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Virological rebound among re-suppressed patients after failing second-line HIV therapy in Johannesburg, South Africa

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in partial fulfilment of the requirements for the degree of
MSc in Epidemiology in the field of Epidemiology and Biostatistics

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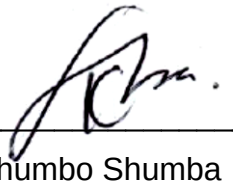
DECLARATIONS:

I Khumbo Shumba declare that this Research Report is my own work, compiled under the supervision of Dr. Dorina Onoya and Cornelius Nattey. It is being submitted as partial fulfilment of the requirements for the Degree of Master of Science in Epidemiology in the field of Epidemiology and Biostatistics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



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DEDICATION:

This research is dedicated to my late parents, Jenkins Sikhohilso Shumba, and Constance Shumba. They both have been my source of motivation throughout this work. May their souls keep resting in eternal peace.

ABSTRACT:

BACKGROUND:

While reports suggest that some HIV-infected patients on a protease inhibitor (PI)-based second-line antiretroviral therapy (ART) achieve viral re-suppression after experiencing virological failure, their risk of experiencing virological rebound (subsequent virological failure) is unclear. This study estimated the incidence rates and investigated predictors of virological rebound among these patients in Johannesburg, South Africa.

METHODS:

A retrospective cohort study design was used to analyse routinely collected adult (≥18years) patient data from Themba Lethu Clinic for patients switched to PI-based second-line ART between 1st January 2012 and 31st December 2016. The analysis was restricted to patients who experienced virological failure and achieved viral re-suppression within six months while on PI-based second-line ART. The follow-up period was 24 months, and censoring occurred at the time of death, transfer to another HIV treatment site, loss to follow-up, or at the dataset closure date (24 months from the viral re-suppression date). Baseline socio-demographic and clinical characteristics were summarised with stratification by sex. Kaplan Meir survival analyses were used to estimate incidence rates and the probability of surviving virological rebound stratified by selected covariates. Predictors of virological rebound were identified using Cox proportional hazard models.

RESULTS:

Out of 2371 patients initiated on PI-based second-line ART, a total of 246 patients (57.4% males and 42.6% females) experienced initial virological failure and achieved viral re-suppression in a median (IQR) of 3.7 (2.8-4.6) months. The probability of surviving virological rebound was significantly higher for patients who received at least five enhanced adherence counselling (EAC) sessions compared to those who received less than five (log-rank test: p-value <0.0001). The overall incidence rate of virological rebound

was 23.20 (95% CI: 18.38-29.27) per 100 person-years. However, the incidence rate was higher for males (Males: 24.39; 95% CI: 17.15-34.68 per 100 person-years vs Females: 22.35; 95% CI: 16.39-30.47). The risk of virological rebound was higher for underweight patients (BMI: <20kg/m^2) compared to those with a normal BMI (BMI: 25-29.9 kg/m^2), (aHR: 3.08; 95% CI:1.16-8.13). Patients with a low CD4 count (<350 cells/μl) had a higher risk compared to those with a higher CD4 count (>500 cells/μl) (aHR: 3.68; 95% CI:1.10-12.33). Receiving at least five adherence counselling sessions seemed to reduce the risk by 89% compared to receiving less than five (aHR: 0.11; 95% CI:0.06-0.20).

CONCLUSION:

Virological rebound is highly incident among HIV-infected patients on PI-based second-line ART who re-suppress after experiencing initial virological failure. Enhancing patient-centred interventions to improve ART adherence, CD4 count, and BMI in these patients, are essential for the reduction of switches to more expensive and limited third-line ART regimens.

Key words: HIV, second-line ART, virological failure, virological rebound

LIST OF ABBREVIATIONS:

3TC: Lamivudine

AIDS: Acquired Immune Deficiency Syndrome

ART: Antiretroviral Therapy

AZT: Zidovudine

BMI: Body Mass Index

CD4: Cluster of differentiation 4

D4T: Stavudine

EAC: Enhanced Adherence Counselling

FBC: Full blood count

HIV: Human Immunodeficiency Virus

HR: Hazard Ratio

LTFU: Loss to follow up

NNRTI: Non-nucleoside reverse transcriptase inhibitor

NRTI: Nucleoside reverse transcriptase inhibitor

PI: Protease inhibitor

r: Ritonavir

RLS: Resource-limited setting

TB: Tuberculosis

TE: Therapy Edge-HIV™

TDF: Tenofovir

TLC: Themba Lethu Clinic

WHO: World Health Organisation

VL: Viral load

DEFINITION OF TERMS:

