Predictors of adherence among antiretroviral therapy naïve patients on first-line regimen at Themba Lethu Clinic inJohannesburg: Results from a prospective cohort study

By

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DECLARATION

I, Mouhamed Abdou Salam Mbengue declare that this report is my unaided own work, compiled under the supervision of Prof Charles S Chasela and Dr Denise Evans. The report is being submitted to the University of the Witwatersrand in partial fulfillment of a degree of Master of Science in the field of Epidemiology and Biostatistics. It has not been submitted before for any degree or examination at any other university.

Signature

Mouhamed Mbengue

Date: November 2017

DEDICATION

I dedicate this report to my family whose love and constant support encouraged me and gave me determination during my studies in South Africa.

ABSTRACT

Introduction

Viral load is the most reliable indicator of poor adherence to anti-retroviral therapy (ART). However, this assay is difficult to implement in resource-limited settings due to financial and technical constraints.

Laboratory markers, combined with the patient's demographic and clinical details, have been described as better proxies of adherence than the current self-reported adherence measures. However, the real diagnostic value of these biomarkers remains unknown. Therefore, the aim of this study was to assess the usefulness of a composite marker to identify poor adherence to ART defined as a detectable plasma viral load in HIV-positive patients on first-line regimen at Themba Lethu Clinic (TLC) in Johannesburg, South Africa

Materials and Methods:

This study was retrospective cohort analysis of data collected on HIV-positive ART naïve adults initiating first line antiretroviral regimen at TLC following the 2010 South African antiretroviral treatment guidelines. The data collection was carried out as part of the low-cost monitoring (LCM) study at Themba Lethu Clinic in Johannesburg from February 2012 to 2014. The LCM cohort which aims to look at low cost monitoring of HIV treatment in resource limited settings was initiated in 2009 in Johannesburg, South Africa. The study or treatment outcome was failure to suppress viral load (VL $\geq$ 400 copies/ml) at 6 and at 12 months. Adherence to antiretroviral treatment was assessed using four (4) self-reported adherence (SRA) measures namely: a self-reporting questionnaire, a Visual Analogue Scale (VAS), a pill identification test (PIT) and the Simplified Medication Adherence Questionnaire (SMAQ). The result of each self-reported measure was classified as either positive or negative given a conventional threshold. In our study three (3) self-reported adherence (SRA) measures were

combined into a multi-method approach tool which included self-reports combined with VAS and the pill identification test (PIT).

Continuous variables were summarized by median with interquartile range. Categorical variables were summarized by giving their frequencies. To compare continuous variables, we used an unpaired t-test if the variable was normally distributed. When continuous variables were compared from baseline to the previous 6 months, a paired t-test was done. In the case of skewed distribution, we used a non-parametric variant of the t-test such as the Mann-Whitney U-test. To compare categorical variables, we used cross-tables with corresponding chi-square test or Fisher exact test.

A Modified Poisson Generalized Linear Model (GLM) with robust variance was used to estimate adjusted relative risks (aRR) of failing to suppress viral load at 6 and at 12 months adjusting for age age, gender, self-reported adherence measures, changes in laboratory markers and missed appointments at 6 and 12 months after ART initiation. As there was missing values in the covariatess and the outcome, we performed a multiple imputation technique under missing at random (MAR) assumption in order to compare the robustness of the estimations between the complete case analysis and the imputation model under MAR after imputing missing values. with the imputed dataset.

Additionally, we calculated the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for each self-reported adherence measure using viral load as the reference standard. Thus, we derived two diagnostic risk scores from rounding and adding together the adjusted regression coefficients used to estimate adjusted relative risk and following the Spiegelhalter and Knill-Jones approach, at 6 and at 12 months. The Receiver Operating characteristic (ROC) curves were computed to see the overall discriminative value of each continuous risk score. To assess the clinical usefulness of the continuous riskscores we

dichotomized them from 2 ≥ vs < 1 to 5 ≥ vs < 5 and calculated the sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) at each cut-off, taking detectable viral load as a gold standard.

Results: There were 353 HIV-positive patients initiated on first line ART at TLC for the LCM cohort study. Of these, 80.7% did not suppress viral load after 6 months while 30.1% did not suppress viral load at 12 months. The proportion of patients classified as being highly adherent was 86.7% but this proportion decreased to 60% at 12 months. By 6 months, after adjusting for gender and age, the variables that were significantly associated with detectable viral load included: having missed at least two ARV visits by ≥ 7 days (aRR: 2.35 95% CI: 1.08 -5.11); platelet count < 150 cells/mm^3 (aRR: 2.73 95% CI: 1.04 -7.18) and VAS ≤ 95% (aRR: 1.65. 95% CI: 1.01-2.71). At 12 months, the estimates showed a positive relationship only with age group and unemployment. There were no similarities in the results found using complete case analysis and analysis with imputed datasets. However, the largest standard errors were obtained from the complete case analysis.

At 6 months, the AUC ROC curve was calculated as 0.63 (95% CI, 0.53 - 0.72) while, for the visual analogue scale, the AUC decreased to 0.55 (95% CI, 0.49 - 0.62); for the Simplified Medication Adherence Questionnaire (SMAQ), the AUC decreased to 0.52 (95%CI, 0.45 - 0.60), while for the multi-method approach, it decreased to 0.53 (95% CI, 0.46 - 0.58). The optimal diagnostic accuracy was obtained with the score 5 (≥5 vs <5 Se: 64% and a Sp: 50.0%) followed by a risk score of 4 (Se of 76.0%, Sp of 34.7%). At 12 months, the AUC of the diagnostic risk score was calculated as 0.44 (95%CI, 0.40 - 0.60) while for the three self-reported adherence methods, it decreased to 0.48 (95% CI, 0.40 - 0.60), 0.51 (95%CI, 0.40 - 0.60) and 0.50 (95%CI, 0.41 - 0.59) respectively for the visual analogue scale, the SMAQ and the multi-method approach method respectively.

Conclusion. This study shows that after ART initiation, the 6-month's adherence can be better diagnosed using laboratory markers combined with patient's information and traditional self-reported adherence measures at Themba Lethu Clinic. The advantage of this proposed method is that it is based on routine and accessible informations collected during HIV-positive patient visits, thus incurring no additional cost for its implementation. An external validation of this diagnostic risk score is needed for its translation into clinical practice in resource-limited settings.

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TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS AND ABBREVIATIONS

ACTG	AIDS Clinical Trials Group
AUC	Area Under Curve
3TC	Lamivudine
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral
CASI	Computer-Assisted Self-Interviewing
CD4	Cluster of Differentiation
CDC	Center for Diseases Control and Prevention
CPS	Clinical Prediction Score
d4T	Stavudine
DOH	Department of Health
EFV	Efavirenz
EVP	Events Per Variable
FN	False Negative
FP	False Positive
FTC	Emtricitabine
HE²RO	Health Economics and Epidemiology Research Office
HIV	Human Immunodeficiency Virus
HSRC	Human Sciences Research Council
LPV	Lopinavir
HSRC	Human Sciences Research Council
MAR	Missing at Random
MCAR	Missing Completely at Random
MCV	Mean Corpuscular Volume
MEMS	Medication Event Monitoring System
MNAR	Missing Not at Random
NDOH	National Department of Health
NGO	Non-Governmental Organization
NNRTI	Non-Nucleoside Reverse Transcriptase
NPV	Negative Predictive Value

NRTI	Nucleoside Reverse Transcriptase
NVP	Nevirapine
PI	Protease Inhibitor
PIT	Pill Identification Test
PPV	Positive Predictive Value
ROC	Receiver Operating Characteristics
SMAQ	Simplified Medication Adherence Questionnaire
SRA	Self-Reported Adherence
TDF	Tenofovir Fumarate
TDM	Therapeutic Drug Monitoring
TLC	Themba Lethu Clinic
TN	True Negative
TP	True Positive
USAID	United States Agency for International Development
VAS	Visual Analogue Scale
VL	Viral Load
WHO	World Health Organization

CHAPTER ONE: INTRODUCTION LITERATURE REVIEW, AIMS AND OBJECTIVES

In this chapter, background is provided including the importance of adherence to antiretroviral treatment and its monitoring. In addition, the problem statement and reasons for this research are also explained and the results of literature review are given. The chapter ends with a statement of the research question and a description of the aim and objectives of the study.

1.1 Background

The human immunodeficiency virus (HIV) epidemic remains a major public health problem in the world. In 2012, 36 million people were living with HIV and 2.3 million individuals were newly infected globally (UNAIDS, 2013). Sub-Saharan Africa bears the burden of the epidemic with 25 million people living with HIV and 1.2 million deaths due to Acquired Immunodeficiency Syndrome (AIDS) in 2012 (UNAIDS, 2013).

The scaling-up of antiretroviral therapy (ART) program has reduced the impact of the HIV/AIDS epidemic. Around the world, antiretroviral drugs have reduced mortality and increased life expectancy among HIV-positive persons (UNAIDS, 2013) (Kirk, 2003). In 2012, more than 9 million people were on ART in low and middle income countries (UNAIDS, 2013). This number represented 65% of the global target of 15 million people on ARV before the 2015 deadline (UNAIDS, 2013)

In South Africa, the estimated prevalence of HIV in the general population is 12%, representing 6.4 million people living with HIV and of which 31.2% were on ART treatment (HSRC, 2012).The distribution of the HIV infection differs by gender, age and geographic area. The highest prevalence is found in KwaZulu Natal with 17% while, in Gauteng province the HIV prevalence is around 12.5% (HSRC, 2012).

Since 2004, South Africa has made comprehensive efforts toward achieving free and greater access to antiretroviral treatment in the world. Currently, over 2 million HIV-positive people are receiving antiretroviral therapy in line with the South African ART treatment guidelines in 3,400 facilities across the country (UNAIDS, 2014). In 2014, the coverage of the national ART programme was estimated to be around 50% in South Africa (UNAIDS, 2014).

In South Africa, the main goal of the ART programme is to save life, achieve the best outcome in the most cost-efficient manner, decentralize service delivery in public health facilities and mitigate the impact of the HIV/AIDS epidemic (DOH, 2013). Based on World Health Organization (WHO) recommendations, ART eligibility criteria for HIV adult patients initiating in South Africa requires the following conditions: being either an HIV-positive patient with a CD4 count less than 350 cells/mm^3, or a patient co-infected with tuberculosis or an HIV-positive woman who is pregnant or breastfeeding, regardless of CD4 count level. The same rule applies to HIV-positive patients with opportunistic infections or at stage 3 or 4 of WHO stage classification.

At ART initiation, first-line treatment consists of two nucleosides reverse transcriptase inhibitors (NRTIs) with one non-nucleoside reverse transcriptase inhibitor (NNRTI). Consecutively, different combinations or substitutions involving NNRTI and NRTI can be made when drug toxicity and interactions occur (WHO, 2012). However, the switch to second line ART is only recommended when the plasma HIV-RNA is greater than 1,000 copies/ml during two consecutive measurements made within 2 months. Second-line ARTs generally combine two nucleoside reverse transcriptase inhibitors (NRTIs) with a protease inhibitor (PI) (WHO, 2010) (DOH, 2013).

The number of persons on ART is still increasing the major reason being that the national ART program has extended over a decade and the treatment is now available in many urban and

rural areas. Standard ART regimen is a combination of antiretroviral drugs that need to be taken correctly during the one's entire lifetime to suppress the HIV virus.

Because of this increase in the number of persons initiated on ART, monitoring adherence to is now a key strategy for the success of the ART programme. Poor adherence leads to treatment failure, higher risk of mortality amongst HIV-infected patients and the need to treat patients with costlier second and third-line ART, a situation that reduces the gains from several years of therapy (WHO, 2012). WHO defines treatment adherence as "the extent to which a person's behavior – taking medications, following a diet and/or executing lifestyle changes – corresponds with agreed recommendations from a health care provider" (WHO, 2003). For ART drugs, at least 90% compliance is required for virologic suppression among HIV-positive patients (WHO, 2003). In South Africa, several adherence assessment methods (self-report, visual analogue scale, pill-count and pill-identification, pharmacy records, and the Simplified Medication Adherence Questionnaire) are currently used in clinical practice (Berg & Arnsten, 2006). However, the performance of these methods is limited by their poor sensitivity and specificity when compared to viral load, which is considered to be the gold standard method (Berg & Arnsten, 2006). In addition, these methods tend to over-estimate adherence due to many factors such as recall-bias and lack of standardization (Bartley et al, 2013). Since the viral load is not available everywhere, there is an urgent need to invest in alternative and low-cost methods that could be used to assess and monitor adherence to ART among HIV-positive patients accurately.

1.2 Problem statement

Adherence to an ART regimen is critical for the success of antiretroviral therapy at individual and community level. Many studies have shown that a low level of adherence is the most well-known reason associated with drug resistance, treatment failure and mortality in HIV-positive patients. Conversely, the benefits of high adherence include suppression of viral replication, high level of CD4 cells, prevention of drug resistance, rapid immune reconstitution and slow disease progression (Berg & Arnsten, 2006; de Olalla et al, 2002). It has been shown that, at community level, good adherence improves the long-term impact of ART programmes by increasing life-expectancy among HIV-infected people and reducing HIV-transmission (Mannheimer et al, 2006; Cohen et al, 2011). A good adherence level requires the patient to take at least 95% of the medication prescribed and at the correct time (WHO, 2003). Without accurate monitoring, patients with poor adherence will continue to have high viral loads, treatment failure and drug resistance. As a result, in the long term, non-adherent HIV-positive patients on tri-therapy are four times more likely to die than adherent patients initiated on the same therapy (de Olalla et al, 2002).

While adherence to ART is critical, there is still no consensus on the most suitable method to monitor and assess it accurately. In developed countries, the viral load assay is routinely used to monitor adherence in patients on ART. However, in resource-limited settings this technique is extremely costly ($15-$150 per test) and cannot be performed routinely (Colebunders et al, 2006). Moreover, viral load assays require well equipped laboratories and the ability for the health system to provide highly trained staff. This situation is not the case everywhere in South Africa where only tertiary reference laboratories are able to perform viral load assays (Stevens & Marshall, 2010). Due to technical and financial constraints, primary health care facilities and secondary facilities located in rural areas, which account for more than 75% of the health

system, are not able to perform HIV-viral load assays routinely (Stevens & Marshall, 2010). As an alternative to viral load assays, the adherence assessment methods currently used in clinical settings include self-reporting, visual analogue scale, pill count and pharmacy refill records. However, there is evidence of poor sensitivity and a risk of overestimation when these methods are used to assess and monitor adherence among patients on ART (Bartley et al,2013). In an evaluation study in South Africa using viral load as a gold standard, the sensitivity and specificity of the visual analogue scale for different cut-offs were 66%, 46% and 29% respectively (Meyer et al,2012). For adherence levels assessed with pharmacy-refill data, the sensitivity was 55%, 15%, and 6% (Meyer al. 2012). There is also evidence that self – reported methods are susceptible to recall bias when applied over a long period (Bartley et al‘ 2013).

While most of the traditional indirect methods fail to assess adherence to ART accurately, there is also increasing evidence that routine laboratory biomarkers could provide an alternative. Laboratory markers collected routinely have the potential to assess the virologic failure and adherence to ART accurately (Van Griensven et al,2014; Robbins et al,2010). Studies have shown that individual markers such haemoglobin , mean corpuscular volume, total lymphocyte count, serum lactate, CD-38, and bilirubin can be used as an alternative to viral load assays to predict adherence (Ross-Degnan et al,2010; Steele et al,2002). Furthermore, the main advantage of these routine biomarkers over the traditional methods is that they are easy to collect and are available in routine clinical care. However, despite their potential, many of these biomarkers have been evaluated in the context of retrospective studies. Although they are simple and feasible, predictive models based on retrospective datasets has several limitations. First, they have missing values and may lead to selection bias that would affect their validity. A second limitation is the poor generalizability of the results (Steyerberg et al, 2013). Finally, the added value of each biomarker when they are combined or mixed with other indirect methods is still unknown. Since a prospective design is the best indicated method, the

diagnostic value of these promising biomarkers need to be assessed using a study with a prospective design.

1.3 Justification for the study

Viral load is known as the gold standard to assess adherence in patients on ART. However, this technique is unavailable in many areas due to financial and technical constraints. Despite their wide use, the validity of the current indirect methods based on questionnaires and pharmacy records remains limited. To assess the usefulness of laboratory markers as alternative methods, previous findings from retrospective datasets need to be confirmed with a prospective study. A prospective evaluation of relevant biomarkers will enable us to define an accurate and low-cost method for medication adherence assessment.

In resource-limited settings where viral load is often unavailable, such a new method based on routine data could be useful for clinicians to effectively monitor adherence to ART and address the issue of treatment failure among HIV-positive patients in good time. Furthermore, at the national level, the availability of a simplified, low-cost performant tool will reduce the use of viral load assays; hence improve the sustainability of the national ART program.

1.4 Literature review

The positive impact of highly antiretroviral treatment in reducing mortality and morbidity related to AIDS has been demonstrated and recognized in observational and experimental studies. Research findings have demonstrated the effectiveness of early combination of antiretroviral drugs to prevent the transmission of HIV-1 virus (Cohen et al, 2011).

Adherence to ARV treatment, detectable viral load, and treatment failure and drug resistance are closely linked. Poor adherence to highly active ART regimen is a major cause of HIV drug

resistance and non-adherent patients on triple therapy are about four times more likely to die than adherent patients on the same therapy (de Olalla et al, 2002).

Moreover, the long term benefit of ART is lowered when the patient is non-adherent (Mcmahon et al, 2013). Many factors can negatively affect the change in viral load among patients on first line therapy. These include ART regimen, adherence to treatment, existence of drug resistance and drug metabolism, genetic differences and clinical stage at the beginning of the treatment (Tran et al, 2014). However, studies have shown that the factor most related to viral load change and HIV/AIDS progression is adherence to therapy (Luebbert et al, 2012). The WHO public health strategy recommends a combination of CD4 count with clinical criteria as an alternative method to assess viral load suppression in HIV-positive patients (Gilks et al, 2006). Although not specifically designed to target a single elevated viral load but for assessing virologic failure, defined as two successive measures of viral load greater than 400 copies/ml, the capacity of this strategy to predict virologic failure is poor (Robbins et al, 2010). In South Africa, an evaluation study of the WHO criteria among adults revealed that the CD4 count criteria had a sensitivity of 21% and specificity of 58% in detecting virologic failure and clinical criteria had a sensitivity of 15.2% and specificity of 88.1%. The positive predictive value of the CD4 count and the clinical criteria in detecting virologic failure was 36.8% and 12.8 % respectively (Mee et al, 2008). Another challenge for implementing routine viral load measurement is related to financial cost and technical constraints. While the viral load measurement as a method for monitoring and addressing the issue of adherence appears to be realistic in developed countries, in resource-limited settings the absence of well-equipped laboratories and financial constraints limit its general use in the public health sector, specifically in rural areas (Colebunders et al, 2006). Instead of viral load, many alternative methods for measuring adherence directly are also available and currently used in routine clinical care. These include self-reporting, the Visual Analogue Scale (VAS), the pill count

method, the pharmacy refill records, and Simplified Medication Adherence Questionnaire. While these methods are largely used by the clinicians, they are limited by their lack of sensitivity, specifically in situations of poor adherence (Berg & Arnsten, 2006). Moreover, they also give poor results due to many biases when applied over a long period (Bartley et al, 2013). Additionally, there is evidence that self-reported adherence measures such as VAS may overestimate adherence, especially when questionnaires are administered by health professionals (Giordano et al, 2004). Pill identification tests involve inviting patients to identify the pills in the antiretroviral regimen. One of the inconveniences of this method is related to the loss of sensitivity in treatment experienced patients and for not being a sensitive marker of actual pill intake (Berg & Arnsten, 2006)

Recently, the use of electronic devices for monitoring adherence to ART has been advocated based on research findings in developed countries .The Medication Event Monitoring System (MEMS) (Berg & Arnsten, 2006; Ailinger et al,2008) is an electronic device included with pill containers and it records the removal of the cap of the counter by a patient or another person. This method has been used in the industry and is reliable for recording dose histories. It represents a good proxy for the removal of pills and then to adherence. However, difficulties associated with routine use of MEMS are related to the fact that the patient can open the bottle and not necessarily take the pil. Additionally, the cost associated with this method and its use is high (Berg & Arnsten, 2006).

The best method for assessing adherence to ART should be low cost, brief and non-intrusive so that it could be used many times over the course of the treatment. In addition, it should be reliable and acceptable to the HIV-positive patient while also being sensitive enough to measure change (Evans & Fox, 2013). Estimation methods based on the combination of routine biomarkers with information on adherence to ART and clinical stage of the patients better

predict detectable viral load when compared with the traditional methods based on questionnaires and self-reporting (Evans & Fox, 2013).Moreover, results from observational studies have documented the changes in certain immunological and hematological biomarker levels and their interrelationship with adherence and the treatment outcome (Cosby, 2007).The changes in platelet count, total lymphocytes and their correlation with the viral load have been documented amongst HIV positive adults adults on ART treatment (Cosby et al, 2007; Denue et al, 2013).

Additionally,many studies suggest that changes in mean corpuscular volume for HIV-positive patients taking either Zidovudine *(*AZT) or Stavudine (d4T) may be a useful surrogate marker for adherence to ART (Steele et al, 2002; Meriki et al, 2014). There is increasing evidence from retrospective and observational studies that MCV is one of the hematologic parameters proposed as an early clinical indicator of ART adherence (Mugisha et al, 2012; Kufel et al, 2016).

In a retrospective study, Romanelli et al, (2002) showed that the incidence of macrocytosis is significantly different between adherent and non-adherent patients (78% vs 32.6% $p < 0.001$). This study showed a clear link between the rise of MCV levels and strict adherence to Zidovudine after ART initiation. A rise in MCV is also observed in patients on Stavudine, another thymidine analogue. In certain settings, MCV has been used to assess adherence to ART in HIV-positive patients (Romanelli et al, 2002; Segeral et al, 2010). However, other recent studies (Mugisha et al, 2012; Kufel et al, 2016) have claimed that the long-term effect of Zidovudine or Stavudine based regimens on the MCV remain unclear (Mugisha et al, 2012).

Like mean corpuscular volume, the platelet count is also known as a potential biomarker of poor adherence to ART. More recently, Zetterberg et al (2013) have shown that interrupting ART is associated with an increased risk of thrombocytopenia.

Additionally, however, data from cohort studies has shown that the reintroduction of ART therapy reverses the thrombocytopenia. However, the cut-off level at which the platelet count can be used as biomarker of ART adherence still remains unclear (Meriki et al, 2014).

In a retrospective study, Petersen et al, (2005) used 134 HIV-positive patients to assess the usefulness of bilirubin as a potential biomarker of poor adherence to ART in HIV patients on Atazanavir-based regimens. They found that an increase in bilirubin of more than 0.4 mg/dl correctly classified 81% of patients as having successful ART adherence and treatment response. In another experimental study, Mugo et al (2013) showed that asymptomatic hyperlactatemia (serum lactate concentration $\geq$ 2 mmol/L) was associated with being treated with NRTI/PI and having an undetectable viral load regardless of treatment regimen. Except for patients receiving Zidovudine, studies have shown that, after ART initiation, hemoglobin levels increase amongst HIV-positive individuals with a good adherence level. In studies conducted in Europe (Benito et al, 2004; Ondoa, 2005), a fall in hemoglobin levels and total lymphocytes count below baseline levels after 6 months following ART initiation better predicted the absence of viral load suppression among patients. Studies also revealed that the expression of CD8+CD38+T cell count is an independent marker of plasma viral load in infants treated with first-line ART (Benito et al, 2004; Ondoa, 2005). Studies in Europe and West Africa (Ondoa. 2005; Colebunders et al, 2006; Schreibman & Friedland, 2004; Cosby, 2007) showed that, among positive patients receiving ART, the proportions of CD8+Tcell expressing the activation marker of CD38 was correlated with treatment outcome and virological failure (Ondoa. 2005).

