PERFORMANCE CHARACTERISTICS OF CLINICAL, IMMUNOLOGICAL AND VIROLOGIC CRITERIA IN MONITORING RESPONSE TO ANTIRETROVIRAL THERAPY AMONG HIV-INFECTED PATIENTS IN DAR - ES - SALAAM, TANZANIA

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MMed (Microbiology and Immunology) Dissertation
Muhimbili University of Health and Allied Sciences
December, 2013

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By

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A Dissertation submitted in (Partial) Fulfillment of the Requirements for the Degree of Master of Medicine (Microbiology and Immunology) of Muhimbili University of Health and Allied Sciences

Muhimbili University of Health and Allied Sciences
December, 2013

CERTIFICATION

The undersigned certify that he has read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled **Performance Characteristcs of Clinical, Immunological and Virologic Criteria in Monitoring Response to Antiretroviral Therapy Among HIV-infected Patients In Dar es Salaam, Tanzania** in (partial) fulfillment of the requirements for the degree of Master of Medicine (Microbiology and Immunology) of the Muhimbili University of Health and Allied Sciences

_____

Prof. Said Aboud

(**Supervisor**)

_____

Date

DECLARATION AND COPYRIGHT

I, **Dr Mkhoi Lord Mkhoi** 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 ……..... Date…….....

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to Prof Said Aboud for his supervision, guidance and encouragement from the preparation of the study proposal through the final write-up of the dissertation. I am also grateful and indebted to all members of department of Microbiology and Immunology who gave their constructive criticisms. I would like to extend my deepest thanks to my beloved parents and without forgetting my family and close friends for their encouragement, understanding, sacrifices and acceptance of my absence during the period of preparation of this dissertation.

I also wish to thank the following people:

MDH supported CTC-Dr Nzovu Ulenga, Dr Ali Kaduma, Dr Jacqueline Gabone, Alice Chami, the late Dr. Boniface, Amelbega, Ernest, Dr Elizabeth, Dr Kibao for great support and encouragement.

National Health Laboratory and Quality Assurance Center-Dr Fausta Mosha, Mr Kaigi Marogo for their support and encouragement.

Dr Mabula Kasubi, Betty Mchaki, and all others who extended a helping hand to make this study possible.

DEDICATION

To my family: my mother Esther D Mkhoi and late father J.D Mkhoi for providing me with love, wisdom and unwavering support throughout my life in my determination to find and realize my potential and advance my career . My sister Joyce, Brother Baraka and uncle Dr. Mkama for their love, encouragement and support. My lovely wife Dr. Ndigwake and son Josephat, for being the source of inspiration, their patience, love, support and encouragement has been an invaluable tool throughout the period of this academic endeavor. My newest family Mr. and Mrs. Mallango, brothers in law Sam and Yona and sisters in law Dr Atuganile and Neema for being there for me.

ABSTRACT

Background:

WHO recommends the use of clinical assessment and/or CD4+ T-cell count as surrogate markers to monitor response to antiretroviral therapy (ART) in resource-limited settings. There is limited published data regarding the performance of clinical, immunological and virologic criteria in monitoring the response to ART in the local settings.

Objectives:

To determine the performance characteristics of clinical, immunological and virologic criteria in monitoring response to ART among HIV-infected patients in Dar es Salaam, Tanzania.

Methodology:

Prospective cohort study was carried out from August 2012 to May 2013.Patients initiating ART at Care and Treatment Center at IDC, Amana and Mwananyamala were recruited in the study after obtaining informed consents. Data on socio-demographic characteristics, clinical and laboratory parameters was obtained using standardized case report forms. Blood samples were collected to determine CD4+ T-cell count and plasma viral load at baseline and six months after initiation of ART. Data was analyzed using the SPSS version 17.0. Sensitivity, specificity, positive and negative predictive values of clinical and immunological monitoring were determined using virologic criteria as gold standard. Logistic regression analysis to assess the predictors associated with treatment failures was performed. A p-value of <0.05 was regarded as statistically significant. Ethical clearance and informed consent were obtained prior to the enrolment in the study. Data was analyzed using the SPSS version 17.0.

Results:

A total of one hundred and forty HIV-infected patients were enrolled in the study. The overall mean age (SD) was 40.0 (9.8) years. Majority of patients were females 95 (67.9%). Seventy nine (56.4%) were in WHO clinical stage III at enrollment. The median CD4+ T-cell count (IQR) was 255 (147-255). Of eighty three patients with complete follow up data 34.9% and 31.3% experienced virological and immunological failure respectively. Clinical failure was detected in 7.2% of the patients. The sensitivity and specificity of immunological criteria in detecting virological failure were 34.5%, 95%CI (19.9%-52.7%) and 70.4%, 95%CI (57.1%-83.9%) respectively. The sensitivity and specificity of clinical criteria were 17.2%, 95%CI (7.1%-35.0%) and 98.1%, 95%CI (89.3%-100%) respectively. Patients with CD4 count below 200 cells/µl at enrollment were more likely to experience virologic failure than those with CD4 count equal or above 350 cells/ µl.

Conclusion and Recommendation:

This study further highlights low sensitivity and specificity of immunological and clinical criteria in detecting virological failure in HIV-infected patients in the first six months on ART in Dar es salaam, Tanzania. The low performance of clinical and immunological criteria may lead to misclassification of response to treatment, accumulation of resistance mutations and even poorer treatment outcomes

A CD4 T cell count of < 200 cells/ul was shown to be significantly associated with treatment failure thus HIV-infected patients with such baseline count should have close laboratory monitoring including viral load testing to better improve treatment outcomes.

However more data is required from larger studies to support the findings so that they can eventually translate into policy guidelines.

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ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
AZT	Azidothymidine
AOR	Adjusted Odds Ratio
ARV	Antiretroviral
CD	Cluster of Differentiation
CTC	Care and Treatment Center
DNA	Deoxyribonucleic acid
EFV	Efavirenz
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
IDC	Infectious Disease Center
IQR	Interquartile range
MDH	Management and development for health
NPV	Negative predictive value
PMTCT	Prevention of mother to child transmission
PPV	Positive Predictive Value
TDF	Tenofovir disoproxil fumarate
3TC	Lamivudine
UNAIDS	Joint United Nations Program on HIV and AIDS
VL	Viral load

CHAPTER ONE

1. INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

The World Health Organisation (WHO) 2013 guidelines strongly recommend using viral load testing for detecting virological failure and/or confirming treatment failure among people with evidence of clinical and/or immunological failure. It further recommends that if viral load is not routinely available, CD4 count and clinical monitoring should be used to diagnose treatment failure [1]. However, in most resource-limited settings, access to routine viral load testing to monitor progression of HIV disease is limited or non-existent because of a combination of lack of laboratory capacity, prohibitive cost or logistic constraints [2].

Globally, an estimated 35.3 (32.2–38.8) million people were living with HIV in 2012 [3]. In the same year, 9.7 million people in low- and middle-income countries received antiretroviral therapy, representing 61% of all who were eligible under the 2010 World Health Organization (WHO) HIV treatment guidelines[3]. Improved access to antiretroviral therapy is challenged by lack of viral load testing for monitoring response to antiretroviral therapy especially in middle and low income settings. This is especially true in sub-Saharan Africa, which bears the greatest burden of the HIV epidemic. As a result, evaluation of treatment outcomes and therapeutic decision-making are generally based on clinical and immunological findings [2]. Discordance between recommended immunological and virological criteria for failure diagnosis is nevertheless well documented [4, 5]. In HIV programmes, lack of virological confirmation of failure may result in premature switching to second-line antiretroviral therapy (ART), ultimately leading to unnecessary exhaustion of available treatment options, and use of more complicated antiretroviral (ARV) regimens. Tanzania is a low-income country where viral load testing is not routinely performed, clinical and immunological criteria are used to diagnose treatment failure. It is important to establish data regarding the performance of clinical and immunological criteria in local

settings. The information is important as far as the recent WHO recommendations regarding viral load monitoring are concerned

1.2 LITERATURE REVIEW

1.2.1 Immunologic responses and risk factors for immunological failure

Without intervention, the average rate of CD4 T cell decline is estimated to be 50 cells/uL per year, and the average VL level increase ranges from 30,000 to 50,000 copies/ml [6]. CD4+ recovery following HAART, which is due to redistribution of the cells from tissues, regeneration of naïve T cells, or due to the reduction of immune activation mediated cell death (apoptosis) [7], occurs as a two phase process: In the first phase of two months on ART, rapid increase of CD4+ cells occurs; and in the second phase of the third month and onwards on ART, CD4+ count increase slows down but persists over time [8]. Overall, the long-term status of CD4+ count after HAART depends on the baseline CD4+ count, control of viral replication overtime, the stage of the disease at baseline, duration on treatment [9, 10], as well as on baseline patient factors including higher HIV RNA level, co-morbidities, presence of drug resistant viruses, sub-optimal pharmacokinetics, and potency of the ARV regimen [11]. The time required to reach to normal value of CD4+ counts ranges from two to eight years [4, 12].

