 46 (24.3%) subjects were in stage 1, 2, 3 and 4 respectively. Pulmonary tuberculosis (19%), severe bacterial infection (9.7%), weight loss > 10% (7.4%) and oral candidiasis (6.7%) were the commonest clinical conditions diagnosed among symptomatic participants.

One hundred three (55.7 %) participants had CD4+T-cell count less than 200 and only 27 (14.6%) of them were in stage 4. Among all individuals of the study, 83 (43.9%) and 46 (24.3 %) were in stage 3 and 4 respectively.

In conclusion, this hospital-based cross-sectional study showed a weak correlation between clinical stages of the WHO Staging system and the CD4 + T-Cell Count in adult Zambian HIV-infected patients at UTH. The majority of the patients observed were in stages 3 and 4, of the WHO Staging system and half of the study population had low CD4 +T-Cell Count (less than 200 cells/ul). A larger population-based study including asymptomatic patients with higher CD4 counts would be useful to confirm the findings of this study and help health authorities with formulation of policies and projection of costs of provision of lifelong treatment.

DEDICATION

To God our creator

To my late father, brother and sisters
To my family
To my lovely friend

To victims of HIV/AIDS

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ABREVIATIONS AND ACRONYMS

AIDS	:	Acquired immunity deficiency Syndrome
ART	:	Anti-Retroviral Treatment
CD4 T CELL	:	Lymphocytes important for immunity; helper cells leading Attack against infections
CDC	:	Center for Disease Control
FACSCaliber	:	Fluorescent Activated cell Sorting caliber (machine of CD4+cell count)
HIV	:	Human Immunodeficiency Virus
RBC	:	Red Blood Cell
RPR	:	Rapid Protein Reagen
UNZA	:	University of Zambia
UTH	:	University Teaching hospital
WBC	:	White Blood Cell
WHO	:	World Health Organization

CHAPTER 1: INTRODUCTION

1.0 Background

HIV/AIDS is the most threatening and challenging health problem throughout the world. At the end of 2006, thirty-nine and half million people were estimated to be living with HIV/AIDS worldwide with an estimated 1.7 million newly infected while three million people died of AIDS-related causes in the same year. Sixty-eight percent (68%) of all people living with HIV are in Africa. Sub-Saharan Africa is the most affected region by HIV/AIDS in the world (Global Heath Council, 2008). In Zambia an estimated 14.3% of all adults are infected with HIV (WHO et. al, December 2006).

The University Teaching Hospital (UTH) is a tertiary governmental health institution in Zambia receiving patients from Lusaka and those referred throughout the country. When poor patients present with complications due to advanced HIV/AIDS, they are especially difficult to manage in an environment with limited resources. HIV/AIDS patients in this setting are often sicker and tend to have more complications than those documented in western literature.

Poverty and associated factors affect both the severity of clinical manifestations upon admission to UTH and the ability to adequately diagnose and begin treatment for patients. The World Health Organization (WHO) developed guidelines for such situations where clinical manifestations are presumed indicative of the progression of the disease. Such guidelines are based upon established correlations between CD4+ T-cell count and clinical manifestations. The reliability of these established correlations for African population, particularly those that are poor, has been questioned in literature.

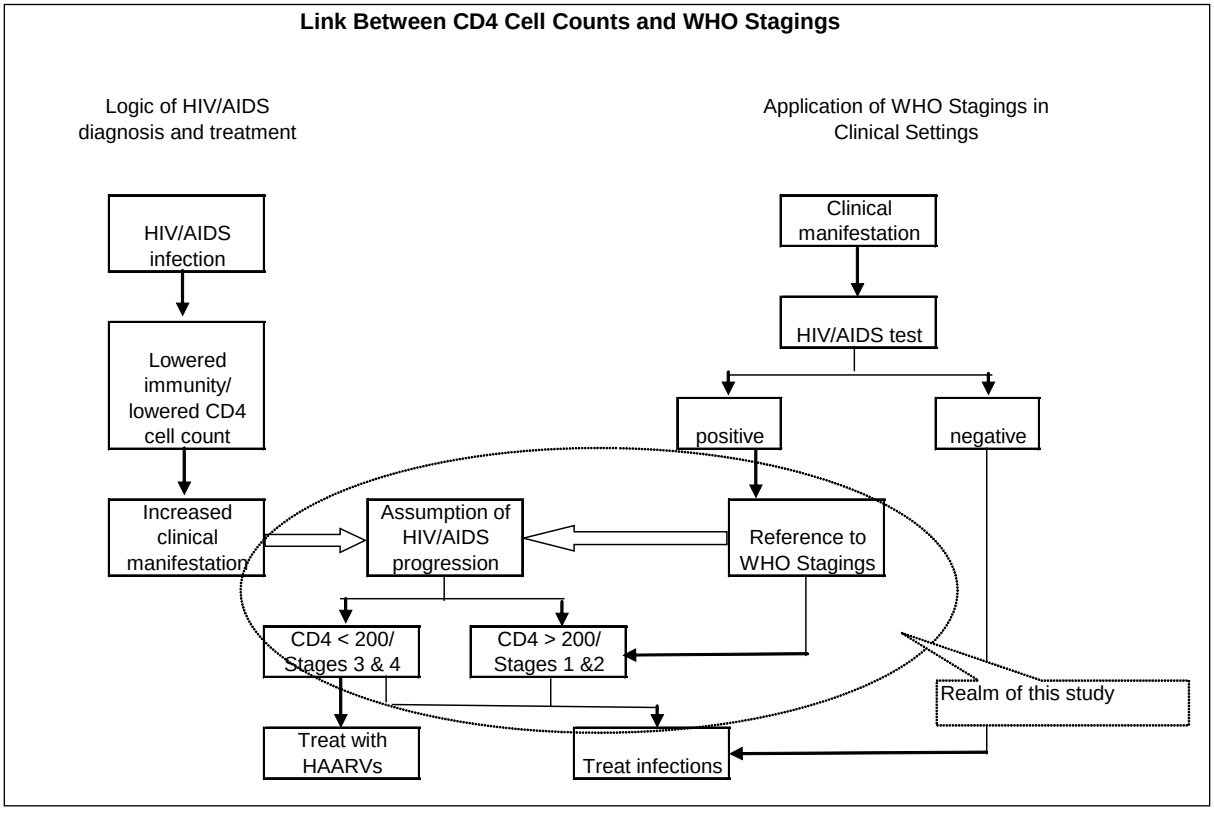
1.1 Statement of the problem

Two important clinical staging systems are currently used throughout the world for HIV infection: the Center for Disease Control (CDC) staging system (1993) and the World Health Organization staging system (2005). The CDC staging (1993, see Table 6, page 26) built on research of largely American and western HIV patients is mostly used in developed countries while the WHO staging (2005) is based largely on the clinical staging is more applicable to poorer countries (fig 1 and Table 7, page 27). The premise here is that the link between CD4+ T-cell counts and clinical manifestations may vary from one region of the world to another. Thus, it is useful to test these relationships in a specific area and among a specific population – adults within Zambia.