Furthermore, immunological and clinical information on missing visits have been previously identified as predictors of adherence to ART treatment in a large retrospective cohort patient analysis in South Africa. This study that used retrospective datasets from South Africa

identified that the change in MCV at 6 months of < 14.5 fL , the number of missed visits (days) and regimen dosing were potential biomarkers of adherence (Brennan et al, 2010). Despite the association between the changes and these biomarkers on the one hand, and their potential usefulness on the other, many of these studies have used a retrospective design. Therefore, the real diagnostic accuracy of these potential biomarkers needs to be confirmed within studies with a prospective design.

1.5 Study aim and objectives

1.5.1 Research question

What are the most significant markers of medication adherence among ART naïve patients on a first line regimen?

1.5.2 Aim of the study

The aim of the study was to determine the usefulness of a composite marker to identify poor adherence to ART, defined as VL ≥ 400 copies/ml in patients on first-line ART at Themba Lethu Clinic (TLC) in Johannesburg, South Africa.

1.5.3 Study Objectives

i. To compare the demographic and clinical characteristics of patients on first line antiretroviral treatment with a detectable viral load (VL ≥ 400 copies /ml) with those with a viral load < 400 copies/ml at 6 and 12 months after ART initiation.

ii. To determine the association between (i) self-reported adherence, (ii) CD4 response, (iii) MCV response, (iv) missed appointment, (v) new condition symptom, (iv) MPR or (vii) drug substitution and a detectable viral load (≥ 400 copies/ml) at 6 and 12 months after ART initiation.

iii. To determine the diagnostic accuracy (sensitivity, specificity, PPV, NPV) of self-reported adherence (e.g. VAS, SMAQ or multi-method) and the composite marker including CD4 response, MCV response, missed appointment and new condition or symptom compared to viral load as the gold standard.

CHAPTER TWO - MATERIALS AND METHODS

2.1 Introduction

This chapter describes the methods used to collect, manage the data and perform the statistical analysis. It also details techniques used in dealing with the missing data in order to ensure the validity of the study results. First, the chapter describes the study design, the setting, how the sample size was arrived at and explains the study population.

2.2 Study design

This is a secondary data analysis of data collected from a prospective cohort of HIV-positive patients. Data were collected from ART naïve patients initiating first-line ART at Themba Lethu Clinic in Johannesburg from February 2012 to April 2014. At baseline, demographic and previous clinical informations were recorded for each HIV-positive patient prior to ART initiation. Clinical information, adherence to antiretroviral drugs, viral load, CD4 count and other routine biomarkers were assessed at 6 and at 12 months after ART initiation. Although, the information was collected prospectively, the object of diagnostic study is cross- sectional (Figure 1) (Collins et al, 2015). Therefore, the effect of the exposure in the outcome must be assessed at a single point during the follow-up (Steyerberg et al, 2013; Collins et al, 2015). For the purpose of our study, we analyzed the data using the time period (T) of 6 months as T=0 (Collins et al, 2015).

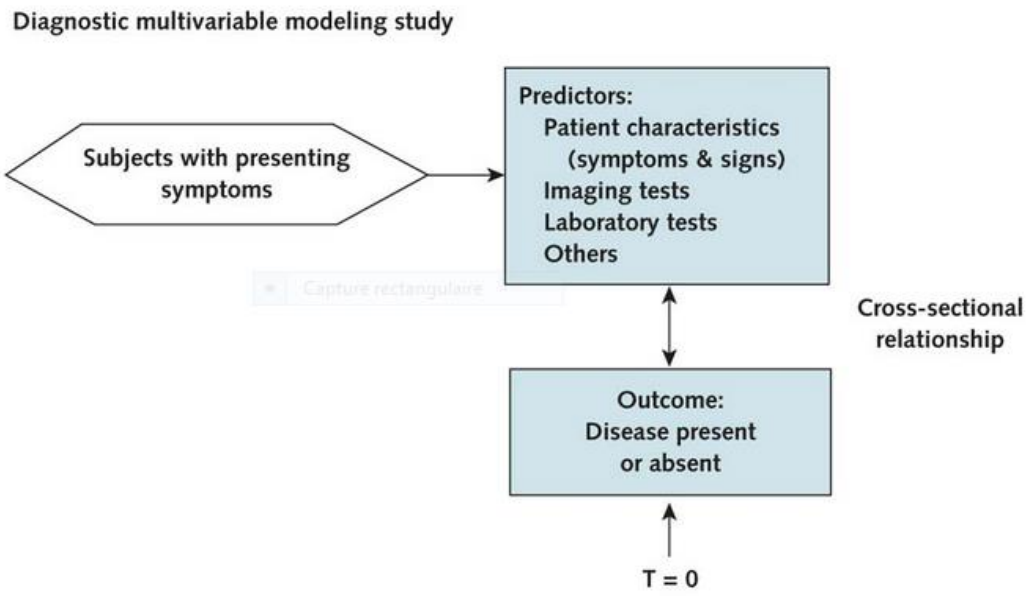

Figure 1 TRIPOD schematic representation of diagnostic study: (Collins et al, 2015).

2.3 Study Setting

The data were prospectively collected at the Themba Lethu HIV Clinic, an ambulatory centre dedicated to the treatment of people living with HIV/AIDS, located in the city of Johannesburg in the Gauteng Province, north central South Africa. The Themba Lethu HIV Clinic cohort database is the result of collaboration between the Non-Governmental Organization (NGO), Right to Care, Boston University, the Clinical HIV Research Unit and the Health Economics and Epidemiology Research Office (HE^2RO) of the University of the Witwatersrand.

The clinic is located inside the Helen Joseph Hospital in Johannesburg, Gauteng Province. Themba Lethu HIV Clinic started in 2004 with the roll out of South Africa's National ART treatment programme and, since then, more than 30000 HIV-positive persons have been enrolled for HIV treatment and care and 21000 of them have been initiated on antiretroviral treatment (Fox et al, 2012) with an average of 176 medical visits per day (Macleod et al, 2012). Patients are mostly from the Johannesburg area, the majority of them are South Africans and some of the patients are from bordering countries (Fox et al,2012).

Themba Lethu HIV clinic is a governmental clinic and operates under the South African National Treatment Guidelines (DOH. 2013). According to the 2013 South African ART treatment guideline (DOH. 2013), adult patients are initiated onto ART when their CD4 count is ≤ 350 cells/mm³ irrespective of WHO clinical stage. Individuals with tuberculosis, pregnant women or breast-feeding women are also eligible to initiate ART treatment. Also, patients with WHO stage III or IV conditions are also eligible for ART treatment, regardless of CD4 count level. Most of the HIV-positive patients (70%) are initiated onto the first-line combination Tenofovir-Lamivudine-Efavirenz. Patients on ART treatment are seen typically during medical visits at month 1, 3, and 6 and 12 months whereas the laboratory monitoring is done every 6 months to one (1) year (Fox et al, 2012). For the LCM study, laboratory markers were done at 0, 6, 12 and 24 months on ART.

According to the 2013 South African ART guidelines (DOH, 2013), the first viral load should be measured at six (6) months and yearly thereafter following initiation of therapy. By Six months, patients with high adherence level should have reached viral load suppression defined as viral load value ≥ 400 copies/ml (2.6 Log_{10}) (DOH, 2013). Additionally, following the 2013 South African ART guidelines, patients have CD4+ cells levels measured every 6 months (DOH. 2012; DOH. 2013). Beside viral load and CD4 count, several other laboratory markers are routinely checked prior to ART initiation and later during clinical visits. TherapyEdge-HIV™ (Advanced Biological Laboratories, SA, Luxembourg) is an Electronic Medical Record (EMR) which has been used at Themba Lethu clinic since 2009 for the management of routine care data (Fox et al, 2012). The application provides a platform to enter and store updated data on demographics, visits, laboratory results such as viral load, CD4 count, medical conditions and antiretroviral treatment (Fox et al, 2012). It also provides a possibility for the staff to enter data or consult patient's clinical history during medical visits in order to ensure a regular update of patient's information (Fox et al, 2012). TherapyEdge-HIV™ is connected to laboratory

results from the NHLS laboratory and also the pharmacy system (Fox et al, 2012). The quality of the TherapyEdge-HIV™ database is optimized with a team dedicated to verifying, checking, correcting errors and regularly cleaning the database (Fox et al, 2012).

2.4 Study population

The study population consisted of HIV-positive men and women ART naïve patients older than 18 years of age and who initiated standard first-line ART based on the 2010 South African National Department of Health ART treatment guidelines (DOH, 2013). These HIV-positive adult patients were recruited and followed up at Thembu Lethu Clinic between February 2012 and April 2014. Data were prospectively collected during the routine visits.

2.4.1 Inclusion criteria

The inclusion criteria were as follows:

- Adult (over 18 years of age) at ART initiation and willing to consent
- HIV-positive patient eligible to initiate first-line according to the 2013 South African National adult ART treatment guidelines (DOH, 2013).
- ART naive

2.4.2 Exclusion criteria

The exclusion criteria included any of the following:

- Participants who were pregnant at enrolment or who became pregnant during the study
- Patients transferred in from other facilities
- Participants who had already started ART at the beginning of the study.

2.5 Study sample

From 2012 to 2014, consecutive HIV-Positive patients on First-line ART treatment at Themba Lethu Clinic who fulfilled the eligibility criteria were prospectively included in the study database of Low Cost Monitoring of HIV in resource-limited settings (LCM). A sample size of 357 patients was obtained at the end of the enrolment period in 2014. For this secondary data analysis, the information available on LCM database was linked to the TherapyEdge-HIV™ electronic database to generate a single dataset with 357 observations.

We estimated that the prevalence of failing to suppress viral load will be 32% at treatment initiation (Fox et al,2013) and this proportion will be around 39% at 6 months after ART initiation (Evans et al, 2014) .With this sample size of N=357 and a two-sided test with α=5%, given a difference of at least 3% in the adherence measure between males et females, and a within group standard deviation of VAS to be 9.03, our study will have more than 80% power (83.16%) for detecting an independent association of failure to suppress viral load (HIV/RNA $\geq$ 400 copies/mL) with poor adherence represented by VAS < 95% adjusted for gender, age, MCV response, missed appointment, new condition symptoms and drug substitution (Vittinghoo et al, 2012; STATA, 2011).

2.6 Data sources and measurement

2.6.1 Data sources

The data used in this study was obtained from 2 data sources, namely the study database of the Low-Cost Monitoring of HIV in resource-limited settings (LCM) and the TherapyEdge-HIV™ database. Individual records from LCM database were linked to routine care data drawn from TherapyEdge-HIV™. The linkage was done using a unique identifier number (TE number) and after removal of personal information.

The LCM study was a prospective cohort study in which patients on first-line ART treatment, upon meeting the eligibility criteria, were successively enrolled since its start in 2010. After enrolment into the cohort, patients come at the clinic every 6 months. At each patient's visit, demographic and clinical information were gathered by the study staff and stored in an Excel spreadsheet (Table 1).

Table 1 Data Source of different informations collected during the study.

	Database source	
Variables	**TherapyEdge-HIV™**	**LCM**
Eligibility criteria	According to DOH guidelines	≥ 18 + not pregnant and not transferred in
Demographic characteristics	Same	Same
ART regimen	Same	Same
Medical visit	1, 3, 6 months and every 6 months thereafter	0, 6, 12, 24 months
Laboratory tests (serum lactate, albumin)	Standard of care according to NDoH guidelines	0, 6, 12, 24 months
Extra laboratory monitoring: Adherence, serum lactate, albumin, total lymphocyte count	As clinically indicated	0, 6, 12, 24 months
Viral load	6, 12 months	0 months
WHO stage at ART initiation	0 month	0, 6, 12, 24 months

MCV: Mean cell volume. ART: Antiretroviral treatment. NDoH: National Department of Health. WHO: World Health Organization. LCM: Low cost monitoring. DOH: Department of Health

2.6.2 Definition and measurement of outcomes and exposures

2.6.2.1 Adherence measures

At Themba Lethu Clinic, adherence to treatment was assessed based as part of the Low-Cost Monitoring of HIV in resource-limited settings (LCM) project at 6 and 12 months of follow-up. Data were collected by the health care workers who administered the tools at during each follow up visit. The assessment method used four (4) self-reported adherence (SRA) measures namely: self-reporting, Visual Analogue Scale (VAS), pill identification test (PIT) and the Simplified Medication Adherence Questionnaire (SMAQ) (Appendix D). Each of these methods has been validated in previous studies (Knobel et al, 2002). The result of each method of adherence measurement was classified as either positive or negative given a conventional threshold.

Previous studies have shown that the validity of applying a combination of tests is higher when compared to a single test to assess adherence to antiretroviral treatment. Therefore, WHO recommends a multi-method approach when measuring a patient's adherence to ART (Steel & Joshi, 2007). Thus, in our study three (3) self-reported adherence (SRA) measures were combined into a single adherence assessment tool. The method to derive a single adherence assessment tool from the three (3) SRA individual methods is described below.

- **Self-report (SR)**

In the self-report, adherence is based on the patient's assessment of the number of pills taken in the last week compared to the actual dose that should have been taken. This method also includes questions that attempt to assess whether the patient did stop or sometimes had difficulties taking the medication correctly as prescribed during the last visit. Thus, the self-report tool also addresses the underlying causes of low adherence. To collect the data on adherence, the health worker used a questionnaire. There are four questions on which the

patient responds with either “yes” or “no”. A patient who answers “no” to all the four questions is recorded as highly adherent, but the one whose answer is “yes” to one of the items is recorded as moderately adherent. When a patient responds “yes” to two (2) or more questions, he or she is rated as poorly adherent.

- **Visual analogue scale (VAS)**

The Visual Analogue Scale is used to assess adherence to the antiretroviral treatment for four weeks. With the visual analogue scale, the patient is asked to mark on a scale of measurement from 0 to 100%, the number or proportion of doses taken to assess his or her adherence to the medication over the past 3 or 4 weeks. Upon the request of the health worker, the patient ranks his or her adherence level on a graduate scale usually a ruler marker from 0 to 100%. The point on which the patient places his or her finger reflects how much of the drug he or she has taken over the previous 3 or 4 weeks and is recorded by the health worker as the patient’s own adherence score. Three levels of score results are defined: 95% or more, 75-94% and less than 75%. A patient who score 95% or more at the VAS is considered as highly adherent while a patient who score between 75-94% at the VAS then the overall adherence is moderate. Finally, when a patient scores less than 75% at the VAS then the overall level of adherence is “low” (Steel & Joshi, 2007).

- **Pill identification test (PIT)**

In this method, the health worker asks the patient during a face-to-face interview to visually identify the different categories of drugs that were dispensed to him or her. For each drug, the patient must give the following information: name of medication, number of pills per dose and the time the patient usually takes the medication. This identification test is performed for each single drug included in the patient’s antiretroviral drug regimen. After the patient has provided the responses, these are collected on a sheet of paper and then classified as true or false by the

health worker. A patient who knows the dose, time and the instructions to the ART regimen is classified as a highly adherent patient, a patient who knows the Dose and Time to take the medicine is classified as moderately adherent, and a patient who only knows the dose or was confused is classified as poorly adherent.

- **Simplified Medication Adherence Questionnaire (SMAQ)**

The SMAQ is a tool that enables the researcher to assess how adherent the patient was to medication during the last weeks or last month using quantitative and qualitative questions (Knobel et al, 2002). In the LCM study, the SMAQ was used to collect information on adherence over the previous 3 months. The SMAQ score ranged from 0 to 7 with 0 corresponding to 100% adherence (Appendix D). For this present study, a patient was considered as positive or non-adherent with the SMAQ adherence tool when a positive response was given to one of the questions, or the patient did not take any medicine over the past weekend, or had missed taking the medicine for more than two days over the past 3 months (Knobel et al, 2002).

- **Multi-method approach**

In our study, the multi-method approach tool included self-reports combined with VAS and the pill identification test (PIT). Overall adherence assessment with the multi-method approach was rated into 3 categories: high, moderate and low. A high level of adherence corresponds to patients who reported "No" to all questions with self-reporting, had a VAS score ≥ 95% and who knows the dose, time and instructions on how to take the drugs. A moderate adherence level was given to patients who responded "Yes" to one question with the self-report, had a VAS value between 75% and 95% and who additionally knows the dose and appropriate time at which he should take the drug. A patient who did not meet the above-mentioned criteria was classified as poorly adherent with the multi- method approach.

2.6.2.2 Baseline and follow-up variables

The baseline demographic and clinical variables were assessed 90 days prior to ART initiation and 7 days after. The clinical follow-up variables were also collected during the medical visits after ART initiation at 6 and 12 months. All the laboratory tests were performed at the same time during the medical visits. The baseline and follow-up variables were categorized into three groups which are described below:

- **Socio-demographics**

- Age in years at the time of ART initiation
- Sex categorized into male or female
- Level of education: illiterate or not yet schooled, primary, secondary, tertiary and beyond
- Current employment status of patients.
- Nationality: South African or foreign national

- **Biological**

In addition to the demographics and adherence measurements, 24 variables (clinical and laboratory markers) were selected as potential predictors of adherence to ART treatment and included in the univariate analysis. These variables were selected following a literature review and from previous studies that assessed factors associated with adherence to ARV treatment. Furthermore, some immunological and clinical markers have been previously identified as predictors of adherence to ARV treatment in diagnostic prediction models using retrospective datasets. The values of each clinical marker at 6 and 12 months were dichotomized using cut-off points suggested from previous studies and for easy use of the diagnostic prediction model in routine clinical practice (Lynen et al, 2009; Segeral et al, 2010; Chauhan et al,2011). The following clinical and biomarker variables were extracted for each patient during the medical

visit, first prior to the ART initiation therapy and then consecutively at 6 and 12 months on ART.

- **Baseline biological variables**

- WHO stage: either stage I/II or stage III/IV based on WHO classification of disease severity
- ART drugs regimen: Tenofovir (TDF)-based regimen or stavudine (d4T)-based regimen or other first-line ARV regimens.
- Tuberculosis: Tuberculosis at ART initiation.
- CD4 (cells/mm^3)
- Body Mass Index (kg/m^2)
- Haemoglobin (g/dL)
- Mean cell volume (MCV) (fL)
- Platelet count (10^2/mm^3)
- Total lymphocyte count (10^3/mm^3)
- Serum lactate (mmol/L)
- Albumin (g/l)
- Mean corpuscular haemoglobin (pg)
- Haematocrit (volume %)
- Red blood cells (million cells/µl)
- Systolic and diastolic blood pressure (mmHg)

- **Biological variable changes over the previous 6 months** (Table 1)

- Failing to increase CD4 count by ≥ 50 cells/mm^3
- BMI < 18.5 kg/m^2
- BMI drop over the previous 6 months of > 2.5%
- Haemoglobin drop over the previous 6 months of > 1g/dl
- Change over the previous 6 months in MCV < 14.5 fL
- Serum lactate below 2 mmol/L
- Serum albumin decreased or unchanged over the previous 6 months

- Platelet count < 150/mm^3 after 6 months
- Total lymphocyte count < 2,000 cells/mm^3
- Mean corpuscular haemoglobin < 2.7 pg
- Number of missed ARVs visits ≥ 7 days

Table 2 Summary of different cut-off values used by type of laboratory markers.

Biomarkers	**Cut-off value**	**References**
CD4 count	Failing to increase by ≥ 50 cells/mm^3 at 6 months; Failure to increase by ≥ 100 cells/mm^3 at 12 months.	Van Griensven et al, 2014; Evans et al, 2014; Robbins et al, 2010
Body Mass Index (BMI)	BMI drop over the previous 6 months of > 2.5%	Messou et al, 2008
Body Mass Index (BMI)	BMI < 18.5 kg/m^2	Evans et al, 2014
Criteria for anaemia	Women: Haemoglobin < 7.4 mmol/L	WHO, 2001
	Men: Haemoglobin < 8 mmol/L	WHO, 2001
Haemoglobin (Hb)	Haemoglobin drop over the previous 6 months > 1g/dl	Van Griensven et al, 2014
Mean corpuscular volume (MCV)	Change over the previous 6 months in MCV < 14.5 fL	Romanelli et al, 2002; Steele et al, 2002 ; Lynen et al, 1999; Colebunders et al, 2006
Serum Lactate	Serum lactate below 2 mmol/L after 6 months	Desai et al, 2003
Albumin	Albumin decreased or unchanged over the previous 6 months	Chauhan et al, 2011
Platelet count	Platelet count < 150/mm^3 after 6 months	De Santis et al, 2011; Zetterberg et al,2013
Total lymphocyte count	Total lymphocyte count < 2,000 cells/mm^3	Lau et al, 2003; Schreibman and Friedland 2004
Mean corpuscular haemoglobin (MCH)	Mean corpuscular haemoglobin < 2.7 pg after 6 months	Evans et al, 2014

2.6.2.3 Study outcomes

The outcome of interest was poor adherence to ART assessed through the failure to supress viral load at periods since ART initiation. This outcome was defined by a single elevated plasma viral load equal to or above 400 copies/ml (HIV-1 RNA copies ≥400 copies/ml) at 6 or 12 months post ART initiation. Viral load is considered as the standard reference method to classify HIV-positive patients on ART as being adherent or non-adherent (Evans et al, 2014). The viral load was assessed at 6 and 12 months, however, during the primary data collection, data on viral load measurement were not available for all patients at these times. The plasma viral load was not available in the LCM database, therefore we used the electronic patient medical record for viral load measures. Viral load between 4-9 months and between 10-14 months were respectively considered as plasma viral load at 6 and 12 months respectively (see data management section). Furthermore, the viral load was also considered as the gold standard to assess the diagnostic accuracy of three measures of adherence based on questionnaires (VAS, SMAQ, Multiple approach method) at 6 and 12 months after ART initiation.

2.6.3 Data management

Data for each patient were entered into the LCM database and stored in an Excel spreadsheet. These data included: age, gender, ARV regimen at ART initiation, adherence to ARV treatment at 6 months and 12 months, body mass index, laboratory results (mean cell volume, haemoglobin, serum lactate, platelet count, mean corpuscular haemoglobin, and the viral load) at 6 and at 12 months. For the secondary analysis, the database was transferred to STATA and transformed from a long to a wide format STATA dataset. We used clinical file number (TE number) to link the LCM study datasets to the Themba Lethu Clinic electronic medical record system, TherapyEdge-HIV™ (TE) where the patient's records are kept and to the National Health Laboratory Services (NHLS) laboratory data (e.g. CD4 counts and viral load at 6 and

12 months from TE) to find, missing, incomplete or erroneous data. Some clinical information such as missing ARV visits and WHO clinical stages (CDC, 2005) were stored in a SAS format Therefore, we also used the SAS system for Windows, version 9.2 (SAS Institute, Cary, North Carolina, USA). We used the software Stat/Transfer version 13 to convert files from a SAS data file to a STATA data file (Stata Statistical Software: Release 13. College Station, TX: STATACorp LP). Then, we used the unique clinical ID number to merge these pieces of information with the patients in the LCM study database. This procedure allowed us to form a single dataset including the baseline characteristics and the follow-up variables at 6 and 12 months in a wide format. All the duplicate observations were dropped and individuals who did not meet the inclusion criteria were identified and numbered at baseline, 6 and 12 months of follow up.