Complete immune recovery following HAART is not usually observed in patients on antiretroviral therapy. Absent or modest improvements in CD4+ counts do occur in 5–27% of the patients on HAART that achieve plasma HIV-1 RNA suppression [13,14,15]. A study conducted at Muhimbili National Hospital found the overall mean CD4+ T cell count increase was 23cells/μL and CD4+ T cell count increased more rapidly within the first three months of ART (Mean of 27 cell/uL per month), relatively slower thereafter and tended to plateau at 10 - 12 months[16]. A steady CD4 increase in the first 7 months, followed by slow increase in subsequent months was noted in patients on ART at Bugando Medical Center in Tanzania [17]. The prevalence of immunological treatment failure in the study was found to be 17.1% (95% CI 17.1%±3.9) an adherence below 95% was strongly associated with immunological

treatment failure (p=0.00001). There was significant association between baseline CD4 of more than 100cell/ µl and immunological treatment failure, p=0.001[18]

Risk factors for failure or incomplete immune recovery include; the degree of CD4+ decline before and at the initiation of the treatment (the steeper the decline the steeper the rise), the rate of decline in viral-load [18], old age [11, 18], co-infection (e.g. HCV, HIV-2, HTLV-1, HTLV 2), medications (ZDV, TDF+DDI), and persistent immune activation [11]. However, others have reported no difference in immunological response related to baseline viral load, HIV risk factor, sex, HCV coinfection and HAART regimen [4]. Several explanations have been given about the mechanisms by which inadequate immune CD4+ recovery occurred in response to HAART. These include; myelosuppressive effects of ARV drugs (e.g. ZDV) [19], thymic involution related to old age [20], and abnormal cell death (apoptosis) due to higher immune activation related to higher background risk of endemic infections [21]

1.2.2 Virologic responses and risk factors for virologic failure

Viral load testing serves as a surrogate marker for treatment response[22] and can be useful in predicting clinical progression[23,24]. The minimal change in viral load considered to be statistically significant (2 standard deviations) is a threefold, or a 0.5 log10 copies/mL change. Optimal viral suppression is generally defined as a viral load persistently below the level of detection (<20 to 75 copies/mL, depending on the assay used). For most individuals who are adherent to their antiretroviral (ARV) regimens and who do not harbor resistance mutations to the prescribed drugs, viral suppression is generally achieved in 12 to 24 weeks, although it may take longer in some patients [25].

The risk factors for virological failure includes sex (although reports are controversial) [26, 27], old age, poor adherence, previous exposure to ART, lower base line CD4+ count, OIs, TB after ART, persistent lower VL, insufficient CD4+ cell gain, clinical symptoms, lower weight than baseline, and emergence of drug resistant viruses [28,29]. Digestive symptoms and poor adherence to ART have also been reported as risk factors for low ARV plasma concentrations [30], which in turn results in sub-optimal virological responses.

1.2.3 Antiretroviral drug regimen options in Tanzania

HIV infection can be treated by antiretroviral (ARV) drugs to prolong and improve the quality of life of HIV-infected individuals. Since 1996, when more extensive use of potent antiretroviral therapy for HIV started, there has been a significant improvement in the safety and tolerability of regimens used for initial treatment [31]. The currently existing and commercially available antiretroviral drugs fall into the following five main categories:-

1. Binding and Fusion Inhibitors: These prevent HIV from entering target cells. Drugs of this class bind to the HIV envelope protein gp41, which is involved in viral entry. These are a new class of antiretroviral drugs (e.g. Enfuvirtide) that are currently not available in Tanzania.

2. Nucleoside reverse transcriptase inhibitors (NRTIs): This was the first group of drugs to be used and was the mainstay of antiretroviral therapy in the country. The primary mechanism of action of this class is inhibition of viral RNA-dependent DNA polymerase (reverse transcriptase) enzyme. The drugs that are available in Tanzania under this class include: Zidovudine (AZT), Stavudine (d4T), Lamivudine (3TC), Abacavir (ABC), Emtricitabine (FTC) and Didanosine (ddI)

3. Non-nucleoside reverse transcriptase inhibitors (NNRTIs): Similar to the NRTIs, NNRTIs also act by disrupting the reverse transcription of viral RNA into DNA which is then incorporated in the cell's nucleus. However, unlike the NRTIs, they are not directly incorporated into the viral DNA; instead they inhibit replication directly by binding to the enzyme reverse transcriptase. Resistance to these drugs develops rapidly, especially when used alone. Drugs under this class that are available in Tanzania are Nevirapine (NVP) and Efavirenz (EFV).

4. Nucleotide reverse transcriptase inhibitors (Nucleotide analogues): Nucleotide analogues resemble monophosphorylated nucleosides, and therefore require only two

additional phosphorylations to become active inhibitors of DNA synthesis. An example of this relatively new class of antiretroviral drugs is Tenofovir (TDF).

5. Protease inhibitors (Pls): PIs competitively inhibit the HIV protease enzyme whose activity is critical for the terminal maturation of infectious virions. This inhibition prevents the maturation of virions capable of infecting other cells. Drugs available in Tanzania that fall under this class are Lopinavir (LPV), Ritonavir (RTV) and Atazanavir (ATV).

Antiretroviral therapy both in naive patients and those who have received treatment before involves the use of a combination of drugs. Triple therapy consisting of 2 NRTI + 1 NNRTI or 2 NRTI + 1 PI or 3 NRTI's is recommended. The default first line regimen for Adults and Adolescents in Tanzania is: Zidovudine (AZT) 300 mg/Lamivudine (3TC) 150 mg twice daily and Efavirenz (EFV) 600 mg once daily at night. For women in the child bearing age, Nevirapine (NVP) 200 mg twice a day is given instead of Efavirenz. This is prescribed to all patients if there is no contraindication.

1.2.4 Sensitivity and specificity of clinical, immunological criteria in detecting virologic failure

Studies from a variety of settings have been conducted to investigate the sensitivity and specificity of clinical and immunologic criteria for the detection of treatment failure in HIV-infected patients on HAART.

Chaiwarith et al., conducted a retrospective cohort study to evaluate the sensitivity and specificity of using CD4+ T-cell count measurement and clinical evaluation to detect ARV treatment failure in HIV-infected patients who had received their first regimen of HAART in Thailand [32]. It was observed that using the combined immunological and clinical criteria to detect antiretroviral treatment failure, the sensitivity was 20.0% and the specificity was 85.9% [32]. Virological failure was detected in 9.2% of patients. Patients with a previous history of opportunistic infection had a greater risk for developing virologic failure (OR = 2.66, 95% CI = 1.1—6.4). The study, although limited by small numbers, was not able to demonstrate that

immunological or clinical criteria can adequately replace virologic criteria for the determination of treatment failure.

During 1856 person-years of follow up in a study involving 2009 patients, 63 patients met the immunological criteria (CD4 counts persistently <100 cells/uL, a fall below the baseline CD4 T-cell count, or a fall of >50% from the peak value) [33]. Thirty five (higher threshold, two measurements =10,0000 copies / ml) and 95 patients (lower threshold) met the virologic criteria, respectively [33]. Sensitivity was 17.1% (95% CI 6.6–33.6%) for the higher and 12.6% (6.7–21.0%) for the lower threshold (=500 copies/mL). Corresponding results for specificity were 97.1% (95% CI 96.3–97.8%) and 97.3% (95% CI 96.5–98.0%), respectively. Positive predictive values were 9.5% (95% CI 3.6–19.6%) and 19.0% (95% CI 10.2–30.9%) and negative predictive values were 98.5% (95% CI 97.9–99.0%) and 95.7% (95% CI 94.7–96.6%) for higher and lower thresholds, respectively [33]. These findings show that the positive predictive value of the WHO immunological criteria for virological failure of ART in resource-limited settings is poor, but the negative predictive value is high. Immunological criteria are more appropriate for ruling out than for ruling in virological failure in resource-limited settings. [33].

A study conducted in Uganda by Moore D et al showed that large numbers of individuals with CD4 T-cell count declines at 6, 12, and 18 months from treatment initiation had plasma viral load of <500 copies/mL. [34]. If the current WHO guidelines were applied to these patients, they would have been mistakenly identified as failing ART and prematurely switched from their primary ART regimen which was effectively controlling viral replication . Therefore the use of CD4 T-cell count monitoring to identify patients who have not achieved virologic suppression on ART can result in substantial misclassification of treatment responses [34].