Laboratory markers (CD4 cell count mostly and HIV viral load) and clinical manifestations are used in the CDC staging while WHO staging considers more clinical manifestations. The link between CD4 cell count, the main indicator of disease progression, and clinical manifestations remain unclear for some populations – particularly in Africa.

Poor patients often appear to be sicker than either the WHO or CDC staging guidelines would indicate. Their clinical manifestations register at the more advanced stage than their CD4 count would indicate. The straightforward match between CD4 positive cell count and clinical manifestations do not appear to be a reliable guide. Figure 1 shows the underlying relationship between CD4 positive cell count and the WHO staging

Figure1.



Since this population is poor but are required to pay for tests due to resource limitations, laboratory testing cannot always be done to back up clinical observations. Without available laboratory testing, doctors must rely on WHO staging. But when such staging is not applicable, treatment indications may be inaccurate and delayed. An assessment of the validity or applicability and relevance of WHO staging for this population is urgently needed.

This study will examine the relation between CD4 positive cell count and clinical manifestations of HIV/AIDS positive patients admitted to UTH. Ascertaining whether the

WHO staging criteria with its underlying assumptions of CD4 positive cell count is a reliable estimate of HIV/AIDS progression for this population will help determine how useful this tool is for initiating treatment.

1.2 Rationale/Justification:

The idea behind CDC staging (1993) was to provide a standardized system with relationship between clinical manifestations, CD4 levels and viral loads. The three measures were correlated to form a fairly accurate picture of the progression of the disease and, hence, its treatment (Philips et al.1991; Stein et al. 1992). The WHO Staging (2005) used these relationships to develop a similar measure that took less time, money and resources but obtained a relatively accurate guide for treatment. Thus, the WHO staging were based largely on the CDC clinical staging. The CDC staging was developed on research of largely American and Western HIV patients. The efficacy of the use of this staging for non-western populations was either not questioned or nor validated.

Yet, there is a known relationship between nutrition and survival in HIV disease (Kelly et al.). It is possible that other relationships exist with medical history, stress, lifestyle and other factors (Hukstaert and Hannet, 1994; Teck et al. 2005). These factors vary among populations and are often linked to poverty. Some studies have found that the progression of HIV/AIDS is related to poverty levels (Fylkesnes et al., 1997). The CDC staging (1993) based purely on three factors is likely not a fully explanatory model. Studies have started to reveal that the CDC staging are not as accurate for people in poor countries as they are for the western population from which they were developed (Anglaret et al., 1997; Attili, 2005; Ghate et al., 2000; Karm et all., 1998; Kassa, 1999).

One of the questions being pursued among HIV researchers, then, is how accurate a guide Are the CDC and WHO staging for treatment of populations with varying poverty levels and ethnic groupings.

This research is being pursued sporadically throughout African and occasionally in other poor countries (French et al., 1999; Urassa et al., 2004; Vajpayee etal.,2003; Vajpee et al., 2005: Whittle et al., 1992). Preliminary finding are beginning to indicate that patients may well present with more severe clinical manifestations than their CD4 cell levels might have predicted using the CDC/WHO stagings. Yet such research is still inconclusive.

There is not enough research to conclude the underlying factors that cause the variability and the smaller studies that have been done are not comprehensive enough to determine specific geographic and poverty parameters. There are a few studies that indicate this may be true for the Zambian population (Katubuluski, Yavwa, 2005; Kelly and Zulu, 2002).

Thus, this study will add to this body of literature by testing the CD4 cell count - clinical manifestation relationship in a particular geographic area and for a rather specific population. That is, it will measure patients within Zambia who are patients at UTH - a

relatively poor population within Zambia. In so doing, it will establish one additional point of known relationships from which broader patterns might be discerned with additional research. Further, it will serve as a guideline for the treatment of this particular population.

The review of existing guidelines and the knowledge on clinical manifestations for adult HIV-infected patients at UTH will permit those patients to be evaluated quickly for optimal access to ART. This may lead to reduced health costs and more effective management of the disease by the government through the ministry of health. This information will be used to suggest to authorities a new approach to HIV- infected adults at UTH with benefit of more efficient health care plus reduce costs.

Further, it will contribute to the growing body of literature on the CD4 cell count/ clinical manifestation relationships for African populations.

1.3 Research Hypothesis

This study postulates that the WHO clinical staging guidelines do not accurately predict CD4+ cell count levels of patients presenting to UTH

1.4 Aims of the study

The objectives of this study are:

1. General objective: to determine whether CD4 cell counts are accurately correlated to clinical manifestations (WHO staging) for the UTH population in Lusaka.

2. Specific objectives:

    a. to determine the common opportunistic infections and their related CD4 + T cell count for adult patients at UTH

    b. to determine if the staging applies equally for subpopulation by age and gender

CHAPTER 2: LITERATURE REVIEW

2.0 Relationship between CD4 cell count and clinical manifestations

The relationship between CD4 cell count and clinical manifestations is well established. As the HIV infection progresses the immune status of the patient deteriorates as indicated by lowered CD4 cell count. A patient with a CD4 positive cell count less than 200 is at high risk for developing various opportunistic infections, while those with a CD4 positive cell count more than 500 have considerable immunity to fight pathogens (Attili, 2005; centers for Disease Control and Prevention, 1997). The depletion of CD4 + T lymphocytes has clinical consequences manifested by clinical conditions associated with HIV infection and CD4+ cell counts such as oropharyngeal candidiasis and Kaposi's sarcoma at CD4+cell count between 200 and 500; Pneumocystis pneumonia at CD4+ level < 200; cryptococcosis at CD4+cell count < 100. This relationship has been used to treat HIV/AIDS throughout the world.