For the analysis, some continuous variables were re-coded into binary or categorical variables. Age was categorized as binary variable code 0/1 using the threshold of 35 years old (>35=1; ≤ 35=0). Haemoglobin levels vary with age, gender at different stages of pregnancy, with altitude, smoking and, in certain cases genetic factors (Hurtado & Merino, 1945; WHO, 2001). We considered haemoglobin levels below which anaemia is present as 7.4 mmol/L and 8.2 mmol/L haemoglobin for non-pregnant women and adult men respectively (WHO, 2001).

To assess adherence to the ART treatment, the variable viral load was re-coded into a binary variable (1-Yes/0-No), patients with a viral load either equal to or above 400 copies/ml were considered as non-adherent (≥ 400 copies/ml and < 400 copies/ml). The CD4 count was re-coded to a four-level categorical variable with the following categories: 0: < 50; 1: 51-100; 2:101-200; 3: 201-350, 4: > 350 cells/mm^3. The ART regimen was recoded to three combination regimens that were more meaningful clinically; individuals were classified into one of the following categories: Tenofovir (TDF)-based regimen, stavudine (d4T)-based regimen and "other" for the regimen that contained neither TDF nor d4T. The TDF-based

regimen was an antiretroviral medicine containing Tenofovir (TDF) with a nucleoside reverse transcriptase inhibitor (NRTI) and a non-nucleoside reverse transcriptase inhibitor (NNRTI), either efavirenz (EFV) or Nevirapine (NVP). The d4T-based regimen included stavudine (d4T) with another NRTI and NNRTI, either EFV or NVP. Body mass index (BMI) was re-coded into two categories: (Yes/No) using a threshold value of 18.5 kg/m^2 (Yes < 18.5; No ≥ 18.5). Nationality was categorized into two groups: either south-African or national foreign. To make the use of the diagnostic model easier, all the immunological and laboratory markers over the previous 6 months were transformed from continued to binary variables (Yes/No). We used the cut-off values known from previous studies diagnostic prediction models found in the literature (Chauhan et al, 2011; Evans et al, 2012; Lau et al, 2003; Lynen et al,2009; Schreibman & Friedland, 2004; Messou et al, 2008). For some laboratory marker variables, we determined the percentage of change (PC) after 6 months as the amount of change during the past 6 months relative to the initial value of that variable at the start of the period.

$$\mathrm{PC} = \frac{(6\text{ month value} - \text{baseline value})}{\text{baseline value}} * 100)$$

2.7 Statistical methods

2.7.1 Exploratory Analysis

We describe the distribution of continuous variables using numerical and graphical methods such as histogram, normal quantile-quantile (or Q-Q) plot. For each continuous variable, the graphics were depicted (histograms and normal Q-Q plot) to compare the sharpness of the distribution to a normal distribution using the functionalities of STATA graphics. Secondly, when there was evidence of skewness of the distribution with the graphical representations, testing for departures from normality was done. The Shapiro-Wilk test for normality was used to assess the evidence of deviation from normal distribution. A p value < 0.05 means the

hypothesis that the variable is normally distributed can be rejected. For variables that were skewed, we used a log-transformation (log_{10}) to conduct the analysis and later the results were expressed on the original scale.

2.7.2 Descriptive Analysis

During the primary data collection, some of the predictors and outcomes were not measured in all patients. Therefore, prior to the descriptive and inferential analysis, we reported the proportion of missing data for the outcome, demographic and clinical predictors at 6 and at 12 months. We used an approach of complete case analysis to handle the missing data. We reported the number of patients available at baseline, at 6 and at 12 months, and if an outcome was missing, the patient was excluded from the analysis. The number included at baseline of participation at baseline, 6 and 12 months was based on the inclusion and exclusion criteria at each stage (Figure 2). Continuous variables were summarized by the mean and the standard deviation, or median with interquartile range. Variables that were normally distributed were summarized with the mean and standard deviation and variables while the non-normal distribution or skewed were summarized with the median and interquartile ranges (IQR). Categorical variables were summarized by giving their frequencies.

2.7.3 Inferential Analysis

To compare continuous variables, we used an unpaired t-test if the variable was normally distributed. When continuous variables were compared from baseline to the previous 6 months, a paired t-test was done. In the case of skewed distribution, we used a non-parametric variant of the t-test such as the Mann-Whitney U-test. To compare categorical variables, we used cross-tables with corresponding chi-square or Fisher exact test.

For self-reported adherence such as the visual analogue scale, the self-report, the SMAQ, and the multiple-approach method at 6 and 12 months, we defined and calculated the sensitivities, specificities, positive predictive values (PPV) and negative predictive values (NPV) comparing their performance with the gold standard defined by a viral load that is detectable (≥ 400 copies/ml). Sensitivity (Se) was defined as the number of true positives over the number of actual positives with viral load. Specificity (Sp) was defined as the number of true negatives (negative with the self-reported adherence methods) over the number of negative with the viral load. Positive predictive value (PPV) was defined as the proportion of patients with a detectable viral load (VL ≥ 400 copies/ml) in those with a positive test result with the self-reported adherence assessment tools. Negative predictive value (NPV) was defined as the absence of a detectable viral load (VL ≥ 400 copies/ml) in those with negative test results using self-reported adherence assessment tools. All statistical tests were two sided and a p value of less than 0.05 was regarded as statistically significant.

2.7.3.1 Modified Poisson regression model

For a follow up study or in a study with common outcomes, the relative risk represents the estimated measure of risk instead of the odds ratio (McNutt, 2003). We planned to use a log binomial regression model to estimate an adjusted relative risk at 6 and 12 months. However, this method failed to converge in STATA. Instead, we used a modified Poisson generalized linear model (GLM) with a robust or "sandwich" variance-estimate that adjusts for clustering between individuals in the dataset model at 6 and 12 months of follow up (Hardin et al, 2012; Yelland et al, 2011). The incidence rate ratio obtained from the modified Poisson generalized linear model can be used to estimate the relative risk in a cohort study (McNutt,2003 ;Newton et al, 2010). This method is an extension of the Poisson generalized linear model available in STATA (Newton et al, 2010). We applied a method of variable selection called purposeful selection of variables (Hosmer et al, 2013). With this method, the selection starts with the

univariate analysis of each independent variable. We used a cut-off value of 0.25 instead of 0.05. Any variable with a value equal to or less than 0.25 in the univariate Poisson regression model was retained for the multivariate model. Thus, the multivariate model included all the significant variables at univariate analysis. The importance of each predictor was assessed using the statistical significance of the regression coefficients or the log likelihood ratio test. Any variable that did not have a significant regression coefficient was removed and a smaller model was set up.

To assess for confounding during the process of multivariable selection, we compared the estimated coefficient in the smaller model with the previous values in the larger model for each variable. Variables, when excluded, changed the coefficient of remaining variables of $\Delta\beta >$ 20% were considered as potential confounders and added back in the model. The variables that were not significant at the univariate analysis were added back to the model and their significance assessed in the presence of other significant variables. Variables that remained significant in the model and that did not add significant contributions were finally removed. With the main effect model, we assessed for interaction among the variables in the model including any possible interaction term to the main effect model. We assessed the statistical significance of each interaction term using a likelihood ratio test and interaction terms that were significant were added back to the model (p value < 0.05). Finally, we added demographic characteristics such as age, sex and predictors well known from previous research but not significant in our model. Subsequently, the goodness of fit of our final model was compared using the values of the Akaike Information Criterion (AIC). The model with a smaller AIC and smaller deviance was considered as a better fitting model. Finally, to test whether our Poisson generalised linear model was correctly specified, we used the link test in STATA post-estimation command. The link test is based on the idea that if a regression is properly specified,

one should be able to find no additional independent variables that are significant except by chance (STATA, 2011).

2.7.4 Elaboration of the diagnostic prediction model at 6 and 12 months

We used the result of the multivariate regression model to develop a new diagnostic prediction model. The appropriate design for a diagnostic prediction model is a cross sectional study (Collins et al, 2015), so we developed two models to estimate the probability that the non-adherence to ART therapy is present or absent at 6 and 12 months. We applied a scoring system based on the Spiegelhalter and Knill-Jones approach currently used in many diagnostic prediction studies. With this method, each adjusted regression coefficient in the final model is rounded to the nearest integer and the result is multiplied by a factor of 10 (Seymour et al, 1990 ; Evans et al, 2013). Summing the risk score gives for each patient its total predicted probability of adherence to ART at 6 and 12 months. We observed the diagnostic accuracy of each scoring system and of the self-reported adherence methods at 6 and12 months by computing the area under the curves (AUC) value of the Receiver Operating Characteristics (ROC) curve (Hanley & McNeil, 1982). Based on the ROC curves, we defined the appropriate cut-off value corresponding to the point with the highest validity. Subsequently, we calculated sensitivity (Se), specificity (Sp), positive predictive values (PPV) and negative predictive values (NPV) of these cut-offs points compared to gold standard of detectable plasma viral load.

2.7.5 Sensitivity Analysis

As with routine clinical data, there was missing data in the LCM database. This may result in the estimation and affect the accuracy of the diagnostic model at 6 and 12 months (Donders et al, 2006). Therefore, we implemented a multiple imputation technique to fill in the missing

values in the predictors and the outcome. We assumed that data are missing at random (MAR) with a pattern that is closed to monotone. In a MAR situation, the probability of a missing value on a predictor is independent of the value of that predictor but depends on the observed values of other variables (Collins et al,2015).Therefore, each of the conditional distribution of the missing values can be estimated from the observed data or other variables. Following our assumption of MAR, we used the approach of the Multiple Imputation by Chained Equations (MICE) (Royston & White, 2011) and applied this technique with the ICE program in R Software (R Foundation for Statistical Computing, 2014).

In multiple imputation, the inclusion of many predictors increases the plausibility and reduces the bias in the imputation model (Donders et al, 006; Steyerberg & Van Veen, 2007; Royston & White, 2011), furthermore, the imputation model should be as large as the model intended to use for statistical modelling (Van der Heijden et al, 2006). Therefore, for a reliable estimation, we included all the variables that were in the univariate analysis and the outcome variables were always included as predictors in the imputation process. To deal with non-normality distribution, continuous variables with missing values and with non-normal distribution were transformed to approximate normality by means of logarithmic transformation in the imputation model. Then, to get the imputed values, each variable was back-transformed to its original scale before fitting the multivariate model for analysis and comparison. To reduce the sampling variability, a total of 10 completed datasets were created with the use of the seed option to ensure that the imputed values are reproducible. We used Rubin's rule to produce an overall estimation of each regression coefficient and model performance measures with the imputed dataset. Finally, we conducted a sensitivity analysis to compare the robustness of the estimations between the case complete analyses where subjects with missing values are excluded from the analysis with the cases where missing data

were filled. Specifically, we assessed whether the complete case analysis (CCA) with the multiple regressions from the imputed dataset were different and whether the multiple imputations improved the performance of the diagnostic prediction model or its validity at 6 and 12 months

2.8 Ethics clearance

The primary study was approved by the Human Research Ethics Committee of the University of the Witwatersrand and informed consent for participation in the primary study was obtained from all participants. The original ethical approval was obtained in June 2010 (Clearance Certificate M10418) and re-approved in 2014. For the secondary data analysis, a protocol was submitted to the Wits Human Research Ethics Committee. This study protocol was approved: clearance Certificate M140918 (Appendix B). There was no personal identification in the dataset that was provided, and the patients are only identified using the unique ID number included in the LCM database and linked to the TherapyEdge-HIV™. For this secondary data analysis, an approval letter from the principal investigator, granting access to the data, was obtained (Appendix C).

CHAPTER THREE - RESULTS

OBJECTIVE 1

To compare the demographic and clinical characteristics of patients on first line antiretroviral treatment with a detectable viral load (VL ≥ 400 copies/ml) with those with a viral load < 400 copies/ml at 6 and 12 months after ART initiation

3.1 Organization of the study cohort and total number of participants

Between 2010 and 2014, of a total of 357 HIV-positive patients that initiated ART for the LCM cohort study, 4 (1.1%) were not eligible and a total of 353 met the inclusion criteria. Of the 4 patients who were not eligible, there were 2 pregnant women and two others were not on first-line regimen. This left 353 eligible patients at baseline from Themba Lethu Clinic (see flowchart (Figure 2). Out of the 353 eligible patients who initiated antiretroviral treatment, 57 (16.1%) individuals were excluded from the analysis at 6 months: 25 were lost to follow up after ART initiation; 17 were transferred out, 12 died and 3 were pregnant before the 6-month visit. Therefore, among 353 patients at baseline data collection, 296 were eligible for the analysis at 6 months and of them 239 (80.7%) had their plasma viral load assessed. Out of 296 patients who reached the 6-month follow-up, 6 (2.0%) patients were excluded: one patient was lost to follow-up and 5 were pregnant women before 12 months. This left 290 participants eligible for the analysis at 12 months, of these, 119 (41.0 %) had a plasma viral load assessed at the end of the study period

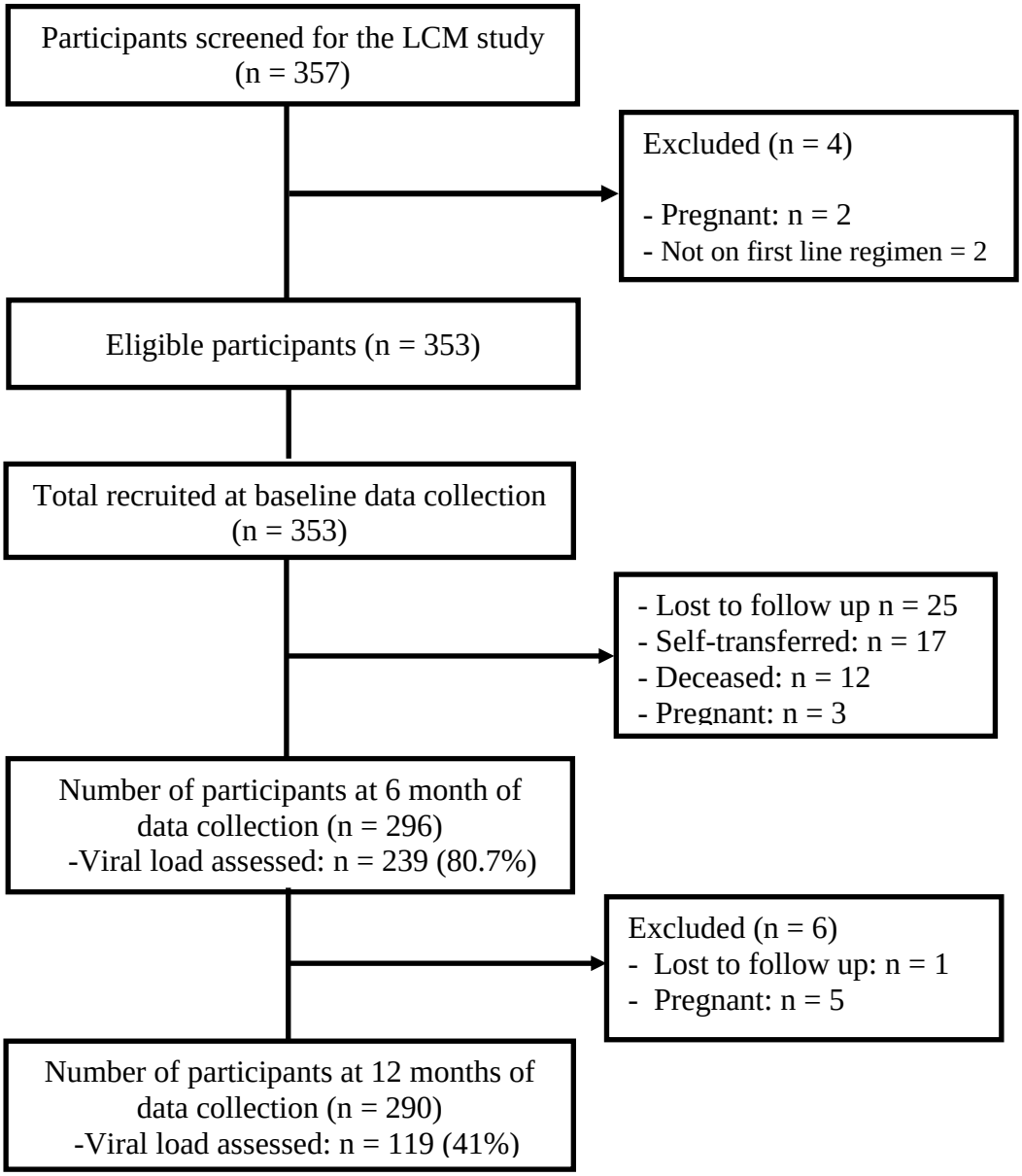

Figure 2 Flowchart of the participants in LCM study.

The flowchart indicates how the final eligible study cohort was obtained from the participants recruited within the LCM study, the total number of patients at 6 and 12 months and the proportion with a viral load (outcome) assessed during the follow-up at 6 and at 12 months.

3.2 Baseline demographic characteristics

Of the 353 patients in the baseline study population, more than half were males with the ratio of male to female being 178 males to 100 female patients and a mean age at ART initiation of 37.6 years (standard deviation = 8). Education level ranged from beyond secondary school (15.0%) to individuals with no education (3.7%). Of the 353 patients at baseline, 85.3% were

South African while the rest where from neighboring countries. Among the patients, only small proportions (4.5%) smoked in the past or were still smoking and 7.0% reported alcohol use. There were minimal missing values at baseline. The maximum percentage was with the variable education with a proportion of 3.1% out of 353 observations (Table 3).

3.3 Baseline clinical characteristics at ART initiation

Of the 353 patients included at baseline, 299 were either WHO stage I or WHO stage II and the majority (87.5%) of patients were initiated on a Tenofovir-based regimen. The median baseline CD4+ count was 196 cells/mm^3 (IQR: 98 - 268.5). Thirty (8.5%) individuals had a CD4+ count above 350 cells/mm^3 and 179 (50.7%) were below 200 cells/mm^3, meaning that half of the patients were under the threshold value below which treatment should not be delayed (DOH, 2013). At baseline, the median albumin level was 40 g/dl (IQR: 28 - 51). Body Mass Index (BMI) values ranged from 13.6 kg/m^2 to 59.4 kg/m^2 and 22 (6.5%) patients were underweighted with a BMI below 18.5 kg/m^2. At enrollment, fewer than five percent had anaemia defined as haemoglobin level < 7.4 mmol/L or < 8 mmol/L respectively for non-pregnant women and adult men. Slightly more than 10% of patients had tuberculosis prior to ART initiation (Table 4).

Table 3 Baseline demographic characteristics of 353 ART- naive patients.

Characteristics	Number (N= 353)	Percentage %
Age at initiation (mean ± standard deviation)	353	37.6 (± 8)
Data missing	00	00%
Age category at initiation		
35 years old	146	41.4%
< 35 years old	207	58.6%
Data missing	00	00.0%
Sex		
Male	226	64.0%
Female	127	36.0%
Data missing	00	00.0%
Employment status at initiation		
Yes	206	58.4%
No	147	41.6%
Data missing	00	00.0%
Level of education		
Beyond secondary school	15	15.0%
Secondary school	257	72.8%
Primary school	25	7.1%
Illiterate/Not yet schooled	13	3.7%
Unknown	32	9.0%
Data missing	11	3.2%
Nationality		
South African	301	85.3%
Non South African	51	14.5%
Data missing	1	0.2%
Smoking*		
Yes	25	7.1%
No	322	91.2%
Data missing	6	1.7%
Alcohol use*		
Yes	16	4.5%
No	331	93.7%
Data missing	6	1.7%

†All data in percentages, or as indicated: mean ± Standard deviation or median with interquartile range (IQR)
*Still smoking or previously smoking *Still alcohol user or previously alcohol user.

Table 4 Baseline Clinical characteristics at ART initiation.

Baseline characteristics	**Total (N)**	**Median (IQR) or proportion%**
CD4 cell count (cells/mm³): median **(IQR)**	352	196 (98 - 268.5)
Data missing	1	0.3%
CD4 cell count (cells/mm³)		
<50	46	13.0%
50- 100	44	12.5%
>101-200	89	25.3%
201-350	143	40.6%
>350	30	8.5%
Data missing	1	0.3%
Body mass index (kg/m²): median (IQR)	339	23.5 (16.3 - 43.8)
Data missing %	14	4.0%
Body mass index (kg/m²) < 18.5		
Yes	22	6.5%
No	317	93.5%
Hemoglobin (g/dl): median (IQR)	352	12.3 (11 - 13.5)
Data missing	1	0.3%
Hemoglobin (mmol/L) of < 8 or 7.4		
Yes	9	2.6%
No	343	97. 4%
Mean corpuscular volume (100 fL): median (IQR)	352	89.1 (84.8 - 92.3)
Data missing	1	0.3%
Mean corpuscular volume of <100 fL		
Yes	348	98.9%
No	4	1.1%
Lactate (mmol/L): median (IQR)	347	2 (1.8 - 2.4)
Data missing	6	1.7%
Mean corpuscular hemoglobin (pg.): median (IQR)	352	29.9 (18.2 - 31.1)
Data missing	1	0.3%
Albumin (g/dl): median (IQR)	352	40 (28 - 51)
Data missing	1	0.3%
Platelet count (10^2cells/mm³): median (IQR)	344	225 (181 - 277.5)
Data missing	9	2.5%
Platelet count < 150 (cells/mm³)		
Yes	30	8.7%
No	314	91.3%
Total lymphocyte count (10^3/mm³): median (IQR)	352	99.7%
Data missing	1	0.3%

Total lymphocyte count ⩽ 2000 cells/mm³		
Yes	70	80.1%
No	281	19.9%
Red bloods cells (10^6 cells/µl): median (IQR)	352	4.1(3.76 - 4 .49)
Data missing	1	0.3%
White bloods cells (10^3 cells/µl): median (IQR)	352	4.1(1.8 - 12.6)
Data missing	1	0.3%
Hematocrit:(10^3 cells/µl): median (IQR)	351	368 (217- 484)
Data missing	2	0.5%
Low hematocrit (volume%) ≤ 50%		
No	312	88.4%
Yes	39	11.6%
Systolic blood pressure (mmHg): median (IQR)	351	76 (70 - 83)
Data missing	2	0.5%
Diastolic blood pressure: (mmHg) median (IQR)	351	117 (109 - 130)
Data missing	2	0.5%
WHO stage at ART initiation (%)		
I or II	299	84.7%
III or IV	48	13.6%
Data missing	6	1.7%
Tuberculosis at ART initiation (%)		
Yes	36	10.2%
No	313	88.7%
Data missing	4	1.2%
Antiretroviral treatment at ARV (%) initiation		
TDF-3TC/FTC-NVP/EFV	309	87.5%
d4T -3TC/FTC-NVP/EFV	39	11.1%
Other first-line regimen	4	1.1%
Data missing	1	0.3%

†All data in percentages, or as indicated: 1 mean ± standard deviation or median with interquartile range (IQR)
TDF : Tenofovir Diproxil Fumarate ; d4T : Lamuvudine. 3TC : Lamivudine ; FTC : Emtricabine ; NVP : Nevirapine ; EFV : Efavirenz. Another Dirst-line regimen. ZDF-EFV-3TC and TDF-3TC-AZT. IQR: Interquartile range. WHO: World Health Organization.

3.4 Comparison of demographic and clinical characteristics of 296 HIV-infected patients with detectable and undetectable viral loads at 6 months

Table 5 shows a distribution of demographic and clinical characteristics by plasma viral loads at 6 months of ART therapy. Out of the 296 patients that reached the 6-month follow-up, 239 (80.7%) had their plasma viral load assessed. Of those 239 patients, 72 (30.1%) did not experience viral load suppression.