1.2.5. Predictors associated with clinical, immunological and virologic failures

Variation in clinical trial endpoint definitions creates a challenge for comparing results between studies [35]. Data on 4143 patients from 5 observational cohorts in Europe and North America who were started on initial 3-drug regimens between 1996 and 2002 indicated that

the annual rate of virologic failure (plasma HIV-1 RNA level >500 copies/mL within 6 to 12 months of starting antiretroviral therapy) declined from 40% to 25% during that period. Risk of virologic failure was lower among patients who were older, had lower baseline viral load, and had an absence of an AIDS diagnosis, and among men who reported having sex with men as a risk factor [36].

In another study which analyzed prospective data from the Swiss HIV Cohort Study on suppression of viral load and progression to AIDS or death among 2674 outpatients (median age 36 years, 27·3% women) who started HAART in 1995–98, viral rebound or failure was defined as two consecutive HIV-1-RNA measurements of more than 400 copies/mL [37]. Outcomes in patients with a history of ART and in treatment-naïve patients were analysed separately. An estimated 90·7% of treatment-naïve patients reached undetectable viral load (<400 copies/mL) by 12 months. Among pretreated patients, estimates ranged from 70·3% treated with one new drug to 78·7% on three new drugs. Two years after reaching undetectable concentrations, an estimated 20·1% of treatment-naïve patients and 35·7–40·1% of pretreated patients had viral rebound [37].

Virologic failure, defined as the failure to reduce viral load to <500 copies/mL, was observed in 51 out of 274 (19%) HIV-infected patients on triple-drug combination therapy, including a protease inhibitor and two nucleoside analog reverse transcriptase inhibitors (NRTIs) in a study that was conducted at a University hospital in Switzerland [38]. Independent risk factors for initial failure included higher baseline viral load, addition of a protease inhibitor to an unchanged NRTI regimen, use of saquinavir hard-gel capsules and longer duration of prior NRTI treatment [38].

In a previous study conducted in North America, Sixteen cohorts representing over 60 sites contributed data on all individuals who initiated combination ART [39]. Individuals who experienced virologic failure (defined as HIV RNA level >1000 copies/mL), received modified therapy, and subsequently had a second episode of virologic failure, were identified. Of 42,790 individuals who received therapy, 7159 (16.73%) experienced a second virologic failure. The risk of second virologic failure decreased from 1996 (56 cases per 100 person-

years) through 2005 (16 cases per 100 person-years; $P < .001$). The cumulative mortality after onset of second virologic failure was 26% at 5 years and decreased over time [39]. A history of AIDS, a lower CD4+ T cell count, and a higher plasma HIV RNA level were each independently associated with mortality. Similar trends were observed when analysis was limited to the subset of previously treatment-naïve patients [39].

Another study conducted previously at nine San Diego County HIV clinics in North America where 103 individuals with an unknown duration of infection received standard drug-resistance testing (performed at Monogram Biosciences [San Francisco, CA]). Twenty-six (25%) of the individuals harbored HIV strains that were resistant to at least one class of ARV agents (NRTIs, NNRTIs and PIs) [40]. These findings show that there was high prevalence of drug-resistant HIV among ARV-naïve patients receiving medical care in San Diego County, California, whose duration of HIV infection was unknown [40].

In a previous study conducted between December 2004 and April 2005 among 279 persons receiving ART at 3 private clinics in Mumbai where quantitative HIV-1 RNA level was determined for 200 participants, 127 (63.5%) had virologic suppression (RNA level, <400 copies/mL) [42]. Independent correlates of suppression were a regimen containing = 3 ART drugs (AOR 5.52), first ART regimen (AOR 3.28), adherence to therapy =95% (AOR 5.70), female sex (AOR 3.19), and a physical component score = 50 (AOR 1.07) [41].

In a retrospective cohort study conducted in South Africa, a total of 456 patients on NNRTI-based ART for a median of 44 months (range 12–99 months; 1,510 person-years), were enrolled in the study in 2008. After a median of 15 months on ART, 19% (n = 88) and 19% (n = 87) had failed virologically and immunologically, respectively. A cumulative adherence of 95% to drug-refill visits was significantly associated with both virologic and immunologic failure ($p<0.01$). These findings show that adherence to drug-refill visits works as an early warning indicator for both virologic and immunologic failure [42].

In a cluster-randomized equivalence trial conducted in Uganda, 859 patients (22 clusters) with WHO stage IV or late stage III disease or CD4 T-cell counts fewer than 200 cells/ µL who

started ART between February 2005 and December 2006 were randomly assigned to home and 594 (22 clusters) to facility care. During the first year, 93 (11%) receiving home care and 66 (11%) receiving facility care died, 29 (3%) receiving home and 36 (6%) receiving facility care withdrew, and 8 (1%) receiving home and 9 (2%) receiving facility care were lost to follow-up. 117 of 729 (16%) in home care had virologic failure versus 80 of 483 (17%) in facility care. Rates per 100 person-years were 8·19 (95% CI 6·84–9·82) for home and 8·67 (95% CI 6·96–10·79) for facility care [43].

In a study conducted in rural Uganda, 1133 participants enrolled in the Rakai Health Sciences Program ART program between June 2004 and September 2007 were followed for up to 44.4 months (median follow-up 20.2 months; IQR 12.4–29.5 months). WHO immunologic failure criteria were reached by 125 (11.0%) participants. A virologic failure (endpoint defined as HIV-1 viral load of >400 copies/mL on two measurements) was reached by 112 participants (9.9%). Only 26 participants (2.3%) experienced both an immunologic and virologic failure endpoint (2 VL>400 copies/mL) during follow-up. Immunological monitoring was performed by CD4 T-cell counts every 3 months during the first year, and every 6 months thereafter. HIV-1 viral loads were performed every 6 months [44].

Ramadhan H.O., et al., reported a virologic failure of 32% individuals in a cross-sectional cohort study to determine predictors of incomplete adherence, virologic failure and antiviral drug resistance among 150 HIV-infected adults receiving ART in Tanzania [45]. Virologic failure was defined as an HIV RNA level >400 copies/mL and was associated with incomplete adherence (AOR 3.6; *P*=.03) and the proportion of months receiving self-funded antiretroviral therapy (AOR 13.0; *P*=.02). These findings show that self-funded treatment was associated with incomplete adherence and virologic failure, and disclosure of HIV infection status was protective against virologic failure [45].

1.3. STATEMENT OF THE RESEARCH PROBLEM

WHO recommends the use of clinical and/or immunological methods for monitoring of patients on ART in resource-limited settings. Clinical and immunologic monitoring have been shown in other settings to have reduced sensitivity and specificity to predict virologic failure and thus causing unnecessary switch of the HIV-infected patients to a second line regimen resulting in emergence of ARV drug resistance and adverse clinical outcomes. Immunological or clinical criteria have been demonstrated to be inadequate in replacing virologic criteria for the determination of treatment failure [4, 5]. In Tanzania, clinical and immunological parameters are used to detect treatment failures in HIV-infected individuals on ART. Clinical and immunological failure in HIV-infected children were found to be 7.4% and 12.9% respectively in a study conducted in Dar es salaam, Tanzania [46]. The same study reported low sensitivity and specificity of clinical and immunological criteria in detecting virologic failure. The performance of these criteria in monitoring the response to ART among HIV-infected adult patients in local setting is not known.

1.4. RATIONALE OF THE STUDY

The possibility of scaling up viral load testing in HIV care and treatment programmes requires established local data regarding the performance of currently used monitoring criteria. Tanzania is a limited resource country where clinical and immunological criteria are used to monitor HIV-infected patients on antiretroviral therapy; viral load monitoring is not routinely done. Current local data on the performance of these criteria using viral load monitoring as a gold standard is limited. This study has provided data on performance of these criteria and put forth recommendations for possible measures to be taken.

1.5. HYPOTHESES OF THE STUDY

1.5.1 There are no differences in sensitivity and specificity between clinical criteria virologic monitoring

1.5.2 There are no differences in sensitivity and specificity between immunological criteria and virologic monitoring.

1.5.3 There are no differences in sensitivity and specificity between combined clinical and immunological criteria and virologic monitoring.

1.5.4 There are no predictors associated with clinical, immunological and virologic failures.

1.6. OBJECTIVES OF THE STUDY

1.6.1. Broad objective

To determine the performance characteristics of clinical, immunologic and virologic criteria in monitoring response to ART among HIV-infected patients in Dar es Salaam, Tanzania.

1.6.2. Specific objectives

1.6.2.1 To determine the sensitivity and specificity of immunological criteria in detecting virologic failure among HIV-infected patients on ART.

1.6.2.3 To determine the sensitivity and specificity of clinical criteria in detecting virologic failure in HIV-infected patients on ART.

1.6.2.3 To determine the sensitivity and specificity of combined clinical and immunological criteria in detecting virologic failure in HIV-infected patients.

1.6.2.4 To determine the proportion of patients who develop clinical, immunologic and virologic failure among HIV-infected patients.