Table 1. CDC Classification System for HIV-Infected Adults and Adolescents

CD4 Cell Categories	Clinical Categories		
	A Asymptomatic, Acute HIV, or PGL	**B** Symptomatic Conditions,#* not A or C	**C** AIDS-Indicator Conditions*
(1) ≥500 cells/µL	A1	B1	C1
(2) 200-499 cells/µL	A2	B2	C2
(3) <200 cells/µL	A3	B3	C3

Key to abbreviations: CDC = U.S. Centers for Disease Control and Prevention; PGL = persistent generalized lymphadenopathy

Table 2. ***CDC clinical classification Category A***

Category A consists of one or more of the conditions listed below in an adolescent or adult (greater than or equal to 13 years) with documented HIV infection. Conditions listed in Categories B and C must not have occurred.
• Asymptomatic HIV infection • • Persistent generalized lymphadenopathy • • Acute (primary) HIV infection with accompanying illness or history of acute HIV infection

Table 3. CDC Classification System: Category B Symptomatic Conditions

Category B symptomatic conditions are defined as symptomatic conditions occurring in an HIV-infected adolescent or adult that meet at least 1 of the following criteria:

a) They are attributed to HIV infection or indicate a defect in cell-mediated immunity.

b) They are considered to have a clinical course or management that is complicated by HIV infection.

Examples include, but are not limited to, the following:

- Bacillary angiomatosis
- Oropharyngeal candidiasis (thrush)
- Vulvovaginal candidiasis, persistent or resistant
- Pelvic inflammatory disease (PID)
- Cervical dysplasia (moderate or severe)/cervical carcinoma in situ
- Hairy leukoplakia, oral
- Idiopathic thrombocytopenic purpura
- Constitutional symptoms, such as fever (>38.5°C) or diarrhea lasting >1 month
- Peripheral neuropathy
- Herpes zoster (shingles), involving≥2 episodes or ≥1 dermatome

Table 4. CDC Classification System: Category C AIDS-Indicator Conditions

- Bacterial pneumonia, recurrent (≥2 episodes in 12 months)
- Candidiasis of the bronchi, trachea, or lungs
- Candidiasis, esophageal
- Cervical carcinoma, invasive, confirmed by biopsy
- Coccidioidomycosis, disseminated or extrapulmonary
- Cryptococcosis, extrapulmonary
- Cryptosporidiosis, chronic intestinal (>1-month duration)
- Cytomegalovirus disease (other than liver, spleen, or nodes)
- Encephalopathy, HIV-related
- Herpes simplex: chronic ulcers (>1-month duration), or bronchitis, pneumonitis, or esophagitis
- Histoplasmosis, disseminated or extrapulmonary
- Isosporiasis, chronic intestinal (>1-month duration)
- Kaposi sarcoma
- Lymphoma, Burkitt, immunoblastic, or primary central nervous system
- *Mycobacterium avium* complex (MAC) or *M kansasii* , disseminated or extrapulmonary
- *Mycobacterium tuberculosis* , pulmonary or extrapulmonary
- *Mycobacterium* , other species or unidentified species, disseminated or extrapulmonary
- *Pneumocystis jiroveci* (formerly *carinii*) pneumonia (PCP)
- Progressive multifocal leukoencephalopathy (PML)
- *Salmonella* septicemia, recurrent (nontyphoid)
- Toxoplasmosis of brain
- Wasting syndrome due to HIV (involuntary weight loss >10% of baseline body weight) associated with either chronic diarrhea (≥2 loose stools per day ≥1 month) or chronic weakness and documented fever≥1 month

This observation of gradual decline in the immunity of the patient as represented by the CD4+ cell count has lead to the use of CD4 cell count as a marker of disease progression (Attili, 2005; Ghani et al. 2001; Philiips,1994; Sheppard et al. 1993; Turner and Taroni , 1996; Weller et al. 1996).Various clinical conditions are related to HIV disease at all levels of CD4 but the most severe conditions are found in patients with low CD4 cell count.

The absolute CD4+cell count at which particular individual experience specific disease syndrome is dependent upon the level of CD4+cell count within the patient (Attili, 2005). Opportunistic infections and malignancies induce chronic immune activation thereby accelerating the loss of CD4 T cells in HIV infected patients (Bentwich et al.,1995; Hazenberg et al.,2003; Mekonne et al.,2003).

Within Zambia, the majority of HIV related untreated infectious diseases have low CD4+cell count. Examples of these are tuberculosis or persistent diarrhea which present with CD4+cell count less than 200 cells per microlitre (Kelly et al., 2002).

It is these established relationships that have allowed researchers to divide disease progression into categories known as stages and related clinical manifestations to these stages.

2.1 WHO staging

Table 5. WHO Clinical Staging of HIV/AIDS for Adults and Adolescents

Primary HIV Infection
• Asymptomatic • Acute retroviral syndrome
Clinical Stage 1
• Asymptomatic • Persistent generalized lymphadenopathy
Clinical Stage 2
• Moderate unexplained weight loss (<10% of presumed or measured body weight) • Recurrent respiratory infections (sinusitis, tonsillitis, otitis media, and pharyngitis) • Herpes zoster • Angular cheilitis • Recurrent oral ulceration • Papular pruritic eruptions • Seborrheic dermatitis • Fungal nail infections
Clinical Stage 3
• Unexplained severe weight loss (>10% of presumed or measured body weight) • Unexplained chronic diarrhea for >1 month • Unexplained persistent fever for >1 month (>37.6°C, intermittent or constant) • Persistent oral candidiasis (thrush) • Oral hairy leukoplakia • Pulmonary tuberculosis (current) • Severe presumed bacterial infections (eg. pneumonia, empyema, pyomyositis,

bone or joint infection, meningitis, bacteremia)
- Acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis
- Unexplained anemia (hemoglobin <8 g/dL)
- Neutropenia (neutrophils <500 cells/µL)
- Chronic thrombocytopenia (platelets <50,000 cells/µL)

Clinical Stage 4

- HIV wasting syndrome, as defined by the CDC (see Table 3, above)
- *Pneumocystis* pneumonia
- Recurrent severe bacterial pneumonia
- Chronic herpes simplex infection (orolabial, genital, or anorectal site for >1 month or visceral herpes at any site)
- Esophageal candidiasis (or candidiasis of trachea, bronchi, or lungs)
- Extrapulmonary tuberculosis
- Kaposi sarcoma
- Cytomegalovirus infection (retinitis or infection of other organs)
- Central nervous system toxoplasmosis
- HIV encephalopathy
- Cryptococcosis, extrapulmonary (including meningitis)
- Disseminated nontuberculosis *Mycobacteria* infection
- Progressive multifocal leukoencephalopathy
- Candida of the trachea, bronchi, or lungs
- Chronic cryptosporidiosis (with diarrhea)
- Chronic isosporiasis
- Disseminated mycosis (eg, histoplasmosis, coccidioidomycosis, penicilliosis)
- Recurrent nontyphoidal *Salmonella* bacteremia
- Lymphoma (cerebral or B-cell non-Hodgkin)
- Invasive cervical carcinoma
- Atypical disseminated leishmaniasis
- Symptomatic HIV-associated nephropathy
- Symptomatic HIV-associated cardiomyopathy
- Reactivation of American trypanosomiasis (meningoencephalitis or myocarditis)

Two global organizations have presented such HIV stages: the Center for Disease Control (CDC) and the World Health Organization (WHO). The CDC uses three parameters for determining the stage of disease progression: CD4 cell count, clinical manifestations and viral loads. The WHO uses only one of these parameters to determine disease progression: The clinical manifestations.