- **Comparison of demographic characteristics at 6 months**

Of the 72 patients with detectable plasma viral load, nearly 42.0% were male, 62.5% of them were aged above 35 years old and more than half of them attained a secondary education. Patients with undetectable plasma viral load at 6 months were more likely to be females (68.0%) or educated and 58% of them were aged above 35 years. The majority of the 72 patients with detectable plasma viral load at 6 months (61.0%), were unemployed. The proportion of patients that did not suppress viral load is slightly higher among smokers compared to non-smokers, being 37.5% and 29.9% respectively.

- **Comparison of clinical characteristics at 6 months**

Table 5 shows that baseline clinical characteristics were almost similar between those who experienced viral load suppression and those who did not. Among the two (2) groups, most of patients were initiated on a TDF-based regimen. Additionally, the clinical characteristics were almost well balanced although HIV-positive patients with detectable viral load appeared more suppressed.

Table 5 Comparison of demographic and Clinical characteristics of 296 HIV-infected patients with detectable viral load at 6 months.

	VL at 6 months		No VL at 6 months	
Variables	**VL ≥ 400 (n = 72)**	**VL < 400 (n = 167)**	**(n = 57)**	**p value**
Age				
> 35 years old	45 (62.5%)	98 (58.7%)	35 (39.6%)	0.381
≤ 35 years old	27 (37.5%)	69 (41.3%)	22 (61.4%)	
Sex				
Male	30 (41.7%)	53 (32.0%)	17 (29.8%)	0.917
Female	42 (58.3%)	114 (68.0%)	40 (70.2%)	
Education				
Beyond secondary school	7 (8.2%)	5 (2.4%)	5 (8.7%)	
Secondary school	47 (67.1%)	120 (73.0%)	41 (71.9%)	0.165
Primary school	7 (9.6%)	13 (8.0%)	2 (3.5%)	
Illiterate/Not yet schooled	4 (6.8%)	6 (4.3%)	6 (10.5%)	
Unknown	6 (8.2%)	19 (12.3%)	3 (5.2%)	
Unemployment				
Yes	28 (39.5%)	66 (39.5%)	24 (42.0%)	0.890
No	44 (61.1%)	101 (60.5%)	33 (58.0%)	
Nationality				
South African	64 (90.1%)	139 (83.2%)	50 (87.1%)	0.434
Non South African	7 (9.9%)	28 (16.7%)	7 (12.9%)	
ART regimen at initiation				
TDF-based regimen	61 (84.7%)	145 (86.8%)	50 (87.7%)	
d4T-based regimen	10 (13.9%)	19 (11.4%)	5 (8.7%)	0.361
Other first line regimen	1 (1.4%)	3 (1.8%)	2 (3.6%)	
WHO stage				
I/II	62 (86.1%)	144 (87.0%)	54 (94.6%)	0.601
III/IV	10 (13.9%)	23 (13.0%)	3 (5.4%)	
CD4 count (cells/mm³)				
< 50	11 (15.2%)	17 (10.1%)	9 (16.3%)	
51-100	12 (16.6%)	21 (12.5%)	5 (7.2%)	0.583
101-200	17 (23.6%)	41 (24.5%)	14 (25.4%)	
201-350	26 (36.1%)	73 (43.7%)	22 (40.0%)	
> 350	6 (8.3%)	15 (9.9%)	07 (10.9%)	

† VL: viral load. † TDF-based regimen: TDF-3TC/FTC-NVP/EFV. † d4T- based regimen: d4T -3TC/FTC-NVP/EFV
† Other first line regimen: other first line regimen with neither TDF nor EFV. WHO: World Health Organization.

3.5 Comparison of demographic and clinical characteristics of 290 HIV-positive patients with detectable and undetectable viral load at 12 months

Table 6 shows the distribution of the baseline demographic and clinical characteristics by plasma viral load status at 12 months. Of the total of 290 patients that completed the 12-month visit, 119 (41.0%) had their plasma viral load assessed and of these, 28.5% did not achieve an undetectable viral load (HIV/RNA ≥ 400 copies/ml) at 12 months.

- **Comparison of demographic characteristics at 12 months**

Table 6 shows that the distribution of the baseline demographics and clinical characteristics were similar between the two groups ($p \geq 0.05$). Among patients with a detectable plasma viral load at 12 months nearly 62.0% were females and there was a similar pattern among those with an undetectable plasma viral load at 12 months. However, the percentage of patients that reached secondary school is slightly higher in the group that suppressed the viral load at 12 months compared to those with detectable viral load.

- **Comparison of clinical characteristics at 12 months**

Similarly, the distribution of clinical characteristics across the two groups is nearly identical. Of the total of 119 individuals with a detectable viral load at 12 months, most (85.0%) of them initiated on a TDF-based regimen. The proportion of patients who initiated a d4T-based regimen is the same (11.7%) in both groups. The distribution of CD4+ count was nearly identical across the two groups and among patients with a detectable viral load at 12 months 87.0% were WHO stage I or II while in the other group this proportion was 82.3

Table 6 Comparison of demographic and clinical characteristics of 290 HIV-infected patients with detectable and undetectable viral load at 12 months.

	VL at 12 months		No VL at 12 months	
Variables	**VL ≥ 400 (n = 34)**	**VL < 400 (n = 85)**	**(n = 171)**	**p value**
Age				
> 35 years old	18 (52.9%)	51 (60.0%)	106 (38.1%)	0.490
≤ 35 years old	16 (47.1%)	34 (40.0%)	65 (61.9%)	
Sex				
Male	13 (38.2%)	28 (32.9%)	60 (35.1%)	0.911
Female	21 (61.8%)	57 (67.1%)	111 (64.9%)	
Education				
Beyond secondary school	5 (14.7%)	6 (7.1%)	3 (1.8%)	
Secondary school	19 (55.8%)	63 (75.0%)	123 (75.4%)	0.006
Primary school	5 (14.7%)	8 (9.5%)	9 (5.5%)	
Illiterate/Not yet schooled	2 (5.8%)	3 (3.6%)	6 (3.7%)	
Unknown	3 (8.8%)	4 (4.8%)	22 (13.5%)	
Unemployment				
Yes	10 (29.4%)	36 (42.4%)	142 (82.1%)	0.990
No	24 (70.6%)	49 (57.6%)	29 (19.9%)	
Nationality				
South African	28 (84.9%)	79 (92.9%)	142 (83.0%)	0.060
Non-South African	5 (15.1%)	6 (7.1%)	29 (19.9%)	
ART regimen at initiation				
TDF-based regimen	30 (88.3%)	7 (87.6%)	150 (87.7%)	
d4T-based regimen	4 (11.7%)	10 (11.7%)	18 (10.5%)	0.770
Other first line regimen	0 (0%)	1 (1.8%)	3 (1.7%)	
WHO stage				
I/II	28 (82.3%)	74 (87.0%)	161 (94.2%)	0.871
III/IV	6 (17.7%)	11 (13.0%)	8 (5.8%)	
CD4 count (cells/ mm^3)				

< 50	5 (14.7%)	12 (14.2%)	17 (10.3%)	
51-100	5 (14.7%)	8 (9.4%)	25 (14.6%)	0.211
101-200	6 (17.6%)	21 (24.7%)	44 (25.7%)	
201-350	13 (38.2%)	33 (38.2%)	73 (42.7%)	
> 350	5 (14.7%)	11 (12.9%)	11 (6.4%)	

† VL: viral load. † TDF-based regimen: TDF-3TC/FTC-NVP/EFV. † D4T-based regimen: d4T -3TC/FTC-NVP/EFV † Other first line regimen: other first line regimen with neither TDF nor EFV.WHO: World Health Organization

3.6 Description of the missing values in the LCM dataset

Table 7 summarizes the availability of each predictor and outcome at 6 and 12 months.

- **Missing values at 6 months**

At 6 months, missing values were present for all laboratory clinical markers as well as the self-reported adherence variables and the plasma viral load. In all the predictors and adherence questionnaire variables, nearly 35% of data were missing at 6 months. Regarding the outcome of viral load, 241 observations were recorded and 55 values (18.6%) were missing at 6 months.

- **Missing values at 12 months**

At 12 months of follow up, out of the 290 patients, 165 (57%) values were missing for the outcome viral load. For the clinical and laboratory markers, of a total of 290 observations, 47 (16.2%) values were missing for each 6-month follow-up variable in the dataset. Missing values were also present for the self-reported adherence variables (VAS, SMAQ, multi-method approach): out of the 290 patients at 12 months 47 missing values were present in each of the 5 adherence parameters (VAS, self-report, pill identification test, multi-method approach of adherence, SMAQ).

Table 7 Description of the missing values in the LCM dataset at 6 and at 12 months

	At 6 months		At 12 months	
Variables	**Observed**	**Missing (%)**	**Observed**	**Missing (%)**
Clinical and laboratory markers				
Body mass Index	190	106 (35%)	243	47 (16.2%)
Haemoglobin	195	101 (34%)	243	47 (16.2%)
CD4 count	195	101 (34%)	243	47 (16.2%)
Total lymphocyte count	195	101 (34%)	243	47 (16.2%)
Platelet count	192	104 (35%)	242	48 (16.5%)
Mean cell volume (MCV)	195	101 (34%)	243	47 (16.2%)
Albumin decreased or unchanged	192	102 (34%)	243	47 (16.2%)
MCH	194	101 (34%)	243	47 (16.2%)
Missing ARV visits	296	0 (0%)	290	0 (0%)
Self-reported adherence				
VAS	195	101 (34%)	243	47 (16.2%)
Self-report	194	102 (34%)	243	47 (16.2%)
PIT	190	106 (35%)	243	47 (16.2%)
Multi-method approach	195	101 (34.1%)	243	47 (16.2%)
SMAQ	195	101 (34.1%)	243	47 (16.2%)
Outcome				
Viral load	239	57 (19.3%)	119	171 (59%)

†VAS: Visual Analog Scale; †PIT: Pill identification test; †SMAQ: Simplified Medication Adherence Questionnaire. MCH: Mean Corpuscular Haemoglobin. LCM: Low Cost Monitoring

3.7 Complete case analysis

In this section, we present the results of the complete case analysis. If the outcome was missing, the patient was excluded from the analysis. In an inferential study, a complete case analysis has relatively low power. Therefore, we later used a multiple imputation technique to fill in missing values and perform a sensitivity analysis to compare our result with those obtained from a multiple imputed dataset.

At 6 months of ART, out of the 296 patients, 239 had their plasma viral load assessed. At 12 months of ART therapy, of 290 patients, 119 had plasma viral load assessed.

OBJECTIVE 2

To determine the association between (i) self-reported adherence measures, (ii) CD4 response, (iii) MCV response, (iv) missed appointment, (v) new condition symptom (and a detectable viral load (≥ 400 copies/ml) at 6 and 12 months after ART initiation.

3.7.1 Self-reported adherence at 6 and 12 months of ART therapy

Table 8 summarizes the results of different methods of adherence assessment. For each method, the percentage of adherent or non-adherent patients is determined based on the total number of responses recorded and missing values are not included.

- **Self-reported adherence at 6 months**

At 6 months, of a total of 195 patients that were assessed by the visual analogue scale test, most of them (86.7%) had a score value greater than or equal to 95.0 %, 11 (5.6 %) had a score value below 75% and 15 (7.7%) had a score value ranging from 75.0 % to 94.0 %. In the multi-method approach which combines visual analogue scale, self-reporting and pill identification test, of a total of 195 patients, 86.7% were classified as being highly adherent, 23 (11.8%) were moderately adherent and 3 (1.5%) patients had low adherence. However, when the Simplified Medication Adherence Questionnaire (SMAQ) was used, the proportion of patients classified as being highly adherent decreased to 78.5%.

- **Self-reported adherence at 12 months**

At 12 months, of a total of 243 patients, 67.0% showed high adherence with the VAS ≥ 95%, 62 (25.5%) were classified as being moderately adherent, and 23 (14.2%) were classified as poorly adherent. With the multi-method approach, the proportion of patients classified as highly adherent

decreased to nearly 60.0%. Like the 6-month results, at 12 months, when the SMAQ was used, the proportion of patients being classified as highly adherent decreased to 60.0%. Thus, based on the results of the self-reported adherence, most of the patients were highly adherent to their ARV drug regimen. However, care should be exercised in the interpretation of these results as there was a significant amount of missing data.

Table 8 Self-reported adherence at 6 and at 12 months on ART.

	At 6 months		At 12 months	
Self-reported adherence	**Total response (N=195)**	**Percentage (%)**	**Total response (N=243)**	**Percentage (%)**
VAS				
95% or more	169	86.7%	153	67.0%
75–94%	15	7.7%	57	23.0%
Less than 75%	11	5.6%	33	10.0%
Data missing	101	34.1%	47	16.0%
Self-report				
High adherence	190	98.9%	242	99.5%
Moderate adherence	2	1.0%	0	00.0%
Poorly adherent	2	1.0%	1	0.5%
Data missing	102	34.5%	47	16.0%
PIT				
Knows the name	193	98.9%	243	100%
Knows the number of pill per dose	188	98.9%	243	100%
Knows the time the medication is taken	188	98.9%	243	100%
Data missing	106	35.8%	47	16.0%
Multi-method Approach				
High adherence	169	86.7%	158	60.5%
Moderate adherence	23	11.8%	62	25.5%
Low adherence	3	1.5%	23	14.0%
Data missing	101	34.1%	47	16.0%
SMAQ				
Positive adherent	153	78.5%	154	63.0%
Non-adherent	42	21.5%	89	37.0%
Data missing	101	34.1%	47	16.0%

VAS: Visual Analogue Scale, †PIT: Pill identification test; †SMAQ: Simplified Medication Adherence Questionnaire

3.7.2 Comparison of the result of self-reported between patients between with detectable viral load and patients with undetectable viral load.

Table 9 shows the comparison of the results of the self-reported adherence between patients with and without undetectable viral load. To determine which measure of adherence is associated with a detectable viral load, the responses were transformed into binary variables. From the 2x2 table, the sensitivity, specificity, PPV and NPV of each self-reported adherence method was also estimated. Although, the results were not globally significant, a low scale on the VAS or any other self-reported measure was associated with having a detectable viral load.

3.7.3 Comparison of the result of self-reported adherence between patients with and with undetectable viral load at 6 months.

At 6 months, a total of 163 patients had a measure of self-reported adherence (VAS, SMAQ, multi-approach method). Patients with detectable viral load demonstrated lower adherence with the VAS tool. When the multi-method approach method which combines VAS, PIT and self-reporting was used, there was a larger proportion of non-adherence with those with detectable viral load than those with undetectable viral load. Similarly, when the SMAQ tool was used to assess adherence, there was a large difference in patients with detectable viral load compared to those with undetectable viral load.

3.7.4 Comparison of the result of self-reported adherence between patients with detectable and patients with undetectable viral load at 12 months.

At 12 months, a total of 106 patients had a measure of plasma viral load and were also assessed with the VAS. A total of 100 patients had a measure of plasma viral load and were also assessed with the SMAQ. The results indicate that when VAS was used, patients with undetectable viral load at 12 months were slightly less-adherent than the group with undetectable viral load. Regarding the results of the multi-method approach at 12 months, patients with detectable viral

loads demonstrated a greater increase in non-adherence to ART compared to those with undetectable viral loads. Using the SMAQ tool, although this was not also statistically significant, there was also a greater increase in non-adherence amongst those with detectable viral load compared to those without detectable viral load.

3.7.5 Crude and adjusted predictors of detectable viral load (n=163) at 6 months after ART initiation, using modified Poisson regression analysis.

Table 9 indicates the performance of the final regression model with the case complete analysis. For each predictor, it shows the adjusted and the unadjusted relative risks considering the effect of other predictors in the final model. The statistical significance is assessed with the P value and 95% CI.

At 6 months' follow up, 7 variables were associated in the final Poisson Regression Model after adjusting for gender and age although these variables were not significant. The variables that were significantly associated with a detectable viral load included missing at least two ARV visits by ≥ 7 days (aRR: 2.35 95% CI: 1.08- 5.11); platelet count < 150 cells/mm^3 (aRR; 2.73 95% CI: 1.04- 7.18) and VAS (aRR: 1.65 95% CI: 1.01- 2.71). The result shows that patients who missed two ARV visits within the first 6 months were two times more at risk for detectable viral load, holding all other variables constant. After adjustment for other variables effects, the final regression model showed that, in patients with a VAS score < 95%, the risk of detectable viral load was 65% higher than that of patients who scored 95% or more. The multivariate model showed no association between failing to increase CD4 count by ≥ 50 cells/mm^3 and having an absolute change in MCV < 14.5 fL at 6 months after ART initiation.

Table 9 Crude and adjusted predictors of detectable viral load at 6 months (n=163) after ART initiation using a modified Poisson regression analysis.

Viral load ≥ 400 copies/ml				
Baseline characteristics	**RR (95% CI)**	**aRR**	**p value**	**Score[a]**
Age				
≤ 35 years old	Reference			
> 35 years old	1.11 (0.74 -1.67)	1.07 (0.67 - 1.73)	0.751	+1
Gender				
Female	Reference			
Male	1.34 (0.91 - 1.97)	1.46 (0.93 - 2.29)	0.098	+1
Level of education				
Beyond secondary school	Reference			
Secondary school	2.60 (1.00 - 6.00)			
Primary school	1.26 (0.50 - 2.60)			
Illiterate/Not yet schooled	1.51 (0.60 - 3.00)			
Unknown	1.80 (0.70 - 4.00)			
Unemployment				
Yes	1.00 (0.70 - 1.40)			
No	Reference			
Nationality				
South African	1.50 (0.70 - 2.70)			
Non-South African	Reference			
Alcohol drinking				
Yes	1.20 (0.40 - 2.00)			
No	Reference			
Smoking				
Yes	1.25 (0.60 - 2.40)			
No	Reference			
ART regimen initiation				
TDF-based regimen	Reference			
d4T-based regimen	1.00 (0.10 - 4.20)			
Other first line regimen	1.01 (0.05 - 1.50)			
WHO stage at ART initiation				+1
I/II	Reference			
III/IV	1.00 (0.77 - 1.30)	1.10 (0.83 - 1.47)		
CD4 count at ART initiation (cells/mm3)				
<200	Reference			
200-350	0.78 (0.52 - 1.10)			
≥350	0.79 (0.26 - 0.44)			
BMI < 18.5 kg/m²				
Yes	1.08 (0.30 - 3.50)			
No	Reference			
BMI drop from baseline > 2.5 kg/m²				
Yes	0.96 (0.60 - 1.50)			
No	Reference			

Hemoglobin drop from baseline ≥ 1g/dL				
Yes	0.60 (0.16 - 2.00)			
No	Reference			
Failing to increase CD4 count by ≥ 50 cells/mm^3				
Yes	0.85 (0.40 - 1.60)	0.78 (0.40 - 1.60)	0.611	
No	Reference			
Total lymphocyte count < 2000 cells/mm^3				
Yes	0.62 (0.40 - 1.10)			
No	Reference			
Platelet count < 150 cells/mm^3				+3
Yes	2.24 (0.98 - 5.13)	2.73 (1.04 - 7.18)	0.041*	
No	Reference			
MCV change < 14.5 fL				+ 1
Yes	1.23 (0.59 - 2.58)	1.31 (0.60 -2.89)	0.500	
No	Reference			
Missing at least two ARV visits by ≥ 7 days				+ 2
Yes	1.68 (0.73 -3.85)	2.35 (1.08- 5.11)	0.030*	
No	Reference			
VAS score test < 95%				+2
Yes	1.70 (1.03 - 2.81)	1.65 (1.01-2.71)	0.044*	
No	Reference			
Multi-method approach				
Yes	1.50 (0.80 - 2.50)			
No	Reference			
SMAQ				
Yes	1.20 (0.70 - 2.20)			
No	Reference			
Serum lactate ≤ 2 mmol/L				
Yes	1.07 (0.70 - 1.50)			
No	Reference			

[a] The score calculated as the sum of the adjusted relative risks divided by the smallest regression coefficient and the result multiplied by 10 for each predictor rounded to the nearest integer.

RR: Relative risk. †aRR: adjusted relative risk.ARV: antiretroviral therapy. TDF: Tenofovir Fumarate. d4T: Stavudine. Other first line regimen: ZDV-EFV-3TC or TDF-3TC-AZT. †WHO: World Health Organization. BMI: body mass index. MCV: mean cell volume. MCH: mean corpuscular hemoglobin. ARV: antiretroviral. VAS: Visual analogue scale. SMAQ: Simplified Medication Adherence Questionnaire. WHO: World Health Organization. *Significant at the 0.05 level.

3.7.6 Crude and adjusted predictors of detectable viral load (*n*=142) at 12 months after ART initiation, using modified Poisson regression analysis

Table 10 indicates the performance of the final regression model with the case complete analysis. For each predictor, it shows the adjusted and unadjusted relative risks, considering the effect of other predictors. The statistical significance is assessed with the p value and 95% CI.

At 12 months of follow up, the finding shows a positive relationship between age group, unemployment, alcohol drinking on the one hand and adherence to ART regimen on the other as assessed by a HIV/RNA ≥ 400 copies/ml. Patients aged above 35 years are 0.86 times less likely to have a detectable viral load compared to patients under 35 years old. Also, those who are unemployed are 0.6 times less likely to have a detectable viral load compared to those who are employed. However, patients who used to drink alcohol are 1.8 times at risk of having a detectable viral load than patients who do not take alcohol.

Among the clinical markers, the results did not reveal any difference between the biomarker change over the previous 6 months and an adherence to ART therapy as assessed by the viral load at 12 months. Similarly, after adjustment for other variables, the results did not show any difference between patients classified as non-adherent by the traditional methods of adherence and the other groups. After 12 months, when the other variables are considered, having missed more than two ARV visits was not associated with a detectable viral load.

Table 9 Crude and adjusted predictors of detectable viral load (n=142) at 12 months after ART initiation.

Viral load ≥ 400 copies/ml`				
Baseline characteristics	**RR (95% CI)**	**aRR (95% CI)**	**p value***	**Score[a]**
Age				
≤ 35 years old	Reference			+1
> 35 years old	0.80 (0.50 - 1.50)	0.49 (0.20 - 0.90)	0.027*	
Sex				+1
Female	Reference			
Male	1.31 (0.70 - 2.20)	1.49 (0.70 - 3.0)	0.310	
Level of education				
Beyond secondary school	Reference			
Secondary school	1.20 (0.30 - 3.70)			
Primary school	0.60 (0.20 - 1.70)			
Illiterate/Not yet schooled	1.00 (0.30 - 3.10)			
Unknown	1.10 (0.30 - 4.30)			
Unemployment				
Yes	0.60 (0.30 - 1.20)	0.32 (0.10 - 10.0)	0.019*	+0.5
No	Reference			
Nationality				
South African	0.60 (0.30 - 1.30)	0.77 (0.30 - 1.70)	0.540	
Non-South African	Reference			
Alcohol drinking				
Yes	1.80 (0.70 - 4.20)	3.08 (1.00 - 9.30)	0.045*	+3
No	Reference			
Smoking				
Yes	1.50 (0.60 - 3.80)			
No	Reference			
ART regimen initiation				
TDF-based regimen	Reference			
d4T-based regimen	1.00 (0.40 - 2.40)			
Other first line regimen	1.01 (0.40 - 2.40)			
WHO stage at ART initiation				
I/II	Reference			
III/IV	1.3 (0.60 - 2.60)			
CD4 count at ART initiation, cells/mm^3				
<200	Reference			
200-350	0.80 (0.50 - 1.50)			
≥350	1.00 (0.40 - 2.30)			
BMI < 18.5 kg/m^2				
Yes	1.05 (0.30 - 0.40)			
No	Reference			

BMI drop from baseline > 2.5 kg/m²				
Yes	1.2 (0.60 - 2.20)			
No	Reference			
Hemoglobin drop from baseline ≥ 1g/dL				
Yes	1.50 (0.70 - 3.00)			
No	Reference			
Failing to increase CD4 count by ≥ 50 cells/mm³				
Yes	1.10 (0.60 - 2.0)		0.611	
No	Reference			
Total lymphocyte count < 2000 cells/mm³				
Yes	0.70 (0.30 - 1.40)			
No	Reference			
Platelet count < 150 cells/mm³				
Yes	1.45 (0.40 - 6.00)		0.041*	+1
No	Reference			
Absolute MCV change < 14.5 fL				
Yes	2.60 (0.70 - 9.00)	2.70 (0.80 - 9.10)	0.110	+2
No	Reference			
Albumin decreased or unchanged (g/dL)				
Yes	1.75 (0.87 - 3.00)			
No	Reference			
MCH < 2.7 pg. after 6 months				
Yes	0.60 (0.20 - 2.40)			
No	Reference			
Missing at least two ARV by ≥ 7 days				
Yes	1.20 (0.40 - 3.10)			
No	Reference			
Change to VAS score test < 95%				
Yes	1.04 (0.60 - 1.80)			
No	Reference			
Multi-method approach				
Yes	1.20 (0.07 - 2.00)			
No	Reference			
SMAQ				
Yes	1.20 (0.70 - 2.10)			
No	Reference			
Poor self-reported adherence at 6 months				
Yes	2.67 (1.20 - 7.00)	2.10 (0.70 - 5.90)	0.181	
No	Reference	Reference		

[as] the score calculated as the sum of the adjusted relative risks divided by the smallest regression coefficient and the result multiplied by 10 for each predictor rounded to the nearest integer.