Clinical Failure:	The occurrence of a new WHO stage III or IV opportunistic diseases while on antiretroviral therapy (ART)
Human Immunodeficiency Virus (HIV):	A retrovirus that infects cells of the immune system and results in deterioration of the immune system leading to Acquired Immunodeficiency Syndrome (AIDS).
Incidence:	A measure of the probability of occurrence of new cases of disease or health-related event during a specified period within a specific population
Loss to follow-up (LTFU):	Patients with no contact with the health facility for 180 days (6-months) from the last visit.
Immunological failure:	Inability to maintain a high plasma CD4 cell count
Protease Inhibitor:	A class of antiretroviral (ARV) drugs that prevent viral replication by binding to viral protease.
Virological blip:	After achieving virologic suppression, a single detectable HIV RNA level (≥1000 copies/mL) that is followed by a return to virologic suppression
Virological Failure:	The inability to achieve or maintain plasma HIV RNA levels of at least 1000 copies/mL
Virological rebound:	Confirmed plasma HIV RNA level greater than or equal to 1000 copies/mL after achieving virologic suppression on antiretroviral therapy
Viral / Virological supression	A reduction of viral load (HIV RNA) from high (>400 copies/µl) to a viral load of < 400copies/µl or undetectable level (<50 copies/ µl).

LIST OF FIGURES:

LIST OF TABLES:

RESEARCH REPORT OUTLINE:

This research report is divided into four chapters:

CHAPTER 1- INTRODUCTION:

Provides a background to the burden of and factors associated with second-line ART failure, providing insights on virological rebound, which warrant further research in resource limited settings. In addition, the chapter presents detailed peer-reviewed literature on virological failure or rebound while highlighting some of the gaps in this body of literature. The chapter concludes by providing the problem statement, study significance, and objectives.

CHAPTER 2 - METHODS:

Outlines the methods used to address each of the study objectives which includes providing details of the study design, data source, data management, analysis methods, and ethical considerations.

CHAPTER 3 - RESULTS:

Presents the study results, that is, both descriptive and analytical research findings, based on the analysis method used.

CHAPTER 4 - DISCUSSION:

Discusses the research findings while referring to previous literature and HIV treatment and monitoring policies.

CHAPTER 5 - LIMITATIONS:

Presents the present study's limitations and summarises its strengths.

CHAPTER 6 - RECOMMENDATIONS AND CONCLUSION

Presents future research recommendations and provides a conclusion of the study findings.

REFERENCES:

All cited references are presented in the references section at the end of the research report.

APPENDICES: Any additional information is presented in the appendices section.

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CHAPTER 1- INTRODUCTION

This chapter begins by providing a background of the burden of and factors associated with virological failure among patients on PI-based second-line anti-retroviral therapy (ART) in sub-Saharan Africa and South Africa. Next, the chapter gives a critical review of previous literature and, thereafter, outlines the problem statement, followed by the justification of this research study. The chapter ends by stating the research question, objectives, and conceptual framework of the research study.

1.1 BACKGROUND

Despite the global efforts to scale-up access to ART, a high proportion of HIV-infected patients on first-line ART experience treatment failure. Such patients are required to switch to second-line drug regimens (1,2). In sub-Saharan Africa, the number of patients switched to second-line treatment has been increasing over the years (3), from 48% in 2010 to 56% in 2016 (4,5). The region is estimated to have about 0.5-3 million patients on second-line ART by the year 2020 (3). In South Africa, second-line ART is offered based on the WHO recommendations and usually consists of a boosted protease inhibitor (PI) [lopinavir-ritonavir (LP/r) or atazanavir-ritonavir (ATZ/r)] together with two nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs). Most patients on these PI-based drug regimens (henceforth, also referred to as second-line ART) exhibit rapid undetectable viral loads and improved immunity after the switch (6–8). However, a substantial number of these patients tend to experience treatment failure within the first six months (9–11). The risk of experiencing treatment failure is higher, particularly