WHO developed this one-parameter method in response to a need for quick, cheap and easily available diagnosis of HIV/AIDS within poor countries. Most developing countries

have limited resources and the majority of poor patients have limited access to laboratory services. WHO's guidelines are based solely on clinical manifestations (WHO, 2005). The four WHO stages of disease progression, nevertheless, depend upon the known linkages of CD4 cell count and clinical manifestations. Although the stages reference only clinical manifestations, they can be related to CD4 cell counts.

2.2 Applications for African populations

Although the majority of HIV infections occur in sub-Saharan Africa, only limited information is available relative to this occurrence (Mekonnen et al., 2005). There is also little data on CD4+ cell counts in this region. Few studies have analyzed the burden of morbidity in the general urban African population and there is little information about relationship of morbidity to immune damage within the population (Kelly et al., 1996). There is some research on HIV incidence in Zambian context, but most studies are focused on the characteristics of infection and treatment (Fylkesnes et. al.1997; Fylkesnes et. al., 1998; Stringer, 2006).

CD4 cell counts are affected by different factors: physiological, technical, geographical, racial and ethnic origin, age, gender, etc. (Anglaret, et. al., 1997; Vajpayee, et. al., 2005). The reliability of CD4+ cell counts in predicting the progression of the disease varies according to local HIV characteristics. In African populations especially, CD4+ cell counts may indicate a less progressive stage of HIV than clinical manifestations convey. Some studies have observed that the clinical disease stage is a stronger predictor of disease progression than is the CD4+ cell count.

There is not enough research to make a conclusion on the underlying factors that cause the variability and the smaller studies that have been done are not comprehensive enough to determine specific geographic and poverty parameters. There are a few studies that indicate this may be true for the Zambian population.

CHAPTER 3: METHODOLOGY

3.0 Design and site study

This was a cross-sectional study.
This design was chosen because it was well suited for describing variables with their distribution pattern and could be used for examining association. (Stephen B. HULLEY & Steven R.CUMMINGS, 1988)

The study site was University Teaching hospital, a tertiary hospital with predominantly HIV positive patients being admitted into the medical wards as well as being the first center in the roll out of anti-retroviral therapy in late 2002.

3.1 Measurement of variables:

The following variables were measured in this study:

1) Clinical manifestations : all conditions listed in WHO staging system (2005) were considered

2) WHO clinical stages (I, II, III, IV)

3) CD4 T cell count were ranked as followed (* 10^6/l): <50, 50 – 199, 200 - 499, >500 cells/ul

4) Background variables: - gender (M/F) : a nominal variable (dichotomous)
   Some studies have found that there is a significant gender (and racial) differences in immunity (Marc FOCA et al, July 2006)
   There is a consistent relationship between gender related differences in viral load and CD4 cell count (CA Donnelly et al,)
   Lymphocytes subsets varied according to gender in HIV infected

5) Confounding variables:
   There are many confounding variables (HIV variants, host immune system, nutritional status, etc…) on which HIV infection and its predictors depend, but those aspects were not considered in this study due to cost implications.

3.2 Participant selection

3.2.0 Sample size calculation:

Sample size was calculated using the following formula:
Sample Size:

$$ss = \frac{Z^2 * (p) * (1-p)}{C^2}$$

Where:

Z = Z value (e.g. 1.96 for 95% confidence level)
p = percentage picking a choice, expressed as decimal
(.5 used for sample size needed)
c = confidence interval

The confidence interval chosen was estimated using a confidence level of 95 percent and a percentage likelihood of a person falling within the expected category of 50 percent. This percentage was chosen, as the most conservative choice given no previously estimated value. The result was a confidence interval of 6.01.

Using this confidence interval, confidence level and population size, a sample size of 200 was calculated. (Survey system®)

3.2.1 Inclusion criteria:

The sample included patients who met the following criteria:

- Confirmed HIV positive patients tested for HIV infection using current national testing guidelines, who were ARV-naive
- Patients aged 16 years and above
- Patients seen in admission ward ,medical wards and ART clinic

3.2.2 Exclusion criteria:

In order to ensure safety of patients the following were excluded:

- Refused consent to study procedures

In order to try to control for possible confounding effects, the following were excluded:

- Patients who were already on ARVs
- Patients with other chronic illnesses that could affect immunity or were on immunosuppressive therapy
- Patients with pregnancy

3.2.3 Recruitment methods:

Individuals were enrolled in this study over a time period of 3 months (October to December 2008).

Study subjects were recruited from medicine department (admission ward, ART clinic and medical wards). Individuals at all stages of HIV infection including early stages and all sick patients were recruited from medical admission, ART clinic. Individuals at advanced stages of HIV infection and all other sick patients were recruited among hospitalized patients (medical wards) suspected clinically of having AIDS.

Patients were asked to undergo a HIV test, CD4 + T cell count and other laboratory investigations regarding their clinic after a formalized consent for participation to study and pretest counseling. Study subjects were categorized in groups according to their gender and age. All enrolled subjects between October 2008 and December 2008 were included in this study.

3.2.4 Consent procedure:

Patients were asked if they would consent to be part of the study and allow the use of their demographic, clinical and laboratory data notes.

3.3 Collection of data

Data for the study was obtained from adult patients admitted in Medical wards. Participants underwent the following procedures: pre-test counseling after informed consent for HIV testing, Interview on socio-demographic characteristics, medical history, clinical examination and WHO clinical staging (2005) of HIV infection. Clinicians working at all sites of recruitment will used the same guidelines for diagnosis of each stage-specific HIV related condition according to the revised WHO staging 2005. It should be noted that many of the diagnoses were presumptive since laboratory facilities were are limited in this setting.

Data collected included: CD4+ T cell count and clinical data (identity number, history, examination, laboratory test results) after assessment of the exclusion criteria.

3.4 Laboratory methods:

HIV test: We utilised the National guidelines on diagnosing HIV disease. First rapid test was performed with ABBOT® and confirmation was by UNI-GOLD® using serum of blood. ELISA test was used later for conflicting results.