RR: Relative risk. †aRR: adjusted relative risk. ART: antiretroviral therapy. TDF: Tenofovir Fumarate. d4T: Stavudine. Other first line regimen: ZDV-EFV-3TC or TDF-3TC-AZT. †WHO: World Health Organization. BMI: Body mass index. MCV: mean cell volume. MCH: mean corpuscular hemoglobin. ARV: antiretroviral. VAS: Visual analogue scale. SMAQ: Simplified Medication Adherence Questionnaire. WHO: World Health Organization. *Significant at the 0.05 level.

OBJECTIVE 3

To determine the diagnostic accuracy (sensitivity, specificity, PPV, NPV) of self-reported adherence (e.g. VAS, SMAQ or multi-method) and the composite marker including CD4 response, MCV response, missed appointment and new condition or symptom compared to viral load as gold standard.

3.7.7 Sensitivity, specificity, PPV and NPV of the self-reported measures of predicting adherence to ART compared to reference standards of viral load Table 10 shows the ability of each self-reported adherence measure to correctly classify patients into two groups (adherent and non-adherent) as compared to viral load and by using two parameters at 6 and 12 months: sensitivity and specificity.

- **Sensitivity, specificity, PPV and NPV of the self-reported measures of predicting adherence to ART compared to the reference standard of viral load at 6 months**

During the first 6 months on ART, of the 49 patients who had a detectable viral load, 12 were correctly classified as such by the visual analogue scale, giving a sensitivity of 24%, this proportion was 26.0% and 18.0% for the SMAQ, and the multi-method tool respectively. In contrast, 100 of 114 patients who did not have a detectable viral load were classified as negative by the VAS, giving a specificity of 87.0%. This proportion was almost the same with the multi-method approach (Se: 86.0%) but decreased to 79.0% for the SMAQ tool. Of the 46 HIV-infected patients classified as non-adherent by the VAS tool, 12 were confirmed by the viral load, giving a PPV of 46.0% while the NPV increased to 73.0%. The PPV was 37.0% and 35.0% for the multi-method approach and the SMAQ respectively while the NPV for both measures were similar at 71.0%.

- **Sensitivity, specificity, PPV and NPV of the self-reported measures of predicting adherence to ART compared to the reference standard of viral load at 12 months**

At 12 months, for each of the 3 self-reported measures, the sensitivity and the PPV decreased while the specificity and the NPV increased. The sensitivity of the VAS was 37.0% while its specificity was 61.0%. The PPV and NPV for the VAS were 27.0% and 71.0% respectively. When the SMAQ was used, the four parameters were like the values obtained with the VAS tool (Se: 37.0%, Sp: 61.0%, PPV: 27.0%, NPV: 71.0%). Similarly, when VAS was combined with PIT and self-reporting into the multi-method approach, sensitivity was almost the same at 36.0% but the specificity decreased to 62.0%.

Table 10 Comparison of the results of self-reported adherence between HIV-positive patients with detectable and undetectable viral load.

Self-reported adherence	VL≥ 400 (n=49)	VL < 400 (n=114)	Se % (95% CI)	Sp% (95% CI)	PPV % (95%CI)	NPV % (95%CI)
At 6 months						
Non-adherent	12 (24.5%)	14 (12.3%)	24 (17 - 31)	87 (82 - 92)	46 (38 - 53)	73 (66 - 98)
Adherent	37 (75.5%)	100 (87.7%)				
Multi-method						
Non-adherent	9 (18.3%)	15 (13.2%)	18 (12 - 24)	86 (81 - 92)	37 (30 - 44)	71 (64 - 78)
Adherent	40 (81.7%)	99 (86.8%)				
SMAQ						
Non-adherent	13 (26.0%)	24 (21.0%)	26 (19 - 33)	79 (72 - 85)	35 (27 - 42)	71 (64 - 78)
Adherent	36 (73.0%)	90 (79.0%)				
At 12 months						
Non-adherent	12 (34.3%)	24 (33.8%)	37 (28 - 47)	61 (56 - 75)	27 (25 - 43)	71 (60 - 80)
Adherent	23 (65.7%)	47 (66.2%)				
Multi-method						
Non-adherent	20 (62.5%)	20 (29.4%)	35 (28 - 47)	62 (61 - 79)	27 (28 - 47)	71 (61 - 80)
Adherent	12 (37.5%)	48 (70.6%)				
SMAQ						
Non-adherent	12 (37.5%)	19 (27.9%)	36 (28 - 47)	61 (63 - 80)	27 (29 - 48)	70 (62 - 80)
Adherent	20 (62.5%)	49 (72.1%)				

3.7.8 Comparison of changes in laboratory markers between HIV positive patients with and without detectable plasma viral load

Table 11 shows a comparison of laboratory markers over 6 months by detectable plasma viral load at 6 or at 12 months of ART therapy. Overall results at 6 and 12 months show that globally there was no statistical association between laboratory marker changes and the outcome. However, these results must be interpreted with caution as there was a high number of missing values in both the predictors and the outcome.

- **Comparison of changes in laboratory markers between patients with and without a detectable plasma viral load at 6 months**

At 6 months, participants showed differences in total lymphocytes counts, CD4+ response at 6 months and platelet count, however, these differences were not significant. Patients with undetectable viral load showed a higher proportion of individuals failing to achieve CD4 increase ≥ 50 cells/mm^3 by 6 months compared to those with a detectable viral load (20.0% vs.16.3% p = 0.741). Similarly, patients with detectable viral load showed lower proportion of individuals with a total lymphocyte count response < 2000 cells/mm^3 (26.5% vs. 40.4% p = 0.110) when compared to patients who did not suppress viral load during the first six months. Although not statistically significant with the complete dataset, there was a difference in 6-month platelet count in patients with and without detectable viral load (2.8% vs. 0.6%, p = 0.160).

Table 10 Changes in laboratory markers in patients and detectable viral load.

	HIV/RNA viral load (copies/ml) at 6 months			HIV/RNA viral load (copies/ml) at12 months		
Change over the previous 6 months	**VL ≥ 400 (n = 72)**	**VL < 400 (n = 167)**	**p value***	**VL ≥ 400 (n = 34)**	**VL < 400 (n = 85)**	**p value***
BMI < 18.5 kg/m²						
Yes	2 (4.16%)	4 (3.6%)	0.710	2 (6.5%)	4 (5.8%)	0.910
No	46 (95.8%)	106 (96.3%)		29 (93.5%)	64 (94.2%)	
BMI drop of > 2.5 kg/m²						
Yes	23 (50.0%)	57 (53.2%)	0.710	14 (58.3%)	23 (51.2%)	0.600
No	23 (50.0%)	50 (46.8%)		11 (41.7%)	23 (48.8%)	
Haemoglobin drop from baseline of ≥ 1 g/dl						
Yes	2 (4.1%)	9 (7.9%)	0.371	5 (16.0%)	5 (11.4%)	0.300
No	47 (95.9%)	105 (92.1%)		21 (84.0%)	42 (88.6%)	
CD4 count < 200 cells/mm³						
Yes	15 (30.0%)	23 (21.0%)	0.290	8 (21.8%)	8 (11.7%)	0.110
No	35 (70.0%)	91 (79.0%)		26 (78.2%)	63 (88.3%)	
Failing to achieve CD4 increase ≥ 50 cells/mm³						
Yes	8 (16.3%)	21 (20.0%)	0.741	13 (52.0%)	21 (47.8%)	0.820
No	41 (83.7%)	93 (80.0%)		12 (48.0%)	23 (52.2%)	
Total lymphocyte count < 2000 cells/mm³						
Yes	13 (26.5%)	45 (40.4%)	0.110	22 (68.8%)	52 (73.5%)	0.770
No	36 (73.5%)	69 (59.6%)		10 (31.2%)	18 (26.5%)	
Platelet count < 150 cells /mm³						
Yes	2 (2.8%)	1 (0.6%)	0.160	1 (3.1%)	1 (1.5%)	0.610
No	70 (97.2%)	166 (99.4%)		31 (96.9%)	66 (98.5%)	
Mean cell volume < 150 fL						
Yes	37 (49.3%)	89 (53.3%)	0.780	24 (75.0%)	50 (73.0%)	
No	36 (50.7%)	78 (46.7%)		8 (25.0 %)	18 (17.0%)	0.840
Absolute change in MCV < 14.5 fL						
Yes	43 (87.7%)	96 (84.2%)	0.550	23 (92.0%)	33 (75.0%)	0.091
No	6 (12.2%)	18 (15.7%)		2 (8.0 %)	11 (25.0%)	
Albumin decreased or unchanged (g/dL)						
Yes	12 (24.5%)	28 (24.8%)	0.961	18 (72.0%)	22 (50.0%)	0.070
No	37 (73.5%)	85 (75.2%)		7 (18.0%)	22 (50.0%)	
MCH < 2.7 pg after 6 months						
Yes	1 (2.0%)	9 (7.9 %)	0.151	1 (3.2%)	7 (10.3%)	0.490
No	48 (98.0%)	105 (92.1%)		31 (96.8%)	61 (89.7%)	
Serum lactate ≥ 2 mmol/l						
Yes	38 (53.0%)	84 (51.0%)	0.710	13 (25.0%)	17 (12.0%)	0.710
No	34 (47.0%)	83 (49.0%)		39 (75.0%)	120 (88.0)	
Missing at least 2 ARV visits ≥ 7 days (%)						
Yes	3 (4.2%)	3 (1.8%)	0.281	3 (8.3%)	6 (6.7%)	0.750
No	69 (95.8%)	164 (98.2%)		33 (91.7%)	83 (93.3%)	

- **Comparison of changes in laboratory markers between patients with and without detectable plasma viral load at 12 months**

Table 11 shows a comparison of changes from 6 months to 12 months. At 12 months, patients who failed to reach undetectable viral load had a lower increase in CD4 count (52.0% vs. 47.0% p = 0.82). There was also a greater drop from baseline (2.5 kg/m^2) in BMI amongst those with detectable viral load compared to those without (58.0% vs 51.0% p = 0.506). Regarding MCV change from the previous 6 months, table 8 indicates that absolute change in MCV < 14.5 fL was higher in individuals with detectable viral load compared to those without (p = 0.091).

3.7.9 Sensitivity, specificity, PPV and NPV of the laboratory markers for predicting adherence to ART compared to reference standard of viral load

Table 12 indicates the diagnostic accuracy of each laboratory marker to correctly classify patients as adherent or non-adherent when compared to viral load. For each biomarker, the table indicates the observed sensitivity, specificity, positive and negative predictive value compared to reference standard of viral load.

- **Sensitivity, specificity, PPV and NPV of the laboratory markers for predicting adherence to ART compared to reference standard of viral load at 6 months**

At 6 months, platelet count used alone showed the highest sensitivity. When platelet count < 100 fL was used as an indicator to classify patients as adherent or non-adherent, the sensitivity was 67.0%, while the specificity was 68.0%. Table 12 shows that when a BMI drop from baseline was used as a cut-off, the sensitivity decreased to 33.3%, the specificity was 69.7% and the PPV and NPV were 4.2% and 96.3% respectively. However, when failing to increase CD4 count ≥ 50 cells/mm^3 was used as single indicator for adherence to ARV treatment, the sensitivity decreased to 21.0% and the specificity was 65.0%. When change in MCV <14.5 fL was used as cut-off to assess adherence to ART at 6 months after ART initiation, the sensitivity was 31.5% and the specificity was 80%.

- **Sensitivity, specificity, PPV and NPV of laboratory markers for predicting adherence to ART compared to reference standard of viral load at 12 months**

At 12 months, MCV alone demonstrated the highest sensitivity. When mean cell volume < 150 fL after 12 months was used as a cut-off, the sensitivity was 73.0% and the specificity 29.0% while the sensitivity and the specificity of a cut-off based on absolute change in MCV < 14.5 fL were 87.0% and 17.0% respectively. At 12 months after ART initiation, when drop in BMI > 2.5 kg/m^2 was used as a cut-off to classify patient as being adherent or non-adherent, the sensitivity was 51.0% while the specificity was 60.0%. When failing to increase CD4 count by $\geq$ 50 $cells/mm^3$ was used, the sensitivity increased to 55.0% while the specificity decreased to 52.0%.

Table 11 Sensitivity, Specificity, PPV and NPV of the changes in laboratory markers for predicting adherence to ART compared with viral load.

	At 6 months				At 12 months			
Predictors	**Se**	**Sp**	**PPV**	**NPV**	**Se**	**Sp**	**PPV**	**NPV**
Baseline demographics								
Age	63.0%	41.0%	32.0%	71.0%	62.0%	40.0%	31.0%	71.0%
Sex	43.0%	68.0%	31.0%	73.00%	45.0%	68.0%	37.0%	37.0%
Change over 6 months								
BMI <18.5 kg/m²	33.3%	69.7%	4.1%	96.3%	3.0%	97.0%	33.0%	71.0%
BMI drop from baseline of > 2.5 kg/m²	28.7%	68.5%	50.0%	46.2%	51.0%	60.0%	34.0%	75.0%
Haemoglobin drop of ≥ 1g/dL	18.8%	69.0%	4.0%	92.0%	15.0%	91.0%	40.0%	73.0%
Failing to increase CD4 count by ≥ 50 cells/mm^3 28.3%		79.0%	16.0%	81.6%	-	-	-	-
Failing to increase CD4 count by ≥ 100 cells/mm^3	-	-	-	-	64.0%	27.2%	33.3%	57.1%
Total lymphocyte count < 2000 cells/mm³	21.0%	65.0%	26.0%	60.0%	37.0%	55.0%	24.0%	69.0%
Platelet count < 150 cells/mm³	67.0%	68.0%	2.0%	99.0%	00.0%	96.0%	00.0%	70.0%
Mean cell volume < 150 fL	29.3%	69.0%	51.4%	46.7%	73.0%	29.0%	29.0%	73.0%
Change in MCV < 14.5 fL	31.4%	80.0%	91.0%	14.4%	87.0%	17.0%	29.0%	78.0%
MCH < 2.7 pg.	10.0%	68.0%	2.0%	92.0%	7.0%	91.0%	25.0%	71.0%
Albumin decreased or unchanged (g/dL)	24.5%	75.2%	30.0%	70.0%	53.0%	49.0%	21.0%	73.0%
Serum lactate ≥ 2 mmol/L	53.0%	49.0%	31.0%	71.0%	27.0%	87.0%	43.7%	75.0%
Missing at least 2 ARV visits by ≥ 7 days	4.0%	98.0%	50.0%	69.0%	5.0%	91.0%	22.0%	69.0%
Self-reported adherence								
VAS	24.0%	87.0%	46.0%	73.0%	37.0%	61.0%	27.0%	71.0%
SMAQ	26.0%	79.0%	35.0%	71.0%	36.0%	61.0%	27.0%	71.0%
Multi-method approach	18.0%	86.0%	37.0%	71.0%	35.0%	62.5%	27.0%	70.8%

†Se: sensitivity. Sp: specificity. PPV: positive predictive value. NPV: negative predictive value
BMI: body mass index.MCV: mean cell volume. MCH: mean corpuscular hemoglobin. ARV: antiretroviral. VAS: Visual analogue scale.
SMAQ: Simplified Medication Adherence Questionnaire.

3.7.10 Risk scoring from the multivariate model for predicting a detectable viral load at 6 and 12 months

In this section, we used the result of the multivariate regression model to develop a diagnostic risk score to predict adherence to antiretroviral treatment. The Spiegelhalter and Knill-Jones approach was used to generate a scoring risk score (Seymour et al, 1990).

Summing up the risk score for each patient gave the total predicted score of adherence to ART at 6 or 12 months. At 6 months, the diagnostic risk score included ART initiation variables (age, gender, WHO stage) added to clinical follow-up variables and self-reported adherence measures such as VAS < 95%, platelet count < 150 cells/mm^3, absolute MCV change < 14.5 fL Additionally, the diagnostic risk score included the variable missing at least 2 medical or ARV visits. Platelet count had a score of +3 and missed ARV visits and VAS < 95% had a score of +2. All other variables had a score of +1. At 6 months, the total predicted risk score ranged from 0 to 12.

At 12 months, the diagnostic risk score included ART initiation variables such as age, gender and unemployment, alcohol drinking and platelet count as well as absolute MCV change < 14.5 fL. Additionally, the diagnostic risk score included poor self-reported adherence on ART at 6 months as assessed by viral load measure. At 12 months, the total predicted risk score for each patient ranged from 0 to 8.

3.7.11 Diagnostic accuracy of the risk scoring and the self-reported methods to detect viral load in patients at the Themba Lethu Clinic

In this section, we summarize the performance of the diagnostic risk score as well as of each adherence tool at 6 and 12 months. Figure 3 and 4 indicate the diagnostic value of the diagnostic risk score derived from the multivariate model (A), the VAS tool (B), the SMAQ (C) and the multi-method tool (D), at 6 and 12 months over a whole range of possible cut-offs for classifying individuals as patients with good or poor adherence. The AUC represents the

probability that test results from randomly selected pairs of diseased and non-diseased are correctly classified (Bamber, 1975; Handley & McNeil, 1982). With a perfect discriminant test, the ROC curve is more to the upper left corner and had a value AUC equal or close to 1, while an uninformative diagnostic tool, will have an AUC ≤ 0.5.

At the 6-month follow-up visit, the ROC curve for the total diagnostic risk score (Figure 3A) was more on the upper left corner than any other method and the AUC was calculated as 0.63 (95% CI: 0.53-0.72). This AUC was higher than any of the self-reported adherence methods. The ROC curve for the visual analogue scale was less to the upper left corner as the AUC decreased to 0.56 (95% CI: 0.49-0.62) (Figure 3B). When using the SMAQ, the line of the ROC curve (Figure 3C) indicated an uninformative model as the AUC decreased to 0.52 (95% CI: 0.45-0.60). Similarly, the ROC curve was uninformative for the multi-method tool as the AUC also shifted to 0.52 (95% CI: 0.46-0.50) (Figure 3D).

Figure 4 clearly shows that, at 12 months, the diagnostic risk score was uninformative as the ROC curve clearly falls below the discriminant threshold.

At 12 months, the three self-reported adherence methods (VAS, SMAQ, multi-method tool) (Figure 4B, Figure 4C, Figure 4D) performed better than the diagnostic risk score Figure 4A to classify patients as being adherent or non-adherent. Therefore, at 12 months we did not assess the optimal cut-off value for the diagnostic risk score method. At 12 months, the AUC of the total diagnostic risk score was calculated as 0.44 (95% CI: 0.40-0.60) (Figure 4). When the three self-reported adherence methods were used, the AUC of the self-reported adherence measures was 0.48 (95% CI: 0.40-0.60), 0.51 (95% CI: 0.40-0.60) and 0.50 (95% CI: 0.41-0.59) for the VAS, SMAQ and multi-method approach respectively. Similarly, Table 13

summarizes and compares the AUC of the different adherence methods when compared to viral load as the gold standard.

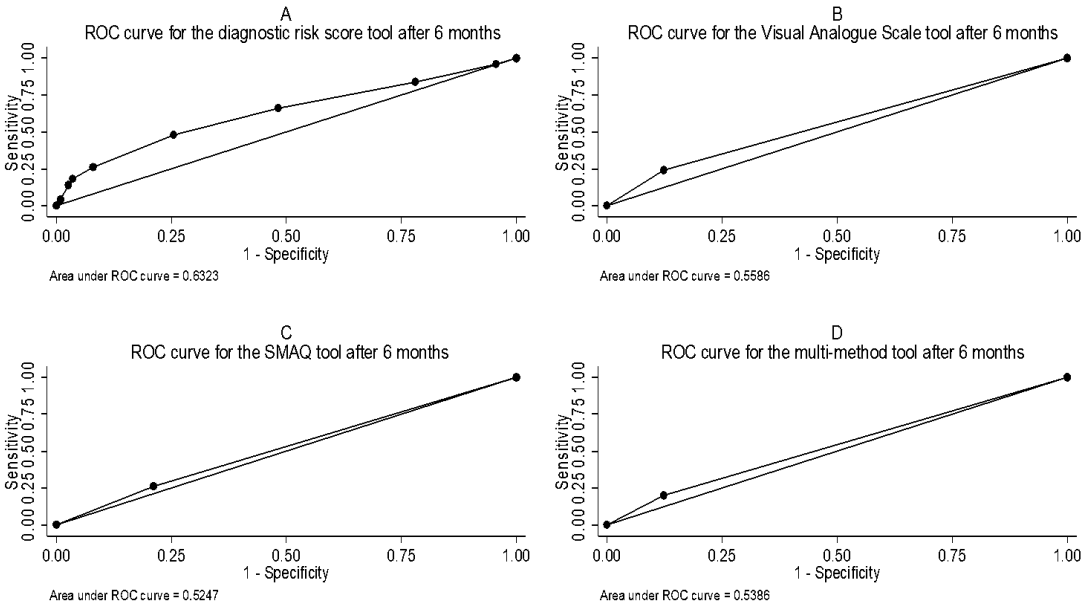

Figure 2 ROC curve for the calculated diagnostic risk score, the VAS, the SMAQ and the multiple approach method at 6 months.