1.6.2.5 To identify predictors associated with virological failure among HIV-infected patients

1.6.2.6 To determine the treatment outcomes of the HIV-infected patients who have been on ART in the past 6 months.

CHAPTER TWO

2.0. METHODOLOGY

2.1. Study setting

The study was carried out at MDH supported care and treatment clinics namely, Infectious Diseases Centre (IDC), Amana and Mwananyamala in Dar es Salaam. Clinical care of all HIV-infected patients at MDH supported HIV CTCs follows national and WHO guidelines. Following enrollment, patients are evaluated monthly if they are on ART (care and treatment) or 4 monthly if not on ART (care and monitoring). At each visit, patients on ART are examined by a physician, undergo adherence and nutrition counseling and receive ART refills. Monitoring laboratory tests include hemoglobin (Hgb), serum creatinine, and CD4 T-cell count which is also performed 6 monthly. Viral load testing is not performed routinely but only in instances where treatment failure is suspected.

2.2. Study Design

This was a prospective cohort study that was conducted between August 2012 and May 2013.

2.3. Study population

The study population consisted of HIV-infected adult patients attending CTC and initiating ART

2.4. Sample size estimation

In estimating the sample size, a 9.9% prevalence of virologic failure documented in a previous Ugandan study by Reynolds et al [44] was used. The minimum number of study participants was estimated by using the following formula:-

$$N = \frac{Z^2 P (1-P)}{E^2}$$

Where;

N= the estimated sample size

P= proportion of a population with virologic failure, 9.9%

E= estimated margin of error which is set at 0.05 (5%)

Z= standard normal deviation set at 95% (1.96)

Therefore

$$N = \frac{1.96 \text{ X } 1.96 \text{ X } 0.099(1-0.099)}{0.05 \text{ X } 0.05} = 137$$

Thus, the minimum number of HIV-infected patients to be included in this study was 137. Adding 10% to cover loss to follow-up, the sample size would be 150 patients. However a total of 140 patients were recruited and 83 patients with complete follow up data were included for analysis.

2.5. Sampling procedure

Convenient sampling was employed and all patients who met inclusion criteria were included in the study.

2.6. Inclusion criteria

HIV-infected patients aged 18 years and above initiating on first line ART regimen and who consented to participate in the study.

2.7. Exclusion criteria

HIV-infected patients below 18 years of age and who were not initiated on ART, and those who did not consent to participate in the study

2.8. Data collection

Case report forms were used to collect socio-demographic (age, sex, residence, marital status), clinical and laboratory data. Physical examination including WHO HIV disease staging, and to detect occurrence of opportunistic infections was performed by attending clinician at baseline and throughout the study period. Signs, symptoms and common opportunistic infections and AIDS defining illness as per CTC 2, were looked for and used to define patient's WHO clinical stage**.** Patients with recurrent or new stage IV disease were considered to have clinical failure during analysis.

2.9. Laboratory investigations

2.9.1. CD4 T cells estimation using BD FACSCalibur system (Becton Dickinson, USA)

Principle of the test: BD FACSCalibur system is based on flow cytometry principle whereby air-cooled argon gas laser emits a monochromatic beam of light fixed at 488 nm at 15 mW of power. As particles or cells flow in single line past the intersection of the light beam, light is scattered in various directions. Fluorochrome labeled monoclonal antibody associated with the cell becomes excited by the laser and a fluorescent emission results. The resulting signals are processed to gather information about the relative size of the cell (forward light scatter, FSC), its shape or internal complexity (side light scatter, SSC) as well as a diversity of cellular structures and antigens (fluorescence)

Sample processing: Blood samples (about 4-5 mls) were collected using EDTA vacutainer tube at entry and 6 months after ART for determination of CD4+T-cell counts and processed within 24 hours after collection. Ten microlitres of liquid antibody reagent (MultiTEST CD3 FITC, CD8 PE, CD45 PerCP and CD4 APC, Becton Dickinson, USA) and 50 µl of whole blood was added to the TruCOUNT tube (Becton Dickinson) containing the reference beads. The tube was vortexed and incubated at ambient temperature in dark for 15 min. The RBCs

were lysed using 450 µl of 1:10 diluted lysing solution (FACSlysing, BD) for 15 min in the dark at ambient temperature. The stained sample was acquired on the FACSCalibur and analyzed using the automated MultiSET software. Per cent and absolute counts of CD4+, CD3+, CD8+ T lymphocytes were then generated by the MultiSET software.

Quality control: As part of quality control procedures for FACSCalibur, the photomultiplier tube voltage, sensitivity and fluorescence compensation settings were optimized using calibrate beads (Becton Dickinson, USA) with FACSComp software on each day before running the samples using channel targeting technique.

2.9.2 Viral load determination using COBAS AmpliPrep/COBAS TaqMan 48 analyzer (Roche Diagnostics, Mannheim, Germany

Principle of the assay: The COBAS AmpliPrep-COBAS TaqMan 48 HIV-1 assay targets a conserved region of the *gag* p41 gene and uses TaqMan differential fluorescence-tagged primers to amplify HIV-1 RNA. This assay detects HIV-1 group M A to D and F to K viruses and several circulating recombinant forms (CRFs).

Sample processing: Blood samples (about 4-5 mls) were collected using EDTA vacutainer tube at entry and 6 months after ART and were centrifuged at 1500 g for 30 minutes to obtain plasma which was then stored at -80 until further analysis. HIV RNA was obtained from a 1.0 ml plasma sample using the COBAS AmpliPrep and RT-PCR amplification and detection was performed using the fully automated COBAS TaqMan 48 instrument.

Quality control: Additional fluorescence primers and probes detect quantification standards, and a partial sequence of the long terminal repeat region acts as the internal control. Positive and negative controls were included in each run to ensure reliability of test results.

2.10 Definitions of treatment failure

Treatment failure can be virologic, immunologic and/or clinical. It results from failure to suppress viral replication, and the development of viral resistance [31]. According to Tanzania national guidelines for the management of HIV and AIDS;

i) Virological failure occurs when there a less than 10 fold drop in viral load after 6-8 weeks of therapy, or when the viral load is detectable after six months of therapy, or when the viral load (VL) is persistently above 5,000 copies/ml.

ii) Immunological failure occurs when there is 50% drop in CD4 count from peak value, or return to pre-ART baseline CD4 count or lower

iii) Clinical failure results from disease progression which presents as the development of opportunistic infections, or malignancy occurring three months or more after initiation of ART

2.11 Data analysis

Data was analyzed using the SPSS version 17.0. Sensitivity, specificity, positive and negative predictive values of clinical and immunological monitoring were determined using virologic criteria as gold standard. Logistic regression analysis to assess the predictors associated with treatment failures was performed. A p-value of <0.05 was regarded as statistically significant. For the purpose of analysis clinical, immunological and virologic failure were based on national guidelines definition of treatment failure.

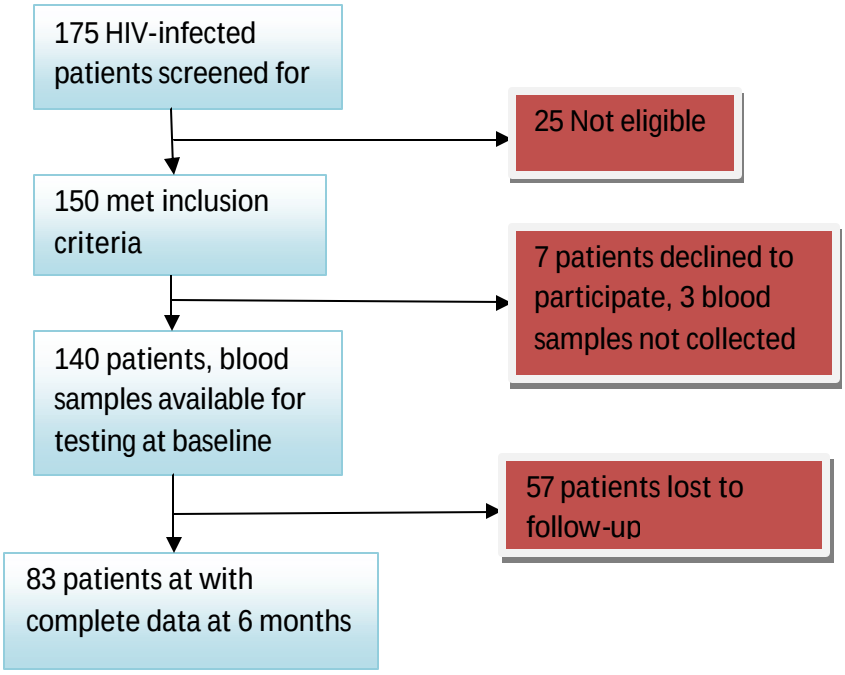
2.12 Ethical considerations

Ethical approval was obtained from MUHAS Senate Research and Publication Committee. Written informed consent was requested from the patients prior to the enrolment into the study. Results for CD4 T cell count and viral load were forwarded to the attending clinicians for routine management of the patients.