CD4 T cell count: Lymphocyte subsets were determined by FACSCalibar drawing 4 ml of blood in EDTA container once. Flow cytometry method was used; blood stained with monoclonal dye and detection was done by the FACSCalibar machine.
For some clinical conditions, specific laboratory tests were used respectively.

Safety laboratory test and other tests

1) For complete blood count (CBC) 3 ml of blood was collected in EDTA container.
The SYSMEX machine version XT 2000I was used with different methods and reagents according to the parameter measured.

WBC and Differential count: flow cytometry method, stromatolyser FB,4DL, 4DS as reagent;
Hemoglobine : cyanmethemoglobine method, sulfolyser method
RBC and platelet: hydro dynamic method, cell pack and FB reagent.

2) For biochemistry (urea, electrolytes, creatinine, Liver enzymes and protein/albumin, lipid profile, uric acid, amylase, LDH). We drew 2-5ml of blood in lithium heparin container (green top) or plain container (red top). For blood sugar 2-3 ml of blood was collected in sodium fluoride and potassium oxalate. The OLYMPAUS automated chemistry analyzer machine was used with olympaus reagent.

These tests contributed to the baseline clinical and laboratory data for our HIV patients before starting the treatment and were also used to help identify some of the opportunistic infections characterizing the clinical stages (WHO).

Confirmation of some diagnoses (clinical conditions listed in WHO staging 2005) were difficult considering our limited resources and lack of laboratory facilities.

3.5 Analysis of data

1) DESCRIPTIVE ANALYSIS:

for clinical conditions the frequency was calculated
for each condition mean & standard deviation of CD4 T cell count were calculated
for background variables mean & standard deviation were calculated for age and frequency for sex.

2) BIVARIABLE ANALYSIS and LINEAR REGRESSION:

were used for the association between WHO clinical stages and CD4 T cell count.

Data were analyzed using Epi Info 6. Proportions were compared using chi-square or Fischer's test where appropriate ($p < 0.05$ was considered statistically significant). Association between WHO clinical stages and CD4+T-cell count was analyzed using ANOVA (Mann Whitney/Wilcoxon and Kruskal Wallis) plus linear regression.

3.6 Ethical considerations

Permission was obtained from the ethical committee after approval of the corrected proposal by the school of medicine.

Authorization was also obtained from UTH administration to enable the use of data/ rooms, and patients' records. We also obtained permission from patients to participate in this study through a consent form (appendix 2). These patients were asked to sign the consent form but could withdrawal from the study at any time and without any relevant consequence to their care.

CHAPTER 4: RESULTS

4.0. Demographic, anthropometric & laboratory characteristics

Individuals were enrolled in this study over a time period of 3 months from October to December 2008.

Data are reported on 216 participants of whom 109 were females (50.5 %) and 107 were males (49.5 %). The mean CD4 + T cell was 228.2 cells/mm^3, the mean BMI 21.2 kg/m^2, and the mean hemoglobin 10.8 g/dL. Complete demographic, anthropometric and laboratory characteristics are displayed in table 1.

Table 6. Anthropometric & laboratory characteristics

	Mean	SD
Age (years)	36.3	9.6
Weight (kg)	61.8	29.6
Height (m)	1.65	0.2
Body mass index (kg/m^2)	21.2	4.8
CD4+T-cell count (cells/mm^3)	228.2	187.8
Hemoglobin (g/dL)	10.5	3.2
ALT (IU/L)	34.2	37.1
Creatinine (umol/L)	106.8	101.6

4.1 Clinical manifestations

Table 7. Clinical manifestations

	Clinical conditions	N	Percentage (%)
1	Pulmonary tuberculosis	41	19.0
2	Asymptomatic	40	18.5
3	Severe Bacterial infection	21	9.7
4	Weight loss > 10 %	16	7.4
5	Oral Candidiasis	15	6.9
6	Extra Pulmonary Tuberculosis	10	4.6
7	Kaposi Sarcoma	10	4.6
8	Cryptococcal Meningitis	9	4.2
9	Fever > 1 month	8	3.7
10	Herpes Zoster	7	3.2
11	HIV Encephalopathy	6	2.8
12	Upper Respiratory Infection	5	2.3
13	Wasting syndrome	4	1.8
14	Diarrhea > 1 month	4	1.8
15	Anemia < 8g/dl	4	1.8
16	Thrombocytopenia	4	1.8
17	Rash	3	1.4
18	Persistent Generalized Lymphadenopathy	3	1.4
19	Moderate weight loss	2	0.9
20	Esophageal candidiasis	1	0.5
21	Pneumocystis Carinii pneumonia	1	0.5
22	Mucocutaneous manifestations	1	0.5
23	Lymphoma	1	0.5
	Grand Total	216	100

A total of 22 clinical conditions were recorded in 176 subjects (81.5 %) and the remaining 40 participants (18.5 %) were asymptomatic (Table 2). Among conditions listed in the WHO staging system, the most common manifestations observed in this study were pulmonary tuberculosis (19.0 %), severe bacterial infection (9.7 %), weight loss > 10 % (7.4%) and oral candidiasis (6.9%).

4.2 WHO clinical stages

Table 8. Distribution of participants by WHO stages and Mean CD4 count for WHO clinical stages

WHO	N (%)	Mean CD4 count	SD
1	44(23.3)	285.8	150.2
2	16(8.5)	212.6	201.5
3	80(43.9)	202.9	196.2
4	45(24.3)	190.1	148.3

Out of 216 individuals enrolled in the study, only 189 participants were considered while 27 subjects were excluded for this analysis due to missing data, i.e. lack of records on WHO staging.

Considering the WHO staging of HIV infection and disease, 44 (23.3%) participants were in stage 1, 16 (8.5%) in stage 2, 83(43.9%) in stage 3 and 46(24.3%) in stage 4. Majority of participants had stage 3 or 4 (68.2% together) advanced HIV disease. Table 3 shows mean CD4 count for each clinical stage.

4.3. WHO Stage and CD 4 +T- Cell Count

Table 9. Distribution of Participant by WHO Stage and Range of CD 4+ T- cell Count

WHO STAGE	CD4 Count Range				
	<50	50 – 199	200 – 499	>500	Total
1	1	12	28	3	44
2	3	6	5	2	16
3	16	39	20	8	83
4	10	18	17	1	46
Total	**30**	**75**	**70**	**14**	**189**

Participants were categorized according to CD4 count range: 30 (16.2%) HIV-positive individuals had less than 50 cells/ul, 75 (39.7%) with CD4+T cell in the range of 50 -199 Cells/ul, 70 (37%) were between 200 and 499 cells/ul and 14 (7.6%) with 500 cells/ul or more. Half of the population examined (55.6%) had a CD4 count of less than 200 cells/ul. These results are shown in table 4.