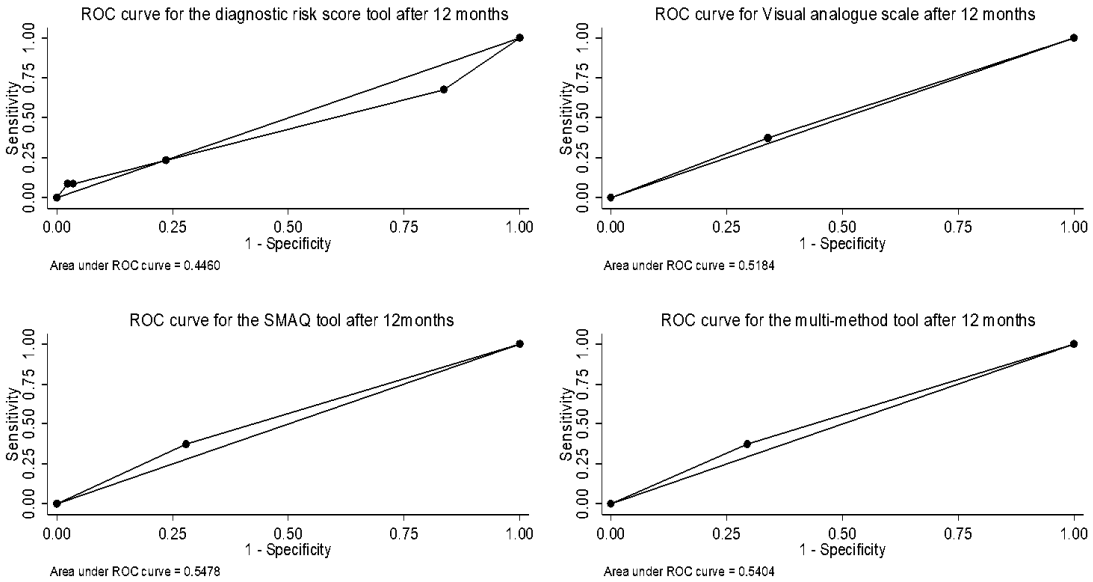

Figure 3 ROC curve for the calculated diagnostic risk score, the VAS, the SMAQ and the multiple approach method at 12 months.

Table 13 AUC ROC curve for the diagnostic risk score and for the self-reported adherence measure among those with a viral load at 6 months.

Method of adherence assessment	Number	AUC	95% CI
At 6 months of ART therapy			
Risk score at 6 months	163	0.63	0.53 - 0.72
Visual analogue scale	163	0.55	0.49 - 0.62
SMAQ	163	0.52	0.45 - 0.60
Multi-method approach	163	0.53	0.46 - 0.58
At 12 months of ART therapy			
Risk score at 12 months	142	0.44	0.40 - 0.60
Visual analogue scale	142	0.48	0.40 - 0.60
SMAQ	142	0.51	0.40 - 0.60
Multi-method approach	142	0.50	0.41- 0.59

*AUC: Area under curve. *SMAQ: Simplified Medication Adherence Questionnaire * ART: antiretroviral therapy

3.7.12 Sensitivity, specificity, PPV and NPV of the diagnostic risk score at different cut-off values compared to reference standard of viral load

Table 14 shows the result of the assessment of the diagnostic accuracy of the 6-month diagnostic risk score to identify detectable viral load, defined by plasma viral load ≥ 400 copies/ml. The sensitivity and specificity of the continuous risk score is defined at each cut-off point. The classification table shows 5 cut-off points derived from the continuous diagnostic risk score used to classify patients as positive or negative using the binary outcome viral load. Our result shows that for the cut-off point of ≥ 2 vs. < 2, of the 72 HIV-positive patients who truly had a detectable viral load at 6 months, 70 were correctly classified as such by the diagnostic risk score with a sensitivity of 97%, while of the 167 patients with undetectable viral load, 162 was classified as such giving specificity of 3%. Additionally, at the cut-off point of

≥ 2 vs. < 2, if the test was positive, the probability that HIV-infected patients was positive (PPV) with the viral load was only 30.2% while, if the test was negative, the probability of having an undetectable viral load was calculated as 71.4% (NPV 71.4%). At the cut-off point of ≥ 3 vs. < 3, the sensitivity of the continuous risk score decreased to 89% but the specificity increased to 14.3% while the PPV and the NPV was almost the same as that of ≥ 2 vs. < 2.

The optimal diagnostic accuracy was obtained at the cut-off points of 5 followed by the cut-off point of 4. Using the cut-off point of 5 (≥ 5 vs. < 5), the sensitivity of the diagnostic risk score increased to 64% but the specificity increased also to 34.7%. Additionally, when using a cut-off point of 4 (≥ 4 vs. < 4), the sensitivity was 76.4% while the specificity was 34.7%. At these two-cut off points, the sensitivity was higher, and the proportion of false positive tests decreased while the predictive values (PPV and NPV) remained almost the same than the previous cut-off points.

Table 12 Diagnostic accuracy of the risk score at different cut-off points at 6 months after ART initiation.

Cut-off points	Detectable viral load (n=72)	Undetectable viral load (n=167)	Se	Sp	1 specificity*	PPV	NPV
Predicted probability ≥ 2	2 (28.5%)	5 (71.4%)	97.2%	3.0%	0.97	30.2%	71.4%
Predicted probability < 2	70 (30.0%)	162 (70.0%)					
Predicted probability ≥ 3	64 (30.0%)	147 (70.0%)	89.0%	14.0%	0.85	31.0%	75.0%
Predicted probability < 3	8 (28.6%)	147 (71.4%)					
Predicted probability ≥ 4	57 (33.0%)	116 (67.0%)	76.4%	34.0. %	0.65	33.0%	72.0%
Predicted probability < 4	15 (22.7%)	51 (77.2%)					
Predicted probability ≥ 5	47 (35.0%)	89 (65.0%)	64.0%	50.0%	0.50	35.6%	75.0%
Predicted probability < 5	25 (24.2%)	78 (75.8%)					

Se: Sensitivity, Sp: Specificity PPV: Positive Predictive Value. NPV: Negative Predictive Value. 1-specificity*: FPR or False Positive Result

3.7.13 Clinical usefulness of the diagnostic risk score in practice at 6 months

More than half (57%) of patients had a risk score ≥ 5 while almost two thirds (75%) had a risk score ≥ 4. Our results indicate that having detectable viral load was more common among patients with a risk score ≥ 5 (35%, 47/136) compared to patients with a risk score < 5 (24%, 25/103). Similarly, compared to patients with a risk score < 4 or a risk score < 3, patients with a risk score ≥ 4 or ≥ 3 had a higher proportion of detectable viral load with 33% (57/173) and 30% (64/173) respectively. Additionally, our result indicates that using the diagnostic risk score derived from the multivariate model, the cut-off ≥ 5 will result in more individuals with detectable viral load being classified as patients with poor adherence compared to other possible cut-off values (cut-off ≥ 4, cut-off ≥ 3, and cut-off ≥ 2).

Furthermore, Figure 5 and 6 also indicate how the 6-month clinical usefulness may be used in routine clinical practice to estimate the probability of poor adherence on ARV treatment so that a decision can be taken on the necessity of performing a viral load or not. This decision support can be used at the cut-off score of 5 or 4.

A patient with a total risk score ≥ 5 will be likely considered to be poorly adherent and will be referred for viral load testing. Among those patients referred for further testing, a viral load ≥ 400 copies/ml will confirm the diagnosis. Therefore, those patients identified as poorly adherent will need to receive intensive support such as adherence counseling, therapeutic education or more frequent monitoring. However, when the risk score is below the cut–off score of 5 (< 5), the patients will be classified as having good adherence and no viral load testing is indicated. However, a reassessment of the risk score at the next medical visit to confirm the status of the patient is necessary.

In practice the application of the diagnostic risk score at this threshold of five (≥ 5 vs. < 5) means that among patients with good adherence, nearly 53% (89/167) of them will be classified as patients with poor adherence, thus they are false positive, while, among those classified as patients with good adherence (score <5), 35% (25/72) will have true poor adherence or false negative. When a lower cut-off value of 4 (≥ 4 vs. < 4) is applied to increase the sensitivity (65% vs < 79%), and lower the specificity (46% vs. < 33%), the application of this algorithm could mean that nearly 70% (116/167) of patients with good adherence level will be classified as poorly adherent while the scoring system will miss nearly 21% (15/72) of patients with good adherence.

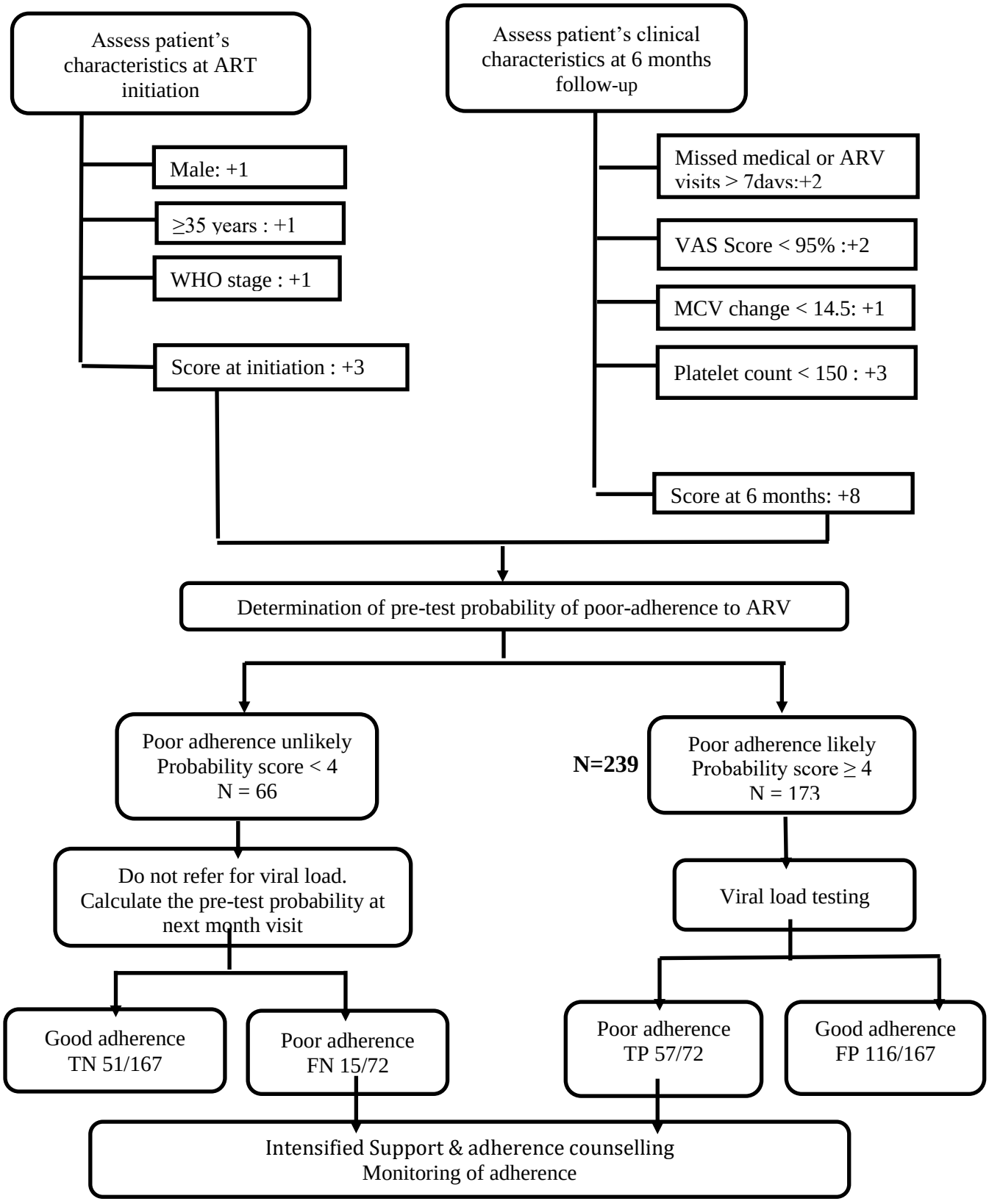

Figure 4 Clinical usefulness of the diagnostic risk score in practice at 6 months with a cut-off value of 5. (TN True negative; TP True positive; FN False negative; FP False positive).

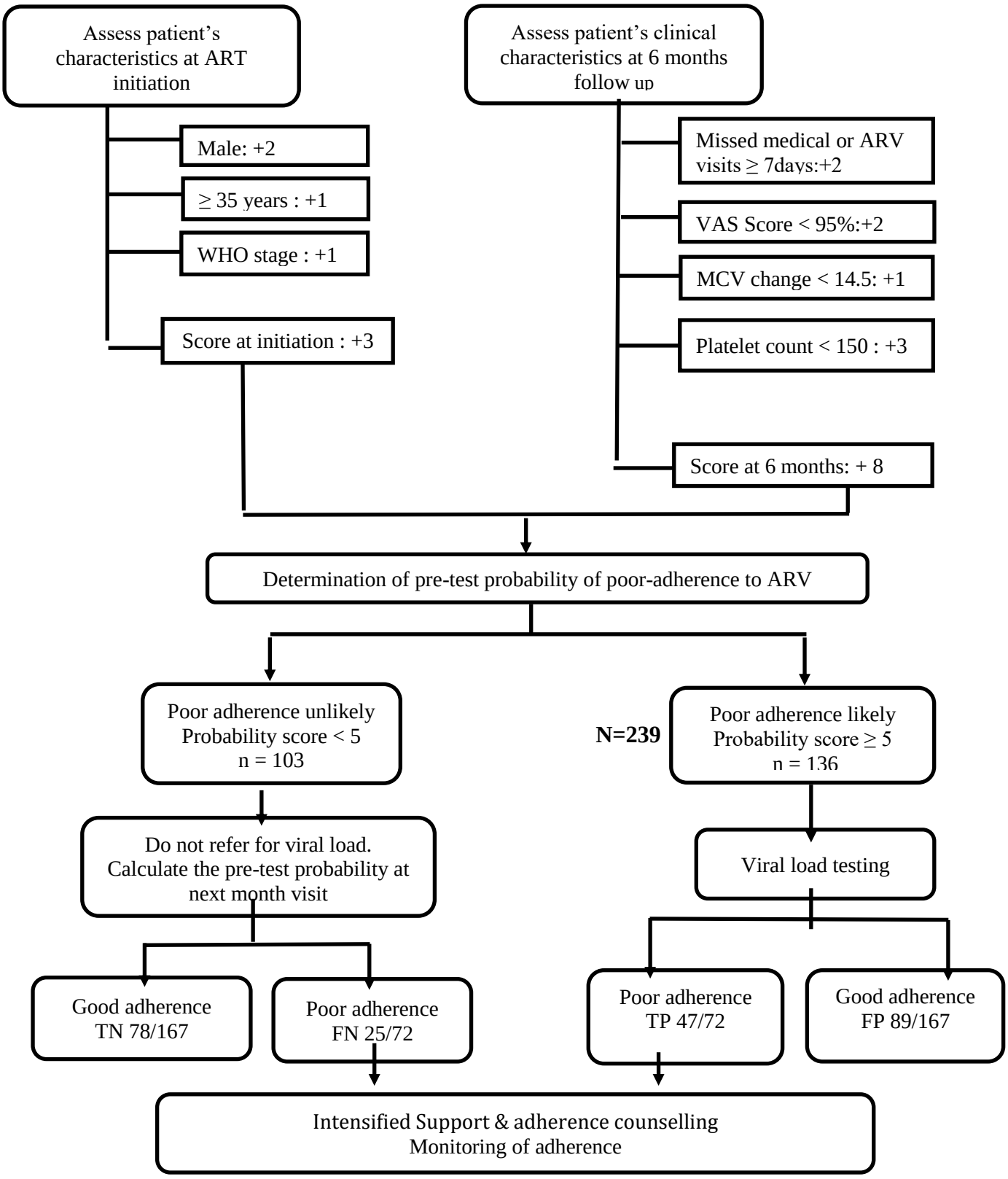

Figure 5 Clinical usefulness of the diagnostic risk score in practice at 6 months with a cut-off value of 4. (TN True negative; TP True positive; FN False negative; FP False positive).

3.8 Sensitivity Analysis

The presence of missing values threatens validity. Therefore, in this section, we explore the reason for missingness and use a multiple imputation technique to fill in all the missing values in the predictors and the outcomes at 6 and 12 months. We compared this approach to the complete case analysis method previously used. First, we used the observed data to explore the missingness mechanism by exploring the relation between missing data and observed values. Secondly, we assessed the impact of missing data on the estimates of the multivariate regression model at 6 and 12 months after ART initiation under the missing at random assumption (MAR).

3.8.1 Relation between missingness of the demographic characteristics and the measured viral load at 6 and 12 months after ART initiation

Table 15 provides a breakdown of the relation between the missingness of the predictor and a detectable viral load at 6 and 12 months using the observed dataset. The result indicates that missingness among the baseline demographic characteristics was minimal. In general, there was no relation between the missingness of the baseline demographic characteristics and the measured viral load at 6 or 12 months.

Table 13 Association between viral load detectable at 6 and at 12 months and missing values in baseline demographic characteristics.

	Viral load at 6 months			Viral load at 12 months		
Demographic baseline	**VL ≥ 400 (n = 72)**	**VL < 400 (n = 167)**	**p value***	**VL ≥ 400 (n = 34)**	**VL< 400 (n = 85)**	**p value**
Age						
Valid	72 (100%)	167 (100%)		34	85	
Missing	00 (00%)	00 (00%)	0.618	0	0	0.568
Sex						
Valid	72 (100%)	167 (100%)		34	85	
Missing	00 (00%)	00 (00%)	0.618	0	0	0.568
Education						
Valid	71(98.6%)	163 (97.6%)	0.618	34	84	
Missing	1 (1.4%)	4 (2.4%)		1	0	0.328
Unemployment						
Valid	72 (100%)	167 (100%)	0.618	34	85	
Missing	00 (00%)	00 (00%)		0	0	0.568
Nationality						
Valid	71 (98.6%)	167 (100%)	0.127	33	85	
Missing	01 (1.4%)	00 (00%)		1	0	0.633
Alcohol drinking						
Valid	71 (98.6%)	167 (100%)	0.127	34	85	
Missing	1 (1.4%)	00 (00%)		0	0	0.633
Smoking						
Valid	71 (98.6%)	167 (00%)	0.127	34	85	
Missing	01 (1.4%)	00 (00%)		0	0	0.633

* Significant at the 0.05 levels

§. P value calculated using Pearson's chi-square test or Fisher's exact test where chi-square assumptions were not met.

3.8.2 Relation between missingness of clinical characteristics and detectable at viral load at 6 months

Table 16 shows the proportion of detectable viral load by missing values on the clinical and laboratory markers at 6 months. There was no association between having a missing value in the clinical and laboratory markers at 6 months and a detectable viral load. For each clinical and laboratory marker, the proportion of HIV-infected patients with detectable viral load was lower in patients whose clinical or laboratory marker was missing. However, none of the association reported in the table 75 was statistically significant ($p < 0.05$).

3.8.3 Relation between missingness of clinical characteristics and a detectable viral load at 12 months

Table 16 shows the proportion of detectable viral load between individuals with and without missing values on the clinical and laboratory markers at 12 months after ART initiation. Similarly, according to the pattern observed for missing values at 6 months, there is no relation between the missing values and a detectable viral load at 12 months.

Table 14 Relation between missingness of clinical and laboratory markers and detectable viral load at 6 and 12 months.

Clinical and laboratory markers	Viral load at 6 months		p value
	VL ≥ 400 (n = 72)	**VL < 400 (n = 167)**	
- At 6 months			
Body mass Index (kg/m^2			
Valid	48 (66.7%)	110 (65.8%)	0.905
Missing	24 (33.3%)	57 (34.2%)	
Haemoglobin(g/dL)			
Valid	49 (68.1%)	114 (68.3%)	0.975
Missing	23 (31.9%)	53 (31.7%)	
CD4 count (cells/mm$^{3)}$			
Valid	49 (68.1%)	114 (68.3%	0.975
Missing	23 (31.9%)	53 (31.7%)	
Total lymphocyte count (cells/mm$^{3)}$			
Valid	49 (68.1%)	114 (68.3%)	0.975
Missing	23 (31.9%)	53 (31.7%)	
Platelet count cells/ mm^3			
Valid	48 (66.7%)	113 (67.7%)	0.880
Missing	24 (33.3%)	54 (33.3%)	
Mean cell volume (fL)			
Valid	49 (68.1%)	114 (68.3 %)	0.975
Missing	23 (31.9%)	53 (31.7%)	
Albumin (d/dL)			
Valid	49 (68.1%)	113 (67.7%)	0.953
Missing	23 (31.9%	54 (32.3%)	
MCH (pg.)			
Valid	49 (68.1%)	114 (68.3%)	0.975
Missing	23 (31.9%)	53 (31.7%)	
Serum Lactate (mmol/l)			
Valid	46 (63.9%)	111 (66.5%)	0.700
Missing	26 (36.1%)	57 (33.5%)	
Missing at least 2 ARV visits ≥7 days			
Valid	72 (100%)	167 (100%)	-
Missing	00 (00%)	00 (00%)	
VAS (%)			
Valid	49 (68.1%)	114 (68.3%)	0.975
Missing	23 (31.9%)	53 (31.7%)	
Multi-method approach (Yes/No)			
Valid	49 (68.1%)	114 (68.3%)	0.975
Missing	23 (31.9%)	53 (31.7%)	
SMAQ (Yes/No)			
Valid	49 (68.1%)	114 (68.3%)	0.975

Missing	23 (31.9%)	53 (31.7%)	
- At 12 months			
BMI (kg/m^2)			
Valid	31(91.2%)	68 (80.0%)	
Missing	3 (8.8%)	17 (20.0%)	0.141
Haemoglobin (g/dl)			
Valid	32 (94.2%)	68 (80.0%)	
Missing	2 (5.8%)	17 (20.0%)	0.058
CD4 count (cells/mm^3)			
Valid	32 (94.2%)	68 (80.0%)	
Missing	2 (5.8%)	17 (20.0%)	0.058
Total lymphocyte count cells/mm^3			
Valid	32 (94.2%)	68 (80.0%)	
Missing	2 (5.8%)	17 (20.0%)	0.058
Platelet count (10^2/mm^3)			
Valid	32 (94.2%)	68 (80.0%)	
Missing	2 (5.8%)	17 (20.0%)	0.058
MCV (fL)			
Valid	32 (94.2%)	68(80.0%)	
Missing	2(5.8%)	17 (20.0%)	0.058
Albumin (g/dL)			
Valid	32 (94.2%)	68 (80.0%)	
Missing	2 (5.8%)	17 (20%)	0.058
MCH (pg)			
Valid	26 (76.5%)	53 (62.3%)	
Missing	8 (23.5%)	32 (37.6%)	0.141
Serum lactate (mmol/L)			
Valid	30 (88.2%)	63 (74.0%)	0.092
Missing	4 (11.8%)	22 (26.0%)	
Missing at least 2 ARV visits ≥7 days			
Valid	34 (100%)	85 (100%)	0.568
Missing	00 (00.0%)	00 (00.0%)	
VAS (%)			
Valid	32 (94.0. %)	68 (80.0%)	
Missing	2 (6%)	17 (20%)	0.058
Multi-method approach (Yes/No)			
Valid	32 (94.0. %)	68 (80.0%)	
Missing	2 (6.0%)	17 (20.0%)	0.058
SMAQ (Yes/No)			
Valid	32 (94.0%)	68 (80.0%)	0.058
Missing	2 (6.0%)	17 (20.0%)	

†BMI: body mass index. †MCV: mean cell volume. †MCH: mean corpuscular hemoglobin. †VAS: Visual analogue scale. †SMAQ: Simplified Medication Adherence Questionnaire *Significant at 0.05 level

†BMI: body mass index. †MCV: mean cell volume. †MCH: mean corpuscular hemoglobin. †ARV: antiretroviral †VAS: Visual analogue scale. †SMAQ: Simplified Medication Adherence Questionnaire.

*Significant at 0.05 level

3.8.5 Comparison of complete case analysis and imputed resssults

There were no considerable differences in the results when comparing the complete case analysis to the imputed analysis although some minor similarities were noted. For this reason, we compared the results obtained from the case complete analysis with the results obtained from the multiple imputed dataset under the MAR assumption for estimation of predictors of detectable viral load.