CHAPTER THREE

3.0. RESULTS

Figure1. Participants' flow chart



3.1. Baseline characteristics of the study population

The median age of study participants at HAART initiation was 38 years (IQR: 32-45 years) and 67.9% were females. The mean baseline BMI was 23 kg/m^2 and 84.3% of the participants had BMI equal to or above 18.5 kg/m^2. Many patients were in WHO clinical stage III at the time of ART initiation and tuberculosis accounted for 6% of the study population. The baseline median CD4 T-cell count was 255 cells/µL (IQR: 147-255) and 75.7% had CD4 T-cell count below 350 cells/µL. The baseline median viral load was 1.15X10^5 copies/mL (IQR:4.26X10^4-3.73X10^5). TDF/3TC/EFV was the most commonly prescribed regimen amongst participants initiating HAART.

Table1: Baseline characteristics of study participants (N=140) initiating ART in Dar es salaam, Tanzania

Variable	%
Age, years	
18-27	12(8.6)
28-37	53(37.8)
38-47	49(35)
=48	26(18.6)
Age, median years (IQR)	38(32-45)
Sex	
Female	95(67.9)
Male	45(32.1)
Marital status	
Single	55(39.3)
Married/Cohabiting	66(47.1)
Divorced/separated	7(5)
Widowed	12(8.6)
Body Mass Index(BMI)	
<18.5	22(15.7)
=18.5	118(84.3)
BMI, Mean (s.d)	23.2(4.9)
ARV drug regimen at initiation	
TDF/3TC/EFV	102(72.9)
AZT/3TC/NVP	22(15.7)
ABC/3TC/EFV	7(5.0)
AZT/3TC/EFV	4(2.9)
Others	5(3.6)
Haemoglobin concentration(g/dL)*	
=12	41(45.6)
8-11.9	46(51.1)
<8	3(3.3)
Haemoglobin, Median(IQR)	11.7(10-12)
Tuberculosis	
Positive	8(6)
Negative/Not sure	132(94)
CD4 cell count (cells/uL)	
<200	51(36.4)
200-349	55(39.3)
=350	34(24.3)
CD4 cell count, Median(IQR)	255(147-255)
Viral load (copies/mL)	
0-50,000	34(24.3%)
50,000-100,000	10(7.1%)
>100,000	96(68.6%)
Viral load, Median(IQR)	1.15E(0.426E-3.75E)

E=X10^5 * Only 90 participants had Hb results due to reagents stock out

7.2. Participants characteristics and treatment outcome at six month follow up

Of 83 patients with complete follow up data, 6(7.2%) and 26(31.3%) had clinical and immunological failure respectively. Twenty nine (34.9%) had virologic failure based on detectable VL at six month and 6(7.2%) had virologic failure based on >400 copies/mL threshold. Twenty seven (32.5%) had combined immunological and virologic response. Adherence of more than 95% was reported for 78 (94%) with complete follow up data.

Table 2: Participants characteristic at six month follow up (N=83) on ART

Variable	%
Sex	
Female	53(63.9)
Male	30(36.1)
Age (years)	
18-27	5(6)
28-37	67(80.7)
38-47	0(0)
=48	11(13.3)
Age, median year (IQR)	38(32-46)
Regimen	
TDF/3TC/EFV	57(68.7)
AZT/3TC/NVP	16(19.3)
Others	10(12.0)
Adherence	
Good*	78(94)
Poor	5(6)
Body Mass index (kg/m^2)	
<18.5	15(18.1)
18.5-25	37(44.6)
26-30	23(27.7)
>30	8(9.6)
WHO clinical stage	
WHO stage 1 or 2	77(92.8)
WHO stage 3 or 4	1(1.2)
Deaths	5(6%)
CD4 cell count (cells/uL)	
<200	16(19.3)
200-349	30(36.1)
=350	32(38.6)
CD4 cell count, median (IQR)	302(213-478)
Immunological failure (IF)	**26(31.3)**
Combined IF and CF	**27(32.5)**
Viral load (copies/mL)	
<20	54(65.1%)
20-400	19(22.9%)
401-1000	6(7.2%)
>1000	4(4.8%)
Viral load, median(IQR)	20(0-84)

*Defined as less than 2 missed days in 30 or 3 days in 60, equivalent to =95% adherence

Table 3: Performance characteristics of clinical, immunological and combined clinical and immunological monitoring/criteria compared to virologic monitoring in HIV-I infected patients (N=83) on ART

Monitoring criteria	Sensitivity	Sensitivity	PPV	NPV
Clinical	17.2% (7.1%-35.0%)	98.1% (89.3%-100%)	83.3% (41%-98.9%)	68.8% (53.1%-73.4%)
Immunological (VL>20 copies/mL)	34.5% (19.9%-52.7%)	70.4% (57.1%-80.9%)	38.5% (22.4%-57.5%)	66.7% (53.7%-77.6%)
Immunological (VL>400copies/mL)	33.3% (30%-50%)	68.8% (74%-90%)	7.7% (5%-18%)	93% (86%-96.8%)
Combined Clinical and Immunological	17.2% (7.3%-35%)	100% (94.3%-100%)	100% (59.9%-100%)	74.4% (58.2%-78.4%)

7.6. Predictors associated with virological failure

In univariate analysis, being married or cohabiting and baseline BMI of =30 were significantly associated with reduced risk of treatment failure (OR=0.467, 95%CI=0.247-0.880 and (OR= 0.278, 95%CI=0.103-0.748) respectively. Current history of TB and baseline CD4 T cell count between 200 and 349 cells/uL were also associated with decreased risk of treatment failure (OR=0.563, 95%CI=0.351-0.901) and (OR=0.476, 95%CI=0.224-1.011)

In a multivariate analysis, patients with baseline CD4 T cell count of < 200 cells/uL had a higher risk of experiencing treatment failure (OR=7.125, 95%CI=1.374-36.946)

Table4: Predictors associated with virological failure in HIV-infected patients on ART (N=83)

Variable	Univariate analysis			Multivariate analysis		
	OR	**95%CI**	**P-value**	**AOR**	**95%CI**	**P-value**
Sex						
Male*						
Female	0.500	(0.234-1.068)	0.740	0.524	(0.156-1.762)	0.297
Age (years)						
18-27*						
28-37	1.000	(0.290-3.450)	1.000	2.004	(0.286-14.030)	0.484
38-47	0.600	(0.290-1.300)	0.198	2.137	(0.409-11.153)	0.368
=48	0.600	(0.260-1.200)	0.138	0.985	(0.204-4.747)	0.965
Marital status						
Single/separated*						
Married/Cohabiting	0.467	(0.247-0.880)	0.019	0.441	(0.147-1.321)	0.144
Regimen						
TDF/3TC/EFV*						
AZT/3TC/NVP	0.727	(0.430-1.230)	0.235	1.618	(0.337-7.767)	0.548
Others	0.333	(0.108-1.034)	0.057	0.678	(0.098-4.702)	0.694
Baseline CD4 (cells/uL)						
<200	1.125	(0.574-2.206)	0.732	7.125	(1.334-36.946)	**0.019**
200-349	0.476	(0.224-1.011)	0.050	2.700	(0.537-13.590)	0.228
=350*						
No Imm. Failure*						
Imm. Failure	0.933	(0.359-2.428)	0.888	2.631	(0.508-13.632)	0.249
WHO clinical stage						
I and II*						
III	0.522	(0.260-1.049)	0.068	0.110	(0.011-1.122)	0.062
IV	0.577	(0.306-1.089)	0.090	0.197	(0.024-1.636)	0.132
Tuberculosis						
Yes	0.563	(0.351-0.901)	0.017	1.488	(0.206-10.767)	0.694
No*						
Adherence at six month				0.126	(0.011-1.429)	0.950

*****Reference category

CHAPTER FOUR

4.0. DISCUSSION

WHO recommends the use of clinical and immunological criteria to monitor response to ART in resource limited setting, viral load measurement is recommended if affordable, when there is suspected treatment failure [1]. The study showed that 34.9% patients had virologic failures. Clinical criteria had the lowest sensitivity (17.2%) but higher specificity (98.1%) compared with immunological monitoring. CD4 count of <200 cells/uL predicted significantly treatment failures than higher counts.

The sensitivity of clinical criteria in detecting virologic failure was low, 17.2% and this finding concurs with previous findings from a neighboring country, Kenya whereby Ferreyra et al reported the sensitivity of clinical WHO criteria to be 18.2% in a retrospective, cross-sectional cohort analysis which was performed for all adult patients (=18 years old) on ART for =12 months, treatment-naive at ART start, attending the clinic at least once in last 6 months. Positive predictive values (PPV) for immunological and clinical criteria to define virological failure were 24.5% and 8.1%, respectively [5]. However in the current study the PPV for clinical criteria was higher (83.3%) the fact that may be accounted for by inherent difference in study design and the relatively shorter duration of the current study. Furthermore, the cut off CD4 T cell count for initiation of ART was 350 cells/uL in the current study as opposed to 200 cells/uL in the Kenyan study.