The lowest CD4+T cell count was 1 cell/ul and the highest 820 cells/ul.

Figure 2. Scatter plot of CD4 count and WHO Stage

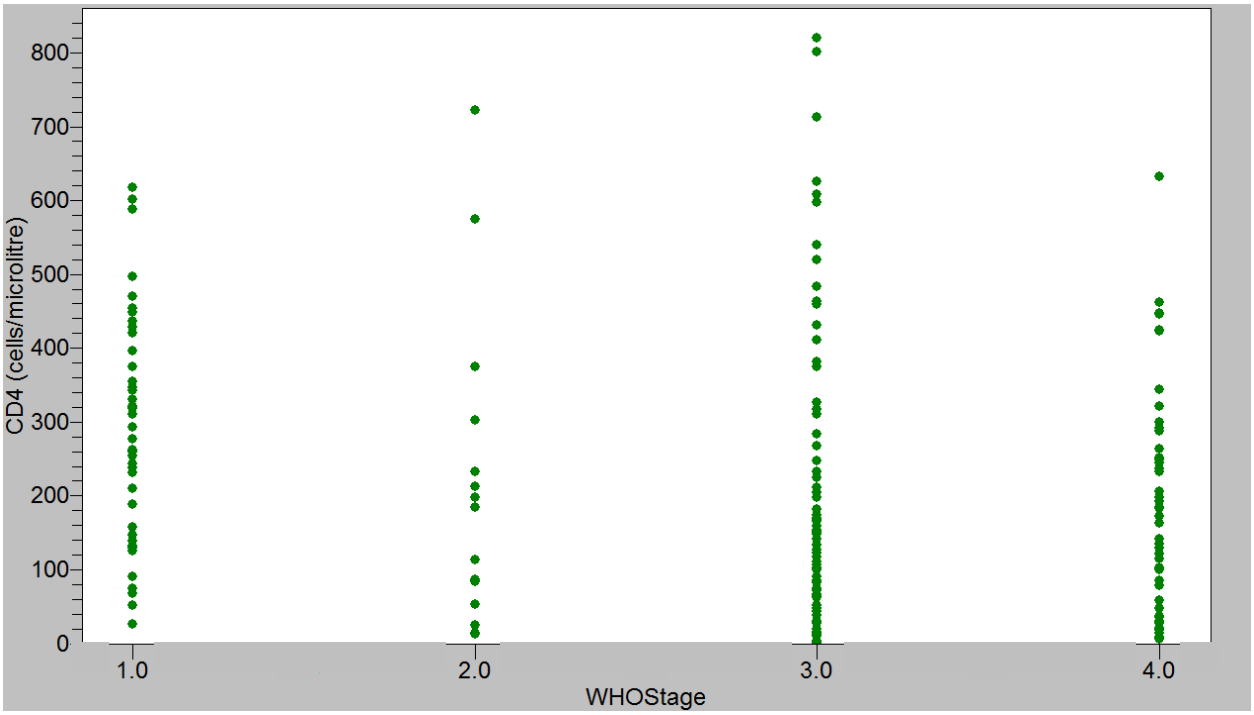


Figure 2 shows the distribution of CD4+T cell counts scattered within each WHO stage. Advanced HIV clinical stages 3 and 4 appear to have a higher concentration of CD4+T cell counts less than 200 cells/ul.

4.4. Bivariable analysis and linear regression

4.4.1. Mean WHO clinical stages/ CD4+T cell count

Mean CD4 counts at each WHO stage were compared using one-way ANOVA with 3 degrees of freedom, and the p value for difference between means was 0.04.

4.4.2 Linear Regression

Table 10. Bivariate linear regression model for CD4+T cell count vs. WHO stage

WHO stage	coefficient	Std error	P value
1, reference	285.8	26.5	
2	-73.2	51.3	0.15
3	-82.9	32.9	0.01
4	-95.8	37.2	0.01

$r^2 = 0.04$

A bivariate linear regression model was constructed. WHO stage was treated as separate dichotomous (dummy) variables, and WHO stage 1 was the referent value (Table 5).

In comparison to WHO stage 1, the regression of CD4+T cell count in clinical stage WHO 2 shows a p value of 0.15 while in clinical stages 3 & 4 shows a p value of 0.01. The correlation coefficient r^2 is 0.04: only 4% of the variation in CD4+T cell count can be accounted for by WHO stage.

Considering the above results (ANOVA & linear regression) the statistical analysis shows that there is an association between clinical stages (WHO) and CD4+T cell count in this study with respect of some limitations.

CHAPTER 5: DISCUSSION

Despite the magnitude of the HIV/AIDS problem, there is paucity of published data on correlation CD4+T-cell count and clinical manifestations in African settings. This cross-sectional study on relationship between WHO clinical stages and CD4+T-cell count was conducted at UTH in the medical department among adults Zambians HIV infected.

The WHO staging system for HIV infection and disease is based on combination of 32 conditions (revised version) divided in four stages and biological markers essentially CD4+T-cell count. Among 22 clinical conditions observed, pulmonary tuberculosis, weight loss >10%, severe bacterial infection and oral candidiasis were the commonly diagnosed manifestations. These findings differ slightly with studies done in Ethiopia and Uganda where oral candidiasis, pulmonary tuberculosis, and wasting syndrome were the commonest manifestations. These findings were obtained despite limited facilities available for the diagnosis of HIV- related conditions suggesting that the WHO staging remains useful in developing countries. Also simple clinical information such as weight and height should be recorded regularly to permit the use of body mass index in these patients for whom weight loss is one of the commonest manifestations.

The CD4 T lymphocyte is the immune system cell that HIV infects and destroys, and CD4+T-cell count reflects the state of the immune system and represents a marker of progression of HIV infection; it is also a measure of the relative risk of developing opportunistic infections. Therefore CD4 estimation is the backbone of AIDS control program in developing countries. The mean CD4+T-cell count in this study was 228. One of the striking results of the study was the low CD4+T-cell count observed in these HIV-positive Zambians individuals: 103/189 (54.5%) subjects had CD4+T-cell count less than 200 cells/ul, a count that defines having AIDS in the 1993 revised CDC classification . We noticed low CD4+T-cell count in some participants of stage 1 and 2 of the WHO staging while interestingly some patients of advanced WHO stage (3 or 4) had high CD4+T-cell count more than 200.

The main aim of this study was to evaluate the association between WHO clinical stages and CD4+T-cell count in adults HIV-infected Zambians at UTH. The bivariate linear regression analysis shows that there is an association between clinical stage and CD4 count. But considering the degree of correlation, only a small proportion of CD4+T cell count can be accounted for by WHO clinical staging. We therefore found a weak correlation between WHO clinical stage and CD4+T-cell count in adults Zambians HIV-infected at UTH.