Table 17 indicates the effect of imputing the missing values in the outcome and the predictors on the LCM database and the performance of the diagnostic model using VL at 6 months as gold standard. The case complete analysis included 119 cases although there were 290 patients with the imputed dataset. The result in Table 18 displays the estimation of the adjusted relative risk with the final regression model to complete case analysis versus the final model fit to the imputed dataset under the missing at random assumption. In both models, the variables age (>35 years), gender, MCV change < 14.5 fL are not significantly associated with detectable viral load at 6 months. However, the largest standard errors are obtained more from the case complete case analysis. In the case of the variables, VAS < 95%, platelet count < 150 cells/mm^3 and missing at least 2 ARV visits by more than 7 days, the adjusted relative risk was significant in the complete case analysis, whereas these variables were no longer significant with the imputed dataset. However, none of these variables were statistically significant.

Table 15 Results of the multiple imputation for estimation of the predictors of ART at 6 months after ART initiation.

	Analysis with complete cases dataset			Analysis with imputed dataset (k=10)		
Variables	**RR**	**Standard error**	**P value**	**RR**	**Standard error**	**P value**
Age category at ART initiation						
≤ 35 years old	Reference			Reference		
> 35 years old	1.1 (0.6 - 1.7)	0.328	0.752	1.1 (0.7 - 1.6)	0.221	0.380
Gender						
Female	Reference			Reference		
Male	1.46 (0.9 - 2.3)	0.931	0.098	1.2 (0.8 -1.8)	0.250	0.283
VAS < 95%						
Yes	1.65 (1.01 - 2.71)	0.417	0.044*	1.4 (0.8 - 2.3)	0.342	0.193
No	Reference			Reference		
Platelet count < 150 cells/mm^3						
Yes	2.73 (1.40 - 7.10)	1.346	0.041*	2.3 (1.0 - 5.2)	0.960	0.042
No	Reference			Reference		
Absolute change in MCV < 14.5 fL						
Yes	1.31 (0.60 - 2.90)	0.530	0.670	1.3 (0.6 - 3.1)	0.500	0.486
No	Reference			Reference		
Missing at least 2 ARV visits by ≥ 7 days						
Yes	2.40 (1.08 - 5.10)	0.937	0.030*	1.5 (0.6 - 4.1)	0.769	0.371
No	Reference			Reference		
Failing to increase CD4 count by ≥ 50 cells/mm^3						
Yes	0.84 (0.45 - 1.60)	0.273	0.611	0.7 (0.3 - 2.3)	0.361	0.611
No	Reference			Reference		
WHO stage at ART initiation						
I/II	Reference			Reference		
III/V	1.10 (0.83 - 1.40	0.161	0.486	1 (0.7 -1.4)	0.146	0.740

RR: relative risk

Table 16 Results of the multiple imputation for estimation of the predictors of ART at 12 months after ART initiation.

Variables	RR	Standard error	p value	RR	Standard error	P value
Age category at initiation						
≤ 35 years old	Reference			Reference		
> 35 years old	0.6 (0.31 - 1.32)	0.234	0.237	0.8 (0.45 - 1.4)	0.231	0.489
Gender						
Female	Reference			Reference		
Male	0.9 (0.47 - 2.08)	0.371	0.977	0.9 (0.53 -1.6)	0.260	0.870
Score at VAS < 95%						
Yes	0.9 (0.43 - 1.9)	0.325	0.809	1.1 (0.43 -2.78)	0.460	0.820
No	Reference			Reference		
Platelet count < 150 cells/mm^3						
Yes						
No	Reference			Reference		
Absolute change in MCV < 14.5 fL						
Yes	3.6 (0.68 - 18.97)	3.057	0.131	2.4 (0.55-11.01)	1.710	0.255
No	Reference			Reference		
Missing at least 2 ARV visits by ≥7 days						
Yes	0.9 (0.29 - 6.05)	1.231	0.960	1.3 (0.29 - 6.05)	0.950	0.681
No	Reference			Reference		
Failing to increase CD4 count by ≥ 50 cells/mm^3						
Yes						
No	Reference			Reference		
Self-reported adherence at 6 months						
Yes	2.1 (0.70 - 5.99)	1.120	0.188	3.1 (1.05 - 9.11)	1.528	0.041
No	Reference			Reference		

RR: relative risk

CHAPTER FOUR- DISCUSSION

4.1 Introduction

In line with the objectives and the study findings, this chapter gives a summary of the results and explores the scientific contribution of our study. The study limitations are discussed by identifying threat to validity and the generalizability of the results. Finally, the chapter gives an overview of the implications of our findings and the potential for further research in this area.

4.2 Summary of results

The study aimed to identify the usefulness of a composite marker of poor adherence assessed by failure to achieve virologic suppression which is defined as a plasma viral load ≥ 400 copies/ml at 6 months on ART in patients on first-line regimen at Themba Lethu Clinic, in Johannesburg, South Africa. This is a secondary analysis of data prospectively collected from HIV-postive patients initiated onto standard government first-line ART regimen, from February 2010 to April 2014.

At baseline, 353 patients were enrolled and the mean age at ART initiation was 37.2 years. Most of the patients (87.5%) initiated on a Tenofovir-based regimen. In terms of viral load suppression at 6 and 12 months, of 239 patients, 30% failed to suppress viral load (HIV/RNA ≥ 400 copies/ml) by 6 months. Patients with undetectable plasma viral load during this period were more likely to be female, educated and older compared to those with detectable viral load. In terms of viral load suppression, at 12 months, of 119 patients, 28.5% did not supress viral load. Among them, more than half were female and the percentage of patients that reached secondary school was slightly higher in the group that suppressed viral load at 12 months

compared to those with detectable viral load. The distribution of CD4 count between the two groups was similar.

To reduce sampling bias, we assessed how the patients in LCM cohort study should be adequately representative of the population of patients at TLC which are likely to receive the clinical prediction score (CPS). Therefore, we compared the demographic characteristics (age, sex), other medical conditions unrelated to the outcome at 6 and 12 months (Appendix E). Our results indicate that the baseline demographic characteristics of the patients who initiated ART were well balanced between the groups. Similarly, on the basis of clinical characteristics prior to ART initiation, the characteristics are nearly the same although there was less people with a CD4 count < 50 cells/mm^3 in the LCM cohort database.

Results of complete case analysis showed a consistent trend of patients having detectable viral load being classified as non-adherent with any of the self-reported adherence measures (albeit not statistically significant). Using viral load as gold standard, the analysis showed that all the three self-reported adherence measures (VAS, SMAQ, and multi-method approach) yielded a high proportion of false negative results during the first 6 and 12 months on ART. Adjusted multivariate modified Poisson regression model with robust variance estimates, showed that patients who missed at least two medical or ARV visits, had a platelet count < 100 fL and a visual analog scale < 95%, were at increased risk of failing to achieve viral load suppression by 6 months on ART. At 12 months, only being older than 35 years, being unemployed and alcohol consumption were associated with failure to suppress viral load. The current ART regimen was not associated with failure to suppress viral load, neither at 6 nor at 12 months.

At 6 months of ART, the ROC curve of the continuous diagnostic risk score was more discriminant than any of the three self-reported adherence measures and different cut-off points yielded less false negative results when compared to viral load. Additionally, both self-reported adherence and risk scores derived from the multivariate model showed high negative predictive values during the first 6 months. However, at 12 months, the ROC curve based on the continuous diagnostic risk score was uninformative as the AUC was clearly below 0.5 while the self-reported adherence measures remained at the same levels.

Our analysis of the missing data shows that the data are not missing for reasons related to the predictors or outcome values, thus the hypothesis that data are missing completely at random (MCAR) seems to be more probable although the missing not at random mechanism (MNAR) cannot be ruled out as we didn't have the unobserved dataset. Sensitivity analysis under the MAR assumption did not show similar association, instead, the results showed notable difference between the models fit with CCA compared with model fit with imputed datasets. The findings from the case complete analysis takes priority over the estimation from multiple imputed dataset.

In conclusion, our results show that, during the first 6 months of ART, a combination of clinical information, laboratory data and self-reported adherence measures can be used to better monitor adherence instead of self-reported methods in patients on first-line regimens.

4.3 Viral load suppression during the first 6 and 12 months on ART

In the following sections, the study findings are compared with relevant knowledge, other findings from previous studies and possible explanations of our results. Furthermore, the chapter discusses the strengths and study limitations.

Of 239 patients, 72 (30.1%) did not achieve an undetectable viral load (HIV/RNA≥ 400 copies/ml) at 6 months. At 12 months, 119 (41.0%) had their plasma viral load assessed and among them 28.5% did not achieve an undetectable viral load. Similar levels of viral load suppression in HIV-positive patients has been described by Fox et al (2012) at Themba Lethu Clinic.

In a meta-analysis study on the prevalence of viral suppression after 12 months of antiretroviral therapy in low and middle-income countries, McMahon et al, (2013) found that the proportion of patients showing viral suppression was 84.0%. Our results support findings from previous studies (Donnelly et al, 2005; Anastos et al, 1999; Sterling et al, 1999; Rezza et al, 2000) that demonstrated that viral load may varies by gender, race, age and probably depends on the mode of transmission. In a Italian cohort study, (Sterling et al, 1999) reported lower median plasma viral load in women added to the fact that viral load increased more rapidly overtime among HIV-positive women compared to HIV-positive men.

4.4 Laboratory markers changes and viral load response

- **CD4 count response**

Our results showed that there was no association between baseline CD4 count and viral load suppression during the first 6 or 12 months of ART treatment. We also compared the change in CD4 count response between patients with and without viral load suppression. Although not statistically significant, patients who suppressed viral load were less likely to have a poor CD4 response compared to patients who suppressed viral load during the first 6 months, while at 12 months on ART, the reverse situation was observed. When "CD4 < 200 cells/mm^3 was used as a cut-off point to assess adherence to ART compared to viral load as gold standard, the sensitivity and the specificity at 6 months were 28.6% and 78.9% respectively, while at 12

months, when "failure to increase by > 100 cells/mm^3" was used, the sensitivity increased to 64%.

Our results confirm findings from previous studies that CD4 count response is a poor predictor of HIV treatment outcome in adult and children on antiretroviral therapy (Badri et al, 2008) (Moore et al,2008). Badri et al, (2008) showed that CD4 count changes was associated with viral load but may have a very limited utility in identifying virologic failure in individual patients. However, using routinely collected health data from South Africa, Evans et al (2014) showed that CD4 when combined with baseline and clinical information increased the sensitivity and specificity of a clinical prediction score (CPS) used to target viral load failure defined as two consecutive HIV-RNA (400 copies/ml) following suppression below this level (Evans et al, 2014).

- **Total lymphocyte count change**

Our 6 months analysis showed that HIV-positive patients with detectable viral load had greater absolute lymphocyte count response compared to individuals who suppressed viral load and the same trend was observed at 12 months after ART initiation. When an absolute lymphocyte count < 2000 cells/mm^3 was used as cut-off point compared to viral load as gold standard, the sensitivity at 6 and 12 months was very low at only 21% and 37% respectively while the specificity was 65% and 55% respectively. This low diagnostic value of the TLC may be partially. Findings from previous studies (Schreibman & Friedland, 2004; Denue et al,2013; Lau et al, 2003) that showed that total lymphocyte count is not a proxy indicator of early viral load suppression, but rather a marker of prognosis and long term survival during HIV infection (Lau et al, 2003). Data from previous studies showed that total lymphocyte count is a marker of disease staging and opportunistic infection. A study by Schreibman and Friedland (2004) conducted in 831 HIV-positive patients indicated that, when used as a surrogate marker of CD4

count, 98% of those with total lymphocyte count of < 1000 had a CD4 cell count < 200 and a specificity of 98%. However other studies have shown that no consensus cut-off point is actually found (Schreibman & Friedland, 2004) (Lau et al, 2003)

- **MCV change**

Several authors (Steele et al, 2002; Segeral et al, 2010; Cosby, 2007) have reported the effect of antiretroviral treatment on the haemoglobin level and macrocytosis defined as mean corpuscular volume (MCV) exceeding 100 fL. A Study from retrospective databases and prospective cohorts has provided evidence of a strong relationship between adherence to ART and MCV (Segeral et al, 2010). In contrast, our findings did not show any relation between MCV and viral load suppression at 6 or at 12 months. The absence of an association between macrocytosis as calculated by the MCV and the outcome may be explained by the fact that macrocytosis during ART is mainly observed in patients strictly adherent to AZT regimen and only partially in those receiving stavudine (d4T), another thymidine analogue. In our study, most of the patients (87.5%) were initiated on a TDF-based regimen. This is because in the 2013 South African national treatment guidelines, the combination TDF+FTC/3TC+EFV/NVP is the first-line preferred regimen for all new patients needing treatment. Based on these guidelines, AZT is only used when there is contraindication to TDF such as renal diseases or the use of other nephrotoxic drugs (aminoglycosides) while d4T is only used when there is contraindication to TDF and AZT. Other factors that may have affected the association between MCV and detectable viral load in this study may be the smaller sample size and the fact that in our study we transformed the MCV variable from a continuous to a categorical variable. Regarding the diagnostic accuracy of MCV taken alone, although this variable was not significantly associated with viral load suppression during the first 6 or 12 months on ART, our results indicate that this biomarker has a highly discriminant value long-term. Using the

change in MCV < 14.5 fL as a cut-off value, the sensitivity was poor at 6 months (31.4%) but it increased to 80% at 12 months. However, the specificity of this biomarker remained poor at 6 and 12 months. Our results seem to suggest that in the multifactorial nature of adherence to ART treatment, the discriminative utility of the MCV as a marker of viral load suppression is poor at 6 months but has a high discriminant value at 12 months. However, this situation may be explained by the fact that at 6 months we assessed the diagnostic accuracy of this biomarker with only incident cases whereas at 12 months we have HIV-positive patients who have been longer on ART (6 vs. 12 months). People who were diagnosed as having detectable viral load at 6 months may be more likely to die shorter before the 12 months follow-up. Therefore, the 12 month's prevalent cohort is more prone to survival bias which impacts on the discriminative value of the biomarker at 12 months (Miller et al, 2012).

- **Serum lactate**

In this study, we did not find a significant association between serum lactate and a detectable viral load at 6 or 12 months. Our findings did not confirm previous studies that suggest that an elevated serum lactate is associated with treatment with NRTI and an undetectable viral load. In their study among 251 HIV-positive patients in the US, Desai et al (2003) suggested that elevated lactate levels are useful in assessing adherence. They found that asymptomatic hyperlactatemia defined as a serum lactate concentration > 2mmol/L was associated with an undetectable viral load regardless of treatment regimen. The fact that asymptomatic hyperlactatemia occurs mostly on adult HIV-infected patients initiated with stavudine and didanosine may explain why we did not observe this. In our study, more than 75% of patients were initiated on TDF-based regimen.

- **Platelet count response**

The present study showed a statistically significant association between platelet count and a detectable viral load. At 6 months, after adjustment for other variables effects, the final modified Poisson regression model showed that patients with platelet count < 150 cells/mm^3 were 2.7 times more likely to have a detectable viral load. However, this was not confirmed with the multivariate model at 6 months. At 6 months, platelet count used alone showed the highest sensitivity; when platelet count < 150 cells/mm^3 was used as an indicator to classify a patient as adherent or non-adherent, the sensitivity was 67%, and the specificity 68%. This finding at 6 months is in line with another study by Zetterberg et al (2013). In their study, platelet counts were retrospectively collected from 2206 HIV-positive patients from visits at study entry and during follow-up. They reported that platelet count decreased significantly in interrupted-ARV groups while they remained stable in the group with viral suppression. However, the same study (Zetterberg et al, 2013) showed that the reintroduction of ARV therapy reversed the thrombocytopenia. Two factors may explain the effect of adherence to ART on the platelet: first the HIV replication itself and secondly the fact that platelet count is itself an inflammatory marker (Zetterberg et al, 2013)

- **Haemoglobin and Albumin levels changes**

Regarding the usefulness of haemoglobin and albumin as prognostic markers of detectable viral load or adherence to antiretroviral treatment, our study did not find any association between the 6 months changes of these biomarkers and having a detectable viral load at 6 or 12 months. However a study in India among 122 adults HIV-positive patients aimed at studying the usefulness of haemoglobin and albumin as prognostic markers for ART, the changes in haemoglobin and albumin levels increased after ART initiation and were found to be strong prognostic markers of HIV disease progression at pre-, one and two year post treatment

(Chauhan et al, 2011). Anaemia and hematologic disorders are the most frequent diseases in HIV-infected patients on antiretroviral treatment (De Santis et al, 2011). However, anaemia is more related to disease progression or as an adverse event for patients who have been initiated on zidovudine based regimen (Cosby et al, 2007) (De Santis et al,2011) .

- **Body mass index and MCH changes**

Regarding the usefulness of body mass index (BMI) and MCH as possible diagnostic markers of failure to suppress viral load, our study did not find any association between the 6 months changes of these biomarkers and having a detectable viral load at 6 or 12 months. However, findings from other studies have found a positive correlation between BMI evolution and severe immunodeficiency but only in a long term. Additionally, high BMI change, alone or in combination with high CD4 gain may not reflect optimum adherence to treatment in the context of resource limited settings (Messou et al, 2008).

Regarding MCH, in a cohort study with patients who initiated antiretroviral therapy and had concurrent HIV/RNA biomarker ≥ 4 months after ART, Lau et al (2010) found that MCH and change in MCH were the strongest predictors of HIV/RNA ≥ 500 copies/ml. However, this difference with our results could be explained by the fact they only assessed routinely collected clinical markers in addition to CD4 and without additional information on adherence (Lau et al, 2010). Another study by Bison et al (2008) has suggested that inclusion of adherence data is more likely to change the diagnostic value of MCH for detecting viral load suppression or treatment failure (Bisson et al, 2008).

4.5 Missing ARV visit or medical visit and plasma viral load response

Our results show that patients who missed two ARV visits within the first 6 months are two times more at risk for detectable viral load, holding all other variables constant. This result is in line with another study in South Africa and in developed countries. Brennan et al (2010) analysed data from 4476 HIV-infected patients initiating ART at Themba Lethu Clinic in Johannesburg and found that missing medical or ARV visits was a marker of poorer outcome. Specifically, the study showed that HIV-positive patients who missed three or more medical ARV visits were more likely to fail to suppress their viral load during the first 6 months. It is likely that missing visits is a marker of poorer adherence which potentially could lead to treatment failure and drug resistance.

4.6 Self-reported adherence (SRA) and viral load response

- **Visual Analogue Scale**

Our findings show that over 80% of HIV-infected patients were reported to be highly adherent to ART during the first 6 months when adherence was assessed with the Visual Analogue scale, but this percentage decreased to nearly 60% at 12 months. Not only that, we additionally found that, during the first 6 months, patients identified as adherent with the VAS tool were more likely to have a suppressed viral load. The high percentage of HIV-infected patients classified as adherent by VAS is consistent with what has been reported from previous findings (Deschamps et al, 2008; Kiwuwa-Muyingo et al, 2012).

The results in our study confirm previous studies that found that self-reported adherence is associated with undetectable viral load and better outcome (Raboud et al, 2002; Bandsberg et al, 2006). However, our study shows that, when viral load was taken as the gold standard, the VAS demonstrated low sensitivity and low positive predictive values while its specificity and

negative predictive values were high at 6 and 12 months. Thus, our results imply that, within the first 6 months of ART, using VAS to assess adherence in HIV-infected patients will lead to nearly 75% of patients with truly detectable viral load being missed and classified as negative, added to the fact that when VAS is used as a first screening test, the probability that a patient classified as non-adherent having a detectable viral load is only 46%. The same trend was observed at 12 months of ART.

These findings reveal, although self-reported adherence measures can be associated with detectable viral load, using VAS as a single tool to assess adherence to ART may have major limitations. Our results confirm the limit to validity attributed to the VAS and support other findings (Kabore et al, 2015; Chkhartishvili et al, 2014; Berg & Arnsten, 2006) from developed and resource limited settings that reported a higher misclassification probability and overestimation of adherence with the VAS tool.

The fact that patients who reported adherence with the VAS or other self-reported adherence measures were more subject to the issues of recall bias and social desirability can explain this overestimation and misclassification when compared to viral load. In the LCM project, patients were provided with financial or conditional economic incentives to come to medical visits and this situation may have translated to a higher report of adherence level during medical visits. Similarly, many studies (Galaraga et al, 2014; Sorensen et al, 2007) have demonstrated that economic incentives to be successful at improving patient's adherence to medical treatment. In this context, while not all, some patients may know that if they stop taking their medication, they may risk financial constraints and these events were more likely to occur during routine medical or ARV visits at TLC. Therefore, our results may not be fully reproducible in routine clinical practice where economic incentives are not used.

However, some authors (Langebeek et al, 2014; Segeral et al, 2010) have suggested that given the complex factors surrounding ARV therapies and adherence, economic incentive and remuneration fear may not fully explain the high self-report adherence. Additionally, other studies suggest that conditional incentives may only reflect adherence level in the short term follow up. In their meta-analysis that included 15351 HIV-infected patients from 65 countries, Langebeek et al (2016) studied the relation between self-report adherence and plasma HIV-RNA in adult non-pregnant patients on ART. Their results demonstrated that an adherence threshold below 95%, social desirability and lack of confidence of the patient may affect the accuracy of the relationship between viral load and self-report adherence (Langebeek et al, 2014) (Segeral et al, 2010).

The issue of reporting bias is known from previous research findings and some authors have even suggested the use of anonymous Computer Assisted Interviews (CASI) to avoid face-to-face interviews between HIV-infected patients and healthcare workers (Berg & Arnsten, 2006) (Arnsten et al, 2001).

- **Simplified Medication Adherence Questionnaire**

HIV-infected patients assessed with the SMAQ tool showed high levels of adherence but very low sensitivity when compared to viral load as gold standard. Our findings show that the diagnostic accuracy of the SMAQ is almost similar of that of the VAS at 6 and 12 months. The fact that SMAQ was also a tool based on retrospective questions and was not anonymous, suggest that performance could also be affected by the issues of recall bias and social desirability. This may explain the similarities of the results between the SMAQ and the VAS. Contrary to our findings, the validation study of the SMAQ carried out by Knobel et al (2002) in Spain in a large cohort of HIV-infected patients, recorded high levels of diagnostic accuracy

for the SMAQ tool. During this validation study, the SMAQ showed 72% sensitivity and 91% specificity when compared with the medication-event monitoring (Knobel et al, 2002). Not only that, non-adherence assessed by the SMAQ was the only factor associated with a failure to suppress viral load after adjusting with other factors (Knobel et al, 2002). The apparent discrepancies between our diagnostic accuracy rates for SMAQ adherence may be due to different drug in the ARV regimen and the gold standard used during the validation study in Spain. In our study, the viral load was considered as the reference standard whereas in the validation study by Knobel et al (2002), they used an electronic device and adherence was recorded as the number of doses recorded by the electronic system over the total number prescribed during the follow-up (Knobel et al, 2002). Additionally, in their study, the HIV-infected patients in whom adherence was assessed were initiated on a Nelfinavir-based PI regimen which is different to NRTI and NNRTI regimens used in standard first-line ART in South Africa.

- **Multi-method approach**

Our findings showed that during the average period of six months, the multi-method tool which combined VAS, PIT and self-report questionnaire into a single adherence measure was not more accurate than the simple self-reported adherence such as VAS and SMAQ. In contrast, another study from Steel (2007) has shown that the validity of applying a combination of tests is higher compared to a single test to assess adherence to antiretroviral treatment (Steel et al, 2007). This multi-tool approach has also been recommended by WHO since 2003 (WHO, 2003). In a large evaluation study carried out in South Africa by Steel and colleagues (2002), a multi-method approach was demonstrated to be superior to single measures which overestimated adherence amongst patients on “d4T-3TC-EFV” regimen. This study also demonstrated a strong positive correlation between high viral load and high level of adherence

assessed with the multi-method approach. The fact that in our study, HIV-infected patients were ART naïve prior to treatment initiation and the fact that we adjusted for other variables that might have influenced or confounded the relationship between multi-method approach and viral load may explain these differences. Additionally, even though multi-method tool is indicated to reduce the error associated with single self-reported measures of adherence, evidence is lacking on how to best combine different measures of adherence into a single accurate and reliable tool (Berg & Arnst, 2010).