The current study reports a relatively higher finding regarding the sensitivity of immunological criteria as compared to the one reported by Kanapathipillai et al in a retrospective analysis of monitoring data from adults treated with ?rst-line antiretroviral regimens for >1 year and meeting the WHO immunological failure criteria in an HIV programme in rural Malawi whereby the sensitivity of immunological criteria to detect virologic failure was found to be 28.4% [2]. However the relatively higher threshold of >5000 copies/ml for virologic failure and a relatively longer duration of the study participants in care

and treatment program in Malawian study may explain a slight difference observed from the findings of the current study.

Furthermore, the current study reports relatively higher values for sensitivity of immunological and clinical criteria than those reported by Chaiwarith et al in Thailand, 13.3% and 10% respectively whereby immunological failure was defined as CD4 count decrease of more than 30% from highest value and more than 50 copies/ml threshold was used for virological failure [32]. Thus these two differences may partly explain the slightly different finding from this study which used a stricter threshold for virological failure of twenty copies/ml.

Interestingly the combined immunological and clinical criteria were found to have the same sensitivity of 17.2% as clinical criteria alone. This may be explained by the fact all of the patients who had experienced clinical failure had immunological failure as well. However comparable findings were reported by Chaiwarith et al whereby a 20.0% sensitivity of using the combined clinical and immunological criteria to detect antiretroviral treatment failure was observed [32]. The observed difference from the current study may be attributed to differences in study design whereby close monitoring of patients in a prospective design of the current study might have influenced adherence to treatment. However adherence was not found to be significantly associated with treatment failure in our study (OR=0.226, 95%CI=0.011-1.249).

A lower finding than the current study of immunological failure rate of 23% was reported by Dragsted et al in an analysis of data from EuroSIDA, a prospective, international, observational human immunodeficiency virus (HIV) type 1 cohort. Immunological failure was defined as CD4+ count less than or equal to the pre-HAART value [47]. A large sample size of 2347 and longer follow up of study participants in the EuroSIDA study may partly explain the difference from 31.3% immunological failure rate found in this study.

A CD4 count below 200 cells/ul was associated with more likelihood of experiencing virologic failure (OR=7.12, 95%CI=1.37-36.95) out of eight baseline parameters that were examined as potential predictors for treatment failure in a multivariate regression analysis.

Ramadhan et al reported a similar virologic failure rate of 48 (32%) out of 150 subjects in a cross-sectional cohort study to evaluate predictors of virologic failure among adult (=18 years of age) HIV-infected patients attending the Infectious Diseases Clinic at the Kilimanjaro Christian Medical Centre (Moshi, Tanzania), a referral hospital in northern Tanzania[45]. Virologic failure was defined as plasma RNA levels ? 400 copies/mL. However virologic failure was associated with incomplete adherence (AOR, 3.6; p=0.03) and the proportion of months receiving self-funded antiretroviral therapy (AOR, 13.0; p=0.02) whilst adherence was not significantly associated with virologic failure in the current study. Disclosure of HIV infection status to family members or others was protective against virologic failure (AOR, 0.10; p=0.04).

Relatively comparable finding of 28% virologic failure rate was reported by Parienti et al in a prospective cohort involving 71 patients in a study to identify predictors of virologic failure and resistance in HIV-infected patients treated with nevirapine- or efavirenz-based antiretroviral therapy. Virologic failure was associated with repeated drug holidays (> or =48 h of unplanned drug cessation), depression, younger age, and low adherence to therapy during baseline evaluation. Moreover, repeated drug holidays was the only risk factor for developing a major mutation conferring cross-resistance to the NNRTI class (hazard ratio, 22.5; 95% confidence interval, 2.8-180.3; P<.0001)[48]

The current study reported a mortality rate of 6% which is lower than mortality rate of 13.1% reported by Chalamilla et al 76.4% in HIV care and treatment clinics in Dar es salaam, Tanzania [49]. The bigger sample size, longer duration of follow up and the use of 200 cells/ CD4 T cell cut-off for initiation of ART in the longitudinal analysis by Chalamilla et al may partly explain the difference in mortality rate from the one observed in the current study.

Unexpectedly big loss to follow up (41%) was encountered in the current study; the main reason being study participants rescheduling appointment for their six month follow-up way beyond the study deadline. However big loss to follow up has been reported in other Sub Saharan settings as well whereby up to 77.5% of patients on ART are retained in HIV care and treatment programs after an average follow-up period of 9.9 months [50]

4.1. STUDY LIMITATIONS

Due to limitation of budget and time it was not possible to perform genotypic resistance testing at the time of initiation of antiretroviral therapy in order to detect viral mutations associated with resistance to commonly prescribed classes of antiretroviral drugs. However the main focus of the study was to determine performance characteristics of criteria used to detect treatment failure in local HIV care and treatment clinics. Furthermore, this limitation reflects the true picture of level of care in most care and treatment clinics in Sub-Saharan countries. Unexpectedly high loss to follow up is another limitation of the study whereby 57 out of 140 participants recruited in the study did not turn up at the sixth month follow up visit despite considerable efforts made through phone calls and relatives.

CHAPTER FIVE

5.0. CONCLUSION AND RECOMMENDATIONS

This study further highlights low sensitivity, specificity and positive predictive values of immunological and clinical criteria in detecting virological failure in HIV-infected patients in the first six months on ART in Dar es salaam, Tanzania. The low performance of clinical and immunological criteria may lead to misclassification of response to treatment, accumulation of resistance mutations and even poorer treatment outcome.

A CD4 T cell count of < 200 cells/ul was shown to significantly associated with treatment failure thus HIV-infected patients with such low baseline count should be have close laboratory monitoring including viral load testing to better improve treatment outcome. Furthermore starting ART at relatively higher CD4 T cell count may have better treatment outcome.

Improving access to viral load testing particularly point of care technologies in care and treatment programs is a reasonable approach to improving efficacy of antiretroviral therapy in order to prevent accumulation of resistance mutations, unnecessary switch of drug regimens and to preserve further treatment options.

However more data is required from larger studies to support the findings so that they can eventually translate into policy guidelines

REFERENCES

1. WHO, Consolidated guidelines on the use of antiretroviral drugs treating and preventing HIV infection. Recommendations for a public health approach, June 2013. Available at www.who.int/hiv/pub/guidelines/arv2013

2. Kanapathipillai R, McGuire M, Mogha R, Szumilin E, Heinzelmann A and Pujades-Rodriguez M. Benefit of viral load testing for confirmation of immunological failure in HIV patients treated in rural Malawi. Trop Med Int Health. 2011 Dec;16(12):1495-500.

3. UNAIDS report on global AIDS epidemic, 2013. Available at www.unaids.org/.../unaids/.../2013/gr2013/UNAIDS_Global_Report_2013...

4. Schechter M, Hiromi TS. Discordant immunological and virological responses to antiretroviral therapy. J. Antimicrob. Chemother. (2006)58 (3): p. 506-510.

5. Ferreyra C, Yun O, Eisenberg N, Alonso E, Khamadi AS, Mwau M, et al., "Evaluation of clinical and immunological markers for predicting virological failure in a HIV/AIDStreatment cohort in Busia, Kenya", PLoS One. 2012;7(11)

6. Mellors JW, Munoz A, Giorgi JV, Margolick JB, Tassoni CJ, Gupta P, Kingsley LA, Todd JA, Saah AJ, Detels R, Phair JP, Rinaldo CR. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med 1997;126(12):946-954.

7. Kaufmann G R, Furrer H , Ledergerber B , Perrin L, Opravil M, Vernazza P, et al., The Swiss HIV Cohort Study. Characteristics, determinants, and clinical relevance of CD4 T cell recovery to <500 cells/mL in HIV type 1-infected individuals receiving potent antiretroviral therapy. Clin Infect Dis. 2005;41:361-372.

8. Autran B, Carcelain G, Li TS, Blanc C, Mathez D, Tubiana R, et al., Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Science 1997;277:112-116

9. Lederman MM, Connick E, Landay A, Kuritzkes DR, Spritzler J, St Clair M, et al., Immunologic responses associated with 12 weeks of combination antiretroviral therapy consisting of Zidovudine, Lamivudine and Ritonavir: results of AIDS clinical trials group protocol 315. J Infect Dis 1998; 178:70-79.

10. Egger S, Petoumenos K, Kamarulzaman A, Hoy J, Sungkanuparph S, Chuah J, et al., Long-Term patterns in CD4 response are determined by an interaction between baseline CD4 cell count, viral load, and time: The Asia Pacific HIV observational database (APHOD). J AcquirImmune Defic Syndr 2009;50:513–520.

11. Boyd M A. Current and future management of treatment failure in low- and middle-income countries. Current Opinion in HIV and AIDS 2010;5 (91): 83-89.

12. Fleury S, Rizzardi GP, Chapuis A, Tambussi G, Knabenhans C, Simeoni E, et al., Long-term kinetics of T cell production in HIV-infected subjects treated with highly active antiretroviral therapy proceedings of the National academy of Science USA 200,97 . Proc Natl Acad Sci USA 2000;97:5393-5398.

13. Benveniste O, Flahault A, Rollot F, Elbim C, Estaquier J, Pédron B, et al., Mechanisms involved in the low-level regeneration of CD4 cells in HIV-1-infected patients receiving highly active. J Infect Dis. 2005 May 15;191(10):1670-9.

14. Marco M, De Santis W, Carello R, Leti W, Antonella E, Isgro A, et al., T-cell homeostasis alteration in HIV-1-infected subjects with low CD4 T-cell count despite undetectable virus load during HAART. AIDS 2006;20:2033–2041.

15. Aiuti F, Mezzaroma I. Failure to reconstitute CD4 Tcells despite suppression of HIV replication under HAART. AIDS Rev 2006;8:88–97.

16. S Aboud, M Bakari, A Nyamtema, F Mugusi, R Josiah, E A Aris. Immunological Response to Antiretroviral Therapy in HIV-1 Infected Patients at Muhimbili National Hospital in Dar es Salaam, Tanzania. TMJ 2007; 22 (1): 1-4

17. H.M Jaka, S.E Mshana, A.C Liwa, R Peck, S Kalluvya. Prevalence of immunological failure and durability of first line antiretroviral therapy at Bugando Hospital Mwanza, Tanzania. TMJ 2010; 24:5-8.

18. Carcelain G, Debré P, Autran B. Reconstitution of CD4+ T lymphocytes in HIV-infected individuals following antiretroviral therapy. Current Opinion in Immunology 2001;13:483–488.

19. Huttner AC, Kaufmann GR, Battegay M, Weber R, Opravil M. Treatment initiation with zidovudinecontaining potent antiretroviral therapy impairs CD4 cell count recovery but not clinical efficacy. Aids 2007;21(8):939-946.

20. Teixeira L, Valdez H, McCune JM, Koup RA, Badley AD, Hellerstein MK, et al.,Poor CD4 T cell restoration after suppression of HIV-1 replication may reflect lower thymic function. Aids 2001;15(14):1759-1756.

21. Eggena MP, Barugahare B, Okello M, Mutyala S, Jones N, Ma Y, et al., T cell activation in HIV-seropositive Ugandans: differential associations with viral load, CD4+ T cell depletion, and coinfection. J Infect Dis 2005;191(5):694-701.

22. Murray JS, Elashoff MR, Iacono-Connors LC, Cvetkovich TA, Struble KA. The use of plasma HIV RNA as a study endpoint in efficacy trials of antiretroviral drugs. AIDS. May 7 1999;13(7):797-804.

23. Marschner IC, Collier AC, Coombs RW, et al. Use of changes in plasma levels of human immunodeficiency virus type 1 RNA to assess the clinical benefit of antiretroviral therapy. J Infect Dis. Jan 1998;177(1):40-47.

24. Thiebaut R, Morlat P, Jacqmin-Gadda H, et al. Clinical progression of HIV-1 infection according to the viral response during the first year of antiretroviral treatment. Groupe d'Epidemiologie du SIDA en Aquitaine (GECSA). AIDS. May 26 2000;14(8):971-978.

25. Department of Health and Human Services (DHHS). Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. 2013. Available from: URL: http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf.

26. Fischl MA, Richman DD, Grieco MH, Gottlieb MS, Volberding PA, Laskin OL, et al.,The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A doubleblind, placebo-controlled trial. N Engl J Med 1987;317 (4):185–191.

27. Geretti A, Smith C, Haberl A, Garcia-Diaz A, Nebbia G, Johnson M, et al., Determinants of virological failure after successful viral load suppression in first-line highly active antiretroviral therapy. Antiviral Therapy 2008;13(7):927-936.

28. Ferradin L, Laureillard D, Prak N, Ngeth C, Fernandez M, Pinoges L, et al.,Positive outcomes of HAART at 24 months in HIV-infected patients in Cambodia. AIDS2007;21:2293–2301.

29. Rougemont M, Stoll B E, Elia1 N, Ngang P. Antiretroviral treatment adherence and its determinants in sub-Saharan Africa: a prospective study at Yaounde Central Hospital, Cameroon. AIDS Research and Therapy 2009;6:21.

30. Kranzer K, Houben RMG, Glynn JR, Bekker L-G, Wood R, Lawn SD. Yield of HIV-associated tuberculosis during intensified case finding in resource-limited settings: a systematic review and meta-analysis. Lancet Infect Dis 2010;10:93–102

31. Ministry of Health and Social Welfare, Tanzania. National guideline for the clinical management of HIV and AIDS, 2012.

32. Chaiwarith R, Wachirakaphan C, Kotarathititum W, Praparatanaphan J, Sirisanthana T, Supparatpinyo K. Sensitivity and specificity of using CD4+ measurement and clinical evaluation to determine antiretroviral treatment failure in Thailand. International Journal of Infectious Diseases (2007) 11, 413—416.

33. Keiser O., et al., Accuracy of WHO CD4 cell count criteria for virological failure of antiretroviral therapy. Trop Med Int Health. 2009 Oct;14(10): p.1220-5.

34. Moore DM., et al., CD4+ T-cell count monitoring does not accurately identify HIV-infected adults with virologic failure receiving antiretroviral therapy. J Acquir Immune Defic Syndr. 2008 Dec 15;49(5): p. 477-84.

35. Aldous JL and Haubrich RH, "Defining treatment failure in resource-rich settings",Curr Opin HIV AIDS, 2009. 4(6): p. 459-66.

36. del Rio C, "Current concepts in antiretroviral therapy failure", Top HIV Med, 2006. 14(3): p.102-6.

37. Ledergerber B., et al., Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. The Lancet, 1999. 353(9156): p. 863-868.

38. Paris D., et al., Incidence and predictors of virologic failure of antiretroviral triple-drug therapy in a community-based cohort. AIDS Res Hum Retroviruses. 1999 Dec 10;15(18):1631-8.

39. Deeks SG., et al., Trends in multidrug treatment failure and subsequent mortality among antiretroviral therapy-experienced patients with HIV infection in North America. Clin Infect Dis, 2009. 49(10): p. 1582-90.

40. Smith D.,et al., Clinical utility of HIV standard genotyping among antiretroviral-naive individuals with unknown duration of infection. Clin Infect Dis. 2007 Feb 1;44(3):456-8.

41. Shah B., et al., Adherence to antiretroviral therapy and virologic suppression among HIV-Infected persons receiving care in private clinics in Mumbai, India. Clin Infect Dis. 2007 May 1;44(9): p. 1235-44.

42. El-Khatib Z., et al., Adherence to drug-refill is a useful early warning indicator of virologic and immunologic failure among HIV patients on first-line ART in South Africa. PLoS One, 2011. 6(3): p. e17518.

43. Jaffar S., et al., Rates of virological failure in patients treated in a home-based versus a facility-based HIV-care model in Jinja, southeast Uganda: a cluster-randomised equivalence trial. Lancet, 2009. 374(9707): p. 2080-9.

44. Reynolds SJ. Nakigozi G, Newell K, Ndyanabo A, Galiwongo R, Boaz I, et al., “Failure of immunologic criteria to appropriately identify antiretroviral treatment failure in Uganda”, AIDS, 2009. 23(6): p. 697-700.

45. Ramadhan HO., et al., Predictors of incomplete adherence, virologic failure, and antiviral drug resistance among HIV-infected adults receiving antiretroviral therapy in Tanzania. Clin Infect Dis. 2007 Dec 1;45(11): p.1492-8.

46. Mgelea E. Validity of clinical and immunological monitoring to detect virological failure in HIV-infected children on antiretroviral therapy in Dar es salaam, Tanzania. Master's dissertation, 2012.

47. Dragsted UB, Mocroft A, Vella S, Viard JP, Hansen AB, Panos G, et al., "Predictors of immunological failure after initial response to highly active antiretroviral therapy in HIV-1-infected adults: a EuroSIDA study", J Infect Dis. 2004 Jul 1;190(1):148-55.

48. Parienti JJ, Massari V, Descamps D, Vabret A, Bouvet E, Larouzé B, et al., "Predictors of virologic failure and resistance in HIV-infected patients treated with nevirapine- or efavirenz-based antiretroviral therapy", Clin Infect Dis. 2004 May 1;38(9):1311-6.

49. Chalamilla G, Hawkins C, Okuma J, Spiegelman D, Akum A, Christin B, et al.,"Mortality and Treatment Failure among HIV-Infected Adults in Dar Es Salaam, Tanzania". J Int Assoc Physicians AIDS Care (Chic). 2012 Sep-Oct;11(5):296-304.

50. Rosen S, Fox MP, Gill CJ. "Patient retention in antiretroviral therapy programs in sub-Saharan Africa: a systematic review", PLoS Med. 2007 Oct 16;4(10):e298

APPENDICES

Appendix I: Informed consent form (English version)

TITLE: PERFORMANCE CHARACTERISTICS OF CLINICAL, IMMUNOLOGIC AND VIROLOGIC CRITERIA IN MONITORING RESPONSE TO TREATMENT AMONG HIV-INFECTED PATIENTS IN DAR-ES-SALAAM, TANZANIA

PID No……

Greetings! My name is Dr Mkhoi L. Mkhoi, I am a postgraduate students at MUHAS, investigating on Performance characteristics of clinical, immunological and virological criteria in monitoring response to treatment among HIV-infected patients in Dar-Es-Salaam Tanzania.

Purpose of the study: To determine the Performance characteristics of clinical, immunological and virological criteria in monitoring response to treatment among HIV-infected patients in Dar-Es-Salaam Tanzania.

What Participation Involves:

If you agree to join the study, you will be interviewed using questionnaire, detailed information on social demographic characteristics, clinical assessment, treatment and laboratory investigation will be requested. Blood will be collected at the beginning of the study and during or after 6 months of follow-up

Confidentiality:

All information collected on questionnaires will be entered into computer with identification number. The questionnaires will be handled with secrecy in order to maintain confidentiality

Risks:

We do not expect that any harm will happen to you because of joining this study. Sometimes, a minimal pain may occur during blood collection.

Taking part in this study is completely your choice. If you choose not to participate in the study or if you decide to stop participating in the study you will continue to receive all services that you would normally get from this hospital. You can stop participating in this study at any time, even if you have already given your consent. Refusal to participate or withdrawal from the study will not involve penalty or loss of any benefits to which you are otherwise entitled.

Benefits:

If you agree to take part in this study, you will benefit by knowing the results for viral load test and CD4+ T-cell count of collected blood, and whenever the results have significant clinical implication you will get appropriate care, treatment and support from the clinic

Cost:

No payment will be requested from you as a fee to participate in the study

In Case of Injury:

We do not anticipate that any harm will occur to you or your child as a result of participation in this study. However, if any physical injury resulting from participation in this research occurs, we will provide you with medical treatment according to the current standard of care in Tanzania

Who to Contact:

If you ever have questions about this study, you should contact the study Principal Investigator Dr Mkhoi L. Mkhoi (Muhimbili University of Health and Allied Sciences, P.O. Box 65001, Dar es Salaam). If you ever have questions about your rights as a participant, you

may call Prof. M. Aboud, Chairman of the Senate Research and Publications Committee, P.O. Box 65001, Dar es Salaam. Tel: 2150302-6.

Signature:

Do you agree?

Participant agrees …… Participant does NOT agree ……..

I, _____ have read the contents in this form. My questions have been answered. I agree to participate in this study.

Signature of participant _____

Signature of witness (if mother/caretaker cannot read) _____

Signature of research assistant _____

Date of signed consent _____

Appendix II: Fomu ya Ridhaa (Swahili version)

Nambari ya mshiriki ... ...

Ridhaa ya kushiriki katika utafiti juu ya Utendaji wa vigezo vya kitabibu vya kufuatilia matokeo ya tiba kwa wagonjwa wenye Virusi Vya UKIMWI (VVU) Dar-Es-Salaam, Tanzania.

Salamu! Jina langu ni Dk Mkhoi L. Mkhoi, mwanafunzi wa Uzamili katika Chuo kikuu cha sayansi za afya Muhimbili (MUHAS) . Nafanya uchunguzi juu ya Utendaji wa vigezo vya kitabibu vya kufuatilia matokeo ya tiba kwa wagonjwa wenye Virusi Vya UKIMWI (VVU) Dar-Es-Salaam, Tanzania.

Madhumuni ya utafiti: Kujua Utendaji wa vigezo vya kitabibu vya kufuatilia matokeo ya tiba kwa wagonjwa wenye Virusi Vya UKIMWI (VVU) Dar-Es-Salaam, Tanzania.

Jinsi ya kushiriki:
Kama utakubali kushiriki, nitakuoji maswali machache yanayokuhusu na nitaomba kutoa sampuli ya damu kwa ajili ya uchunguzi zaidi.
Utunzaji siri:

Taarifa zote zitakazokusanywa zitatuzwa kwa siri kwa kutumia herufi na nanbari badala ya jina la mgonjwa.

Madhara/athari:
Hatutarajii kutakuwa na madhara yoyote yanayotegemewa kutokana na utafiti huu. Wakati mwingine maumivu kidogo yanaweza kutokea wakati wa kuchukua sampuli.
Uhuru wa kushiriki:

Kushiriki katika utafiti huu ni hiari yako. Kama utachagua kutokushiriki katika utafiti utaendelea kupokea huduma zote kama kawaida kutoka hospitali hii. Unaweza kuacha kushiriki katika utafiti huu wakati wowote, hata kama baada ya kutoa idhini yako.

Faida za utafiti:

Kama utashiriki katika utafiti idadi ya virusi katika damu yako pamoja na hali ya kinga ya mwili wako vitachunguzwa na kama kutakuwa na tatizo lolote utapatiwa matibabu stahili pamoja na ushauri.

Gharama:

Hakuna malipo kutoka kwenu kama ada ya kushiriki katika utafiti huu.

Taarifa/Mawasiliano:

Endapo utahitaji kupata maelezo zaidi au taarifa yeyote kuhusu utafiti huu,wasiliana na Dk Mkhoi L. Mkhoi, Chuo Kikuu cha Afya na Sayansi za tiba, SLP 65,001, Dar es Salaam. Kama utakuwa na maswali kuhusu haki yako kama mshiriki, unaweza wasiliana na Prof M. Aboud, Mwenyekiti wa Kurugenzi ya Utafiti na Machapisho, SLP 65,001, Dar es Salaam. Tel: 2,150,302-6.

Sahihi:

Je, unakubali kushiriki kwenye utafiti?

Ndiyo... ... ... Hapana ... ...

Mimi _____ nimeelezwa na nimesoma yaliyomo katika fomu hii. maswali yangu yamejibwa .

Nimekubali kushiriki katika utafiti huu.

Sahihi ya mshiriki/mlezi _____

Sahihi ya ushahidi (kama mama / mlezi hajui kusoma) _____

Sahihi ya mtafiti _____

Tarehe ya ridhaa _____

Appendix III: Case Report Form

TITLE: PERFORMANCE CHARACTERISTICS OF CLINICAL, IMMUNOLOGICAL AND VIROLOGIC CRITERIA IN MONITORING RESPONSE TO ART AMONG HIV-INFECTED PATIENTS IN DAR-ES-SALAAM, TANZANIA

i) Patient Identifier Information

Patient ID…… Phone……

Alt. phone……. Current address…….

ii) **Form information**

Date form completed……. Person completing form……..

Phone……

iii) Dermographic information

Gender…… Date of birth…….

Marital status…….. ..

iv) Laboratory data

Laboratory test	**Baseline**	**Follow-up**
CD4 Count (cells/ uL)		
Viral load (copies/mL)		
Haemoglobin (g/dL)		
Alanine transaminase (mmol/L)		
Complete blood count (x 10^9 cells/L)		

v) Clinical assessment

Signs and symptoms check list

S/N	Sign or symptom	Tick where appropriate
1	Cough (productive or non-productive)	
2	Fever	
3	Night sweats	
4	Hemoptysis	
5	Weigh loss	
6	Oral or oesophageal thrush	
7	Pain and/or difficult swallowing	
8	Severe headache	

9	Altered mental status	
10	Persistent passage of loose stools	
11	Multiple vascular nodules appearing in the skin, mucous membranes, and viscera.	
12	Small painful vesicles, Painful ulcers on the mucosa and skin, genital/peri-rectal ulcerations	
13	Involuntary weight loss of >10%	
14	Shortness of breath that has evolved over 2-4 weeks	
15	Chest tightness	
16	Focal paralysis or motor weakness	
17	Others	

Weight…… Height……. BMI……..

WHO stage defining illness (tick where appropriate)

Unexplained severe weight loss		Pulmonary TB	
Persistent oral candidiasis		Kaposi's sarcoma	
Cryptococcal meningitis		Chronic HSV	
Unexplained chronic diarrhoea		Recurrent severe bacterial pneumonia	
Pneumocystis jiroveci pneumonia		Oesophageal candidiasis	
HIV wasting		Unexplained persistent fever	
Pruritic papular eruption		Others……	