Opportunistic infections occur at a low CD4+T-cell count throughout the world but it is interesting to examine why in this study some HIV-positive individuals with advanced WHO clinical stages had high CD4+T-cell count while some of those in early stages had low CD4+T-cell count. Patients with higher CD4+T cell count may develop certain stages 3 and 4 conditions like in tuberculosis and Kaposi sarcoma. In this study, tuberculosis was the most important opportunistic infection, and this could explain,

among other reasons, the higher CD4+T cell count of some patients in advanced stages. We need also to consider confounding factors and to rule out other conditions which depress immunity (diabetes, malignancies, malnutrition ...).

The majority of individuals were in stages 3 and 4 which are the advanced WHO stages while the other participants are categorized in early stages (1 &2). This implies that most of our patients present at hospital with clinical manifestations are in advanced HIV clinical stages. The important proportion of HIV-positive individuals (half of the population studied) with low CD4+T-cell count (<200 cells/ul) and few clinical manifestations would benefit from chemoprophylaxis of opportunistic infections and early work up for initiation of antiretroviral drugs according to the assessment. Pulmonary tuberculosis was the common opportunistic infection documented among participants of this study; this finding suggests a role for tuberculosis chemoprophylaxis (Isoniazid preventive therapy). Strict measures are required to rule out tuberculosis and reduce its mortality including sputum examination and chest X-ray, close supervision and follow up.

WHO staging system was proposed and evaluated in many countries, and some studies have established that WHO clinical stage correlate well with CD4+T-cell count. WHO clinical staging has played an important role in identifying patients in need of ART especially in resource-limited settings but a number of studies reported its limitations. Although CD4 +T-cell count is an important marker for progression to AIDS, this study shows how important WHO staging system is in our environment. Ideally, we need to combine clinical and immunological parameters when making decisions concerning initiation of ART.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.0 Conclusion

This study was conducted to evaluate the association between WHO clinical stages and CD4+T-cell count in adults HIV-infected at UTH in Lusaka. The study shows a weak correlation between WHO clinical stages and CD4+T-cell count. CD4 counts were low in adults Zambians enrolled in the study but had a wide range in the different WHO stages. A majority of the study population was in advanced stages (3 and 4). Pulmonary tuberculosis, severe bacterial infection, weight loss > 10% and oral candidiasis were the common clinical conditions found in this study. This study emphasizes the importance of clinical examination to diagnose in time those conditions and possibly early chemoprophylaxis against opportunistic infections while waiting the initiation of treatment. A wide or longitudinal study will be more useful to confirm these findings that can be used by both medical personnel and authorities taking care of Zambians HIV-infected individuals.

6.1 Limitations

The sample of this study derived from only one hospital (UTH) in one city(Lusaka) of one province (Lusaka); the findings cannot be extrapolated to all Zambians or to other populations. Also the sample has been taken in a relatively short period of time (3 months) limited by financial resources. Confirmations of some diagnoses were difficult considering our limited resources and lack of laboratory facilities. There are many confounding variables (HIV variants, host immune system, nutritional status …) on which the progression of HIV infection depend and it predictors but those aspects were not addressed in this study.

As such, attempts to generalize the findings of the present study outside of a similar sample population must be made with caution.

6.2 Recommendations

1. Health education about HIV/AIDS should continue and the emphasis on early medical examination should be encouraged in Zambian population and other developing countries.

2. Medical workers should be careful with HIV-infected patients whom progression to AIDS is not well determined at first medical visit. Opportunistic infections should be systematically searched/treated and laboratory facilities made available for patients who can't afford them.

3. More research studies are needed in this field, especially on clinical manifestations and CD4+T-cell count in our settings to suggest new guidelines/protocols to health authorities for the well being HIV-infected patients.

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INFORMATION SHEET/CONSENT FORM

Title: ASSOCIATION BETWEEN CLINICAL STAGES (WHO) AND CD4+ T CELL COUNT IN HIV INFECTED ADULTS AT UNIVERSITY TEACHING HOSPITAL IN LUSAKA, ZAMBIA.

Investigator: Dr. Dominique GANYWAMULUME CHIMANUKA

Medical doctor, department of medicine, UTH

Sponsor: Investigator + UTH + other supports

Introduction: you have being invited to participate in a study to find out the relationship between clinical manifestations (WHO) and CD4 + T cell count (standard test of prognosis for progression of HIV disease) in patients with HIV.
You will find in this form information about the study (purpose, procedures, risks and benefits) and a consent form to sign after explanation and free decision in front of witness
In case you disagreed to participate in the study or withdraw, the other health procedures will continue and you will benefit all the cares as others.

Purpose of the study: to find out how relevant is the WHO guidelines in our setting by analyzing the relationship between clinical stages and CD4 + T cell count in HIV infected adults. This information will help to modify or decide early on the treatment of the patients attending UTH knowing the common opportunistic infections and their related CD4 T cell count.

Study procedures: This study is taking place at the university teaching hospital, department of medicine (admission ward and out clinic). Patients will be recruited after a formalized consent. Clinical data will be taken and blood drawn to check HIV status (after consent), CD4 + T cell count and other tests regarding clinical picture.

Risks/ discomforts: Practically no major risk but subjects of the study will experience the discomfort of routine collection of blood.

Benefits: A part of the main purpose, this study will permit patients to benefit free tests regarding their condition plus early intervention according to their severity's condition.

Confidentiality: this study is strictly confidential. Your personal details/name will not be recorded on files or appear in retained data.

CONSENT FORM

Your participation in this study is strictly voluntary. You will not suffer any consequences if you decide not to participate in the study. You may withdraw from the study at any time for any reason without consequences to your health care.

Thank you for considering your participation into the study. If you have any questions, concerns and clarifications, please contact Dr. D. G. CHIMANUKA (0955812979/0978940482) or UNZA research ethics committee secretary 211256067.

I, …… hereby confirm that I have been sufficiently explained to about the nature, conduct benefits and risks of this clinical study. I have also received and /or read and understood the above written information about the study. I am away that my personal details will be anonymously processed into the research report. I understood that I may voluntary at any point withdraw my participation without suffering any consequences. I have been given sufficient time to ask questions, seek clarification and of own free will declare any participation into the research study.
I have received a signed copy of this agreement.

Participant signature or thumbprint Date

Person obtaining informed consent Date

Annex 2

Questionnaire form

Introduction

This is an anonymous questionnaire, and we will not record your address or identity. This information will be kept strictly confidential and no names in any circumstance will be linked to this interview. Feel free to ask for any clarification or to indicate any issue which might make you uncomfortable.

The questionnaire consists of 4 different sections:

Section I: DEMOGRAPHIC VARIABLES.
Section II: HIV TEST & PAST MEDICAL HISTORY
Section III: CLINICAL MANIFESTATIONS & STAGING (WHO)
Section IV: CD4 T CELL COUNT & OTHER TESTS

DATE: _____ / ____/_____ SITE: _____
INTERVIEWER: _____
PATIENT ID: _____ (! study number)

This patient ID number should be written in every page.

Section I: DEMOGRAPHIC VARIABLES.

1. Sex of respondent *(Tick) and age in completed years*

Female	*Male*	*Age*

2. What is your race? *(Tick one answer)*

RACE	Black	Indian	Asian	Colored	White	Other

3. What is your highest educational qualification? *(fill in number of years for each answer)*

Level of Education	None	Primary	Secondary	Technical	University
Years					

4. What is your current marital status? *(Tick one answer)*

Marital status	Married	Divorced/separated	Widow/widower	Never married	Live together

Patient ID:

5. What is your economical status

	Income per month	Tick
1	< 500.000 K	
2	500.000 – 1.000.000 K	
3	1.000.000 – 2.000.000 K	
4	2.000.000 – 4.000.000 K	
5	> 4.000.000 K	

Section II: HIV TEST & PAST MEDICAL HISTORY

6**.** Do you know your HIV STATUS? (Tick one answer) If "NO" then refer for Diagnostic Counseling Testing"

Yes	No

7. How did you decide to have the HIV test done? *Tick one answer)*

1	By myself	
2	Recommended by family/friends/others	
3	Referred by doctor/clinical officer/nurse	
4	I don't know	
5	I was sick (specify):	
6	Other (specify):	

8. Past medical history: specify for some diseases and write year when you developed the disease or how many years you have been smoking/drinking alcohol. (Tick yes or no)

	Disease/condition	YES	NO	YEAR
1	Diabetes			
2	Heart disease:			
3	Hypertension			
4	Kidney disease:			
5	Liver disease:			
6	Lung disease:			
7	Psychiatric illness:			
8	STI (other than HIV):			
9	Smoking (cigarettes/day:)			
10	Alcohol (drinks/week:)			
11	Other:			

Patient ID:

Section III: CLINICAL MANIFESTATIONS & STAGES (WHO)

9. Which clinical condition do you present with? *(Tick one answer)*

a) Stage 1

	Clinical condition	Tick
1	Asymptomatic	
2	Persistent generalized lymphadenopathy	

b) Stage 2

	Clinical condition	Tick
1	Moderate unexplained weight loss <10%	
2	Recurrent upper respiratory infections (specify):	
3	Herpes Zoster	
4	Minor mucocutaneous manifestations (specify):	

c) Stage 3

	Clinical condition	Tick
1	Severe weight loss >10%	
2	Unexplained chronic diarrhea > 1 month	
3	Unexplained persistent fever for >1 month	
4	Oral candidiasis (thrush)	
5	Oral hairy leukoplakia	
6	Pulmonary tuberculosis within the last 2 years	
7	Severe bacterial infection (specify):	
8	Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis	
9	Unexplained anemia (hemoglobin <8 g/dL) – specify Hgb:	
10	Neutropenia (neutrophils <500 cells/µL) – specify N:	
11	Thrombocytopenia (platelets <50,000 cells/µL) – specify:	

Patient ID:

d) Stage 4

	Clinical condition	Tick
1	HIV wasting syndrome	
2	*Pneumocystis carinii* pneumonia	
3	Recurrent severe or radiologic bacterial pneumonia	
4	Chronic herpes simplex infection (specify):	
5	Esophageal candidiasis	
6	Extrapulmonary tuberculosis	
7	Kaposi sarcoma	
8	Central nervous system toxoplasmosis	
9	HIV encephalopathy	
10	Cryptococcosis, extrapulmonary	
11	Disseminated nontuberculosis *Mycobacteria* infection	
12	Progressive multifocal leukoencephalopathy	
13	*Candida* of the trachea, bronchi, or lungs (specify if possible):	
14	Cryptosporidiosis	
15	Isosporiasis	
16	Visceral herpes simplex, cytomegalovirus infection (specify if possible):	
17	Any disseminated mycosis (specify if possible):	
18	Recurrent nontyphoidal Salmonella septicemia	
19	Lymphoma (cerebral or B-cell non-Hodgkin) – specify if possible:	
20	Invasive cervical carcinoma	
21	Visceral leishmaniasis	

10. Which clinical stage best fits with the condition reported?

	Clinical stage (WHO)	Tick
1	Stage 1	
2	Stage 2	
3	Stage 3	
4	Stage 4	

Patient ID:

Section IV: CD4 T CELL COUNT & OTHER TESTS

11. How is your CD4 T cell count? Indicate the figure/date for the measured CD 4 T cell & tick in the rank it fits.

	CD4 T cell rank	*CD4Tcell measured/date*	Tick
1	< 50 cells/ul		
2	50 – 199 cells/ul		
3	200 – 499 cells/ul		
4	>500 cells/ul		

12. Other tests done & results normal (nl) – abnormal(abn)+ details

	Type of test	*Results*
1	*Hemoglobin/ hematocrit*	
2	*Full blood count*	
3	*ALT/AST/ALP/GGT/Biluribine*	
4	*Urea/Electrolytes/ creatinine*	
5	*RPR*	
6	*Viral load*	
7	*Chest X-ray*	
8	*Sputum AAFB*	
9	*Other;*	
10		

OBSERVATIONS:

_____
_____
_____
_____
_____

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

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5 January, 2009
Ref.: 014-12-08

Dr G. Chimanuka
Department of Internal Medicine
University Teaching Hospital
LUSAKA

Dear Dr Chimanuka,

RE: RESUBMITTED RESEARCH PROPOSAL: **"ASSOCIATION BETWEEN CLINICAL STAGES (WHO) AND CD4T CELL COUNT IN HIV INFECTED ADULTS AT UNIVERSITY TEACHING HOSPITAL IN LUSAKA, ZAMBIA"**

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee Secretariat on 27 November, 2008 where changes were recommended. We would like to acknowledge receipt of the corrected version with clarifications. The proposal has now been approved.

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- Any serious adverse events must be reported at once to this Committee.
- Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a Progress Report (Progress Report Forms can be obtained from the Secretariat).

Yours sincerely,

Dr James C. Munthali
CHAIRPERSON

Date of approval: 5 January, 2009 **Date of expiry**: 4 January, 2010