In our study, we used a cut-off of 95% adherence or better as proxy for viral load suppression. This cut-off was based from previous studies that found that this level of adherence would at least induce 80% of viral load suppression. However, since this level of adherence can be sometime very difficult to attain due to different causes that include: lack of social support, demographic factors, drug resistance, pharmacokinetic factors and side effects. Additionally, some authors (Bangsberg, 2006; Viswanathan et al, 2016) have suggested that a lower level of adherence may be required in order to remove all the barriers and improve access to ART. Additionally, it is possible that the advanced pharmacokinetics of new ARV drug and their lowered genetic barriers may influence the level of adherence required for viral load suppression. Thus, a study by Bangsberg et al (2004) showed that the level of adherence required to suppress viral load was lower that usually expected. In a longitudinal cohort study amongst 1552 HIV adult persons including men having sex with men and injection drug users, Viswanathan and colleagues (2016) showed that at adherence levels between 80 and 84%, the odds of viral load suppression were not significantly different than that in patients with $\geq$ 95% adherence levels. Further studies that could confirm this finding in South Africa are needed.

4.7 Diagnostic risk score and viral load

We used the modified Poisson Regression models to derive a continuous risk score at two-time points: during the first 6 and 12 months on ART. The diagnostic risk score performed well at 6 months. However, at 12 months, the continuous diagnostic risk score was uninformative and performed less than the self-reported adherence measures. The fact that more adherent patients at the first 6 months were more likely to survive until 12 months and we measured the performance of the 12 month's risk score with a prevalent cohort and stable patients may explain this situation (Miller et al, 2012) .

During the first 6 months, we found that a predictor score including demographic variables, laboratory and clinical information follow-up, self-reported adherence and the information on missed ARV visits or medical visits performed better than the self-reported adherence measures for predicting detectable viral load. The predictor score at 6 months included age, gender, platelet count response, WHO stage, CD4 count response, VAS score and the missing ARV or medical visits. The AUC to detect failure to suppress viral load was calculated as 0.63, meaning that we should expect that randomly selected patients with detectable viral load would have a higher predicted probability or risk score of about 63% of the time than randomly selected patients without detectable viral load (Hanley & McNeil, 1982). Taken alone, the AUC of the 3 self-reported adherences were 0.56, 0.52 and 0.52 respectively for the VAS, SMAQ and multiple approach method, meaning that they cannot compare between patients with and without detectable viral load, and there is a little increment value gained between these 3 self-reported adherence measures. We considered two cut-off values for the risk score (risk score $\geq$ 5 and risk score $\geq$ 4) for providing the optimal true positive rate while decreasing the false negative rate. For example, the application of the predicted risk probabilities at the cut-off point of $\geq$ 4 (Se 76.4% vs VAS 24.50%, SMAQ 26.5%, multiple-approach method 18.4%) would

result in significant decrease of false negative results than the use of the self-reported adherence measures.

Additionally, if we consider that missing a patient with detectable viral load is more dangerous than incorrect identification of patients with a good adherence to ARV, the cost of false negative is more important than the cost of false positive. Therefore, the cut-off (≥ 5 vs < 5) although not optimal, seems to be the best to classify HIV-positive patients as adherent and non-adherent. The cost of false negative results may include mortality, virologic treatment failure and switch to more expensive second line treatment.

Of the 3 self-reported adherence methods, only a VAS < 95% during the first 6 months and after adjusting for other variables in the modified Poisson multivariate model, was significantly associated with a failure to suppress viral load. The AUC of the VAS at 6 months was only 0.53. In their study among HIV-positive patients in Eastern Europe, Chkhartishvili et al (2014) used a multivariate modified Poisson regression model to evaluate the measures of antiretroviral adherence in HIV-patients in Georgia. In their model, after adjusting for age, gender and other adherence measurements (refill adherence, four-day ACTG questionnaire), the VAS value < 95% was associated with viral load suppression with AUC being 0.64 and 0.72 for viral suppression of < 400 copies/ml and < 50 copies/ml respectively (Chkhartishvili et al, 2014). However, in their study the self-reported adherence was measured only three months after enrollment and they excluded individuals with missing values (Chkhartishvili et al,2014). Thus, it is difficult to compare the validity of the results in a long-term period such as at 6 or 12 months on ART.

Some studies among HIV-positive patients in developed countries have developed risk scores derived from combining self-reported adherence measures and laboratory and clinical markers in order to predict viral load in patients on ART (Lynen et al, 2009; Segeral et al, 2010 ;Van Griensven et al, 2014; Colebunders et al, 2006; Evans et al, 2014). However, most of them used more stringent criteria to target virological failure or first line treatment failure instead of using the cut-off point of > 400 copies/ml for viral load as we did in thïs study. In their study from Cambodia, Lynen et al (2009) used laboratory data, clinical information and self-reported adherence measures (SMAQ and VAS) to develop a scoring system in HIV-patients with suspected first line therapy failure and on treatment for at least 12 months. A risk score >2 including hemoglobin drop >1g/dl, CD4 count response below baseline, 25% drop in CD4 from peak, CD4 <100 cells/mm^3, VAS < 95% adherence had an optimal combination of sensitivity (41.4%) and specificity (92.6%) to target virologic failure which was defined as one viral load >1000 copies per milliliter (Lynen al . 2009). Similar to the results of our study, this study in Cambodia showed that a VAS value < 95% was predictive of having a viral load >1000 copies/ml after adjusting with other variables in the multivariate model (Lynen al. 2009). Similarly, an algorithm developed in Uganda used a scoring system including CD4 count, MCV ≤ 95 fL, percentage adherence ≤ 90%, and clinical information to predict virologic failure. A cut-off score of 3 was chosen and resulted in a sensitivity of 40% in the derivation population and a PPV of 100% (Colebunders et al, 2006). However, in these two studies, the authors did not mention the follow-up period during which the diagnostic risk score may be applicable or discriminative.

In our study, we defined and evaluated the diagnostic risk scores for two follow-up periods and the fact that our study was prospective with ART-naïve HIV-infected patients strengthens the discriminative value of our risk score. Our results implied that, within the first six months, the

diagnostic accuracy applied with risk threshold of 4 or 5 can help to identify HIV-infected patients for therapeutic group support or viral load testing in a more cost-effective manner. At the cut-off score of 5, the risk score for targeted viral load testing at 6 months correctly identified 65% (47/72) of patients with a detectable viral load, while reducing viral load test by more than 40% and this saved 103 viral loads according to current guidelines. In South Africa, Evans et al (2014) used routinely collected electronic health data from ART clinic to identify virologic failure defined as 2 consecutive HIV-RNA ≥ 400 copies/ml. The algorithm developed by Evans et al (2014) was done in a large retrospective dataset and resulted in more variables being included in the final CPS with a combination of age, CD4 count < 100 cells/mm^3, WHO stage III/IV, albumin < 25 g/dl and laboratory and clinical follow up data. Additionally, they found that a score including CD4 criteria identified better patients with treatment failure than a score without CD4 criteria and better than WHO immunologic and clinical criteria (Evans et al,2014).

Table 17 Sensitivities and specificities of different CPS in the literature.

Reference	Outcome	Se	Sp	PPV	NPV
Lynen et al, 2009	Viral load > 1000 copies/ml	41%	92.6%	22.1	96.9
Meya D et al, 2009	Viral load failure < 1000 copies/ml	67%	82%	100%	97%
Keiser et al, 2009	Virologic failure: > 10000 & > 500; 2 measurements	12.6% and 48.1%	86.8 and 97.3%	9.5% and 19.0%	98.5% and 95.7%
Evans et al, 2014	2 consecutives HIV/RNA load > 1000 copies/ml				
Score with CD4 criteria (≥ 4vs.< 4)		57.1 %	50.5%	27.8%	77.9%
Score without CD4 criteria (≥ 4 vs.< 4)		40.9%	52.7%	64.0%	50.0%
This CPS (≥ 5 vs. < 5)	Single elevated viral load > 400 copies/ml	64%	50%	35.6 %	75%

Se: sensitivity Sp: Specificity PPV: Positive predictive value NPV: Negative predictive value

4.8 Plausible reasons for missingness and impact of the missing data

The exploration of the missingness mechanism prior to the imputation model aimed at seeing if the missing data in the predictors or the outcome are predictable from the observed data in the LCM dataset. Our analysis shows that the MCAR mechanism seems to be more consistent with the observed dataset, meaning that the probability for a missing viral load value was not associated with any predictor in the LCM dataset. Similarly, the probability that a value was missing for one of the predictors was not related to failing to suppress viral load at 6 or 12 months. Since the missing data are not related to the values, the observed data in the LCM study can be considered as representative of the LCM study population and the occurrence of the MCAR mechanism doesn't cause bias in the analysis (Carpenter and Kenward, 2013). In this situation, the result from the complete case analysis reflects the analysis of data which is available and doesn't cause bias except that the sample size is reduced (Donders et al, 2006) (Collins et al, 2015). Thus, we can considerer the results from the complete case analysis as unbiased estimates and representative of the study population.

Additionally, the fact that data are MCAR, may explain the reason why the complete case analysis and the analysis from the imputed dataset gave different results at 6 and 12 months.

4.9 Limitations of the study

The results from this study should be considered in light with several limitations. First there were many factors that may have affected the viral load that were not available in the LCM dataset, such as drug resistance, other co-infection or following immunization. Also, because some patients had not undergone a viral load assessment, we used the window period of 6-9 months and 10-12 months to capture the 6 and 12 month's viral load respectively. This approximation reduced the missing data which may have affected the results. Additionally, the

fact that not all patients underwent HIV viral load assays may have led to verification bias and biased estimates.

Secondly, our study only included patients that were initiated at Themba Lethu Clinic and who were not referred from another facility. This type of patient selection might have led to highly selected patients not representative of patients on ARV treatment in South Africa. Moreover, TLC is an HIV specialized center with highly skilled staff and clinical practices in HIV treatment that might differ significantly from other health centres in South Africa. Therefore, our results may not be generalizable to the overall population. Regarding the diagnostic risk score, results from stimulation studies have demonstrated that the statistical power in a prediction model is based on the number of event per variables (EVP) and a minimum of 10 EVP is required for a reliable prediction (Moons et al, 2015; Collins et al, 2015).This rule was not respected during the primary data collection at TLC as we had an average of 9 EVP and 4 EVP for the 6 and 12-month prediction model respectively.

Fourth, in our study, we used viral load suppression as gold standard to assess adherence to antiretroviral therapy because of sample follow up, we could only look at viral load suppression defined as a single measure of HIV/RNA $\geq$ 400 copies/ml.

Finally, the main limitation is related to the inconvenience of using routine clinical care data which resulted in a lot of missing data, some authors suggest that variables with more than 50% of missing data should be excluded from the application of the diagnostic model as they may be more difficult to obtain in routine care data (Steyerberg & van Veen, 2007).

CHAPTER FIVE - CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

The conclusion and recommendation are based on the results and their discussion in the preceding chapter. In this chapter, we also highlight some recommendations and suggest the potential areas where further research is needed.

5.2 Conclusion

Adherence to antiretroviral therapy remains a key aspect for the success of the antiretroviral programmes. Currently, tremendous efforts are being put into improving adherence to ART in patients on first-line regimens in developed and resource-limited settings. However, despite the abundant literature, adherence to ART is not easy to monitor and the bias associated with the use of self-reported adherence measures has been previously reported. Other measures include plasma viral load monitoring, therapeutic drug monitoring, pill count, pharmacy refill data, electronic monitoring device (MEMS). However, these methods are costly and difficult to implement in resource-limited setting. An accurate and low-cost adherence measure based on routine clinical data and laboratory markers available in daily practice may be useful to predict viral load and monitor adherence to ART in patients on first line ART. Our study aimed to identify the usefulness of a composite marker of poor adherence assessed by failure to achieve virologic suppression and defined as a plasma viral load ≥ 400 copies/ml in patients on first-line ART at Themba Lethu Clinic, in Johannesburg, South Africa

In this study, the prevalence of patients with a detectable viral load was 30.1% and 28.5% at 6 and 12 months respectively. By 6 months, the risk factors associated with a detectable viral load were having a six months platelet count <100 cells/mm^3, a visual analogue scale value < 95% and “having missed at least one ART or medical visit”. While at 12 months, only variables

such as being aged older than 35 years, unemployment and alcohol consumption were related to a detectable viral load. More than 50% of HIV-positive patients were initiated on a TDF-based regimen but there was no association between the ART-regimen and the viral load. These findings confirmed that patients initiating ART are more vulnerable to poorer outcome during the first six months on ART, regardless of the first-line regimen they were initiated on. Additionally, our findings highlight the importance of better and regular monitoring of the antiretroviral drug therapy within this first six months.

In the analysis using the case complete analysis, except for platelet count, the changes observed in laboratory markers were not associated with a detectable viral load at 6 and 12 months on highly active antiretroviral treatment (HAART). This may be due to the large amount of missing data in the predictors and outcomes and the smaller sample size in our study. Our results may also suggest that some changes observed in laboratory markers are mostly associated with the long-term prognosis of HIV/AIDS infection rather than being an early predictor of the treatment effect.

Overall, HIV-positive patients reported high-adherence levels at 6 months which decreased slightly at 12 months. In contrast, when the self-reported adherence measures were compared with viral load, their specific sensitivities were very low, resulting in a higher proportion of false negative results. This misclassification may be explained by the overestimation of adherence that is associated with the self-report tools and reported in previous studies. As a result, this high proportion of false negative test may expose HIV-positive patients to an increased risk of virologic and treatment failure and later drug resistance.

We developed two continuous diagnostic risk scores derived from the Modified Poisson regression models at 6 and 12 months. The 6 months diagnostic risk score which combined patient demographic characteristics (age, gender), WHO stage, laboratory markers (platelet

count, MCV and CD4 count, missing ARVs or medical visit) showed a high discriminative value.

Additionally, there was higher sensitivity, specificity and negative predictive values at two different cut-offs (risk score ≥ 5 and risk score ≥ 4) compared with the self-reported adherence measures. However, at 12 months, the risk score derived from the modified Poisson regression model showed overall lower discriminative value (ROC area of 0.4) and was uninformative when compared with self-reported adherence measures. As a result, the high sensitivity and specificity of the risk score during the first six months indicates that this method is an important tool that can be used to reduce the high proportion of patients falsely classified as adherent by the self-reported adherence measures.

On the other hand, the high predictive value means that it is possible to rule-out poor adherence in large numbers of patients, reducing unnecessary stress and burden. Our results indicate that if we consider that missing a patient with detectable viral load is more dangerous than incorrect identification of patients with good adherence to ARV, the cost of false negatives is more important than the cost of false positives. Therefore, the cut-off, although not optimal (≥ 5 vs < 5) seems to be the best to classify HIV-positive patients as adherent and non-adherent. The cost of false negative results may include mortality, restriction of quality of life, virologic treatment failure and therapeutic cost.

Our findings suggest that during the first six months on ART, a diagnostic risk score tool can be used to estimate the probability that an HIV-positive patient has a detectable viral load. HIV-infected patients with a high-risk score (risk score ≥ 5 or risk score ≥ 4) may be helped to get additional support and counselling such as therapy group regimen and information on how to take their ARV drugs. In resource-limited settings, the viral load may only be used to confirm the diagnosis during the first six months. In this study, the predictors used are readily available

in routine care data and the risk score was developed with cut-off values in line with clinical practices. Thus, they are easy to adapt in routine clinical care and in daily practice during medical visits.

5.3 Recommendations

Following our findings and the comparison with previous research, the following recommendations aim to enhance the validity of the diagnostic risk score and its application at Themba Lethu Clinic.

- Include the diagnostic risk score at 6 months in the routine practice at Themba Lethu Clinic for assessing its internal validity and its applicability to HIV-infected initiated receiving care from this center.
- The 6 months prediction model should also be externally validated to ensure its external applicability to other settings to improve its performance before its use in clinical care. Thus, the model can be improved by including new biomarkers or the regression coefficient adjusted before its implementation in clinical care.
- Conduct a more active follow-up of patient's information on self-reported adherence measures and laboratory markers to avoid the occurrence of missing data.
- Provide adequate training to the health professionals at TLC to ensure that all the patient's clinical information is recorded and completed in the TherapyEdge-HIV^{TM} database.
- There may be other markers of adherence to ARV treatment from routine clinical care or laboratory data. Thus, it may be useful to explore their added value in the diagnostic risk score at 6 and 12 months.

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APPENDICES

7.1 Appendix A

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG | FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Mouhamed Abdou Salam Mbengue (Student number: 830582) am a student registered for the degree of **Msc in Epidemiology** in the academic year **2016**

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.

Signature:

Date: **Wednesday, 9 November 2016**

26/04/2015
1

7.2 Appendix B

R14/49 Mouhamed Abdou Salam Mbengue

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140918

NAME: (Principal Investigator)	Mouhamed Abdou Salam Mbengue
DEPARTMENT:	School of Public Health Epidemiology and Biostatistics
PROJECT TITLE:	Predictors of Poor Adherence among Antiretroviral Treatment Naïve Patients on First Line Regimen at Themba Lethu Clinic in Johannesburg: Results from a Prospective Study
DATE CONSIDERED:	03/10/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr C Chasela and Dr D Evans
APPROVED BY:	Professor Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	03/11/2014

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

7.3 Appendix C

Health Economics and Epidemiology Research Office (HE²RO)

Themba Lethu Clinic, Helen Joseph Hospital
Perth Road, Westdene 2092, South Africa
Tel +27 (11) 276-8850, Fax +27 (11) 276-8885

A collaboration between the Wits Health Consortium of the University of the Witwatersrand and the Center for Global Health & Development of Boston University

04 September 2014

INVESTIGATOR(S): Dr Charles Chasela – School of Public Health, University of the Witwatersrand
Abdou Salam Mbengue, MSc student, School of Public Health, University of the Witwatersrand

TITLE OF RESEARCH PROJECT: **PREDICTORS OF POOR ADHERENCE AMONG ART NAÏVE PATIENTS ON FIRST LINE REGIMEN AT THEMBA LETHU CLINIC IN JOHANNESBURG - RESULTS FROM A PROSPECTIVE COHORT**

PERMISSION TO USE STUDY DATABASE

This letter serves to confirm that the abovementioned investigators have been granted permission to use the study database of the **Low cost monitoring of HIV in resource-limited settings** project. This study has approval from the University of the Witwatersrand HREC-Medical (M10418).

Permission has been granted for the **01 September 2014 – 31 December 2015** on condition that the investigators sign

(i) Standard Operating Procedure: Process to be followed for granting access to data for research on the Themba Lethu Clinical Cohort and other Cohorts stored on the TherapyEdge database and

(ii) RTC confidentiality agreement

The investigators also agree to acknowledge the study and funding in any work that emanates from using this data (NIH/CFAR Creative and Novel Ideas in HIV Research CNIHR: Low cost monitoring of HIV in resource-limited settings UAB CFAR grant number P30AI027767).

Yours sincerely,

Dr Denise Evans
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7.4 Appendix D

Patient initials__ __ __

SIMPLIFIED MEDICATION ADHERENCE QUESTIONNAIRE (SMAQ)

To be completed prior to visit:

Current ART regimen

Current regimen start date | d | d | m | m | m | y | y |

To be completed during the study visit:

1. How many of the current drugs prescribed can the patient correctly name? (i.e. 1/3)
2. How often did the patient take their medication in the last month?
   a. almost never ☐
   b. once in a while ☐
   c. half the time ☐
   d. most of the time ☐
   e. all the time ☐
3. If the patient missed doses in the 4 days preceding the study visit, which of the following can be identified as adherence difficulties?
   a. forgetting ☐
   b. running out of medication ☐
   c. trouble taking the medication ☐
   d. missing medication due to pharmacy-related problems ☐
   e. other ☐
4. Do you ever forget to take your medicine? Yes ☐ No ☐
5. Do you always take your medication at the appropriate/correct time? Yes ☐ No ☐
6. Are you careless at times about taking your medicine? Yes ☐ No ☐
7. When you feel better, do you sometimes stop taking your medicine? Yes ☐ No ☐
8. Sometimes if you feel worse, do you stop taking your medicines? Yes ☐ No ☐
9. Thinking about the last week – How often have you not taken your medicine?
   a. never ☐
   b. 1 – 2 times ☐
   c. 3 – 5 times ☐
   d. 6 – 10 times ☐
   e. > 10 times ☐
10. Did you not take any of your medicine over the past weekend? Yes ☐ No ☐
11. Over the past 3 months, how many days have you not taken any of your medicine at all?
    a. ≤ 2 days ☐
    b. > 2 days ☐

7.5 Appendix E: Characteristics of patients in the Themba Lethu Clinical HIV Cohort and in the LCM study.

Characteristics	Pre-ART (n=8217) n (%)	ART (n=21099) n (%)	LCM cohort (n=357) n (%)
Gender			
Female	5222 (63.6%)	13428 (63.6%)	226 (64%)
Male	2995 (36.4%)	7671 (36.4%)	127 (36%)
Nationality			
South African	7608 (92.6%)	19195 (91.0%)	301 (85.3%)
No South African	609 (7.4%)	1,904 (9.0%)	51 (14.5%)
Missing	0 (0%)	2 (0%)	1 (0.2%)
Education level			
No formal education	1151 (14.0%)	634 (3.0%)	13 (3.7%)
Primary school	648 (7.9%)	2946 (14.0%)	25 (7.1%)
Secondary school	2445 (29.8%)	11320 (53.7%)	257 (72.8%)
Tertiary education	160 (2.0%)	744 (3.5%)	15 (15.0%)
Missing	3813 (46.3%)	5357 (25.8%)	11 (3.2%)
Employment status			
Unemployed	4434 (54.0%)	11121 (52.7%)	206 (58.4%)
Employed	3783 (46.0%)	9978 (47.3%)	147 (41.6%)
Missing	0 (0%)	2 (0%)	0 (0%)
Characteristics at ART Initiation			
Age (median IQR)		36 (30.8 - 42.6)	37 (21.0 - 57.0)
BMI (Kg/m^2)			
< 18.5		3140 (14.9%)	22 (6.2%)
18.5 – 24.9		9025 (42.8%)	192 (54.4%)
25 – 29.9		2804 (13.3%)	74 (20.9%)
≥ 30		1302 (6.2%)	51 (14.4%)
Missing		4830 (22.9%)	14 (3.9%)
Median (IQR)		221.7 (19.1 - 25)	23.5 (16.3 - 43.8)
CD4 count category (cells/mm^3)			
< 50		5224 (24.8%)	46 (13.0%)
50 – 100		3231 (15.3%)	44 (12.5%)
100 – 200		5725 (27.1%)	89 (25.3%)
200 – 350		2359 (11.2%)	143 (40.6%)
> 350		705 (3.3%)	30 (8.5%)
Missing		3857 (18.3%)	1 (0.3%)
Median (IQR)		103 (39 - 178)	196 (98 - 268.5)
Haemoglobin (mmol/L)			
Median (IQR)		11.6 (10.0 - 13.1)	12.3 (11.0 - 13.5)
TB			
Yes		2519 (11.9%)	36 (10.2%)
No		8582 (88.1%)	313 (88.7%)
Missing		0 (0%)	4 (1.2%)
HIV viral load (copies/ml)			
≤ 1 00,000		3,197 (7.2%)	NA
> 1 00,000		1,527 (15.2%)	
Missing		16,377 (77.6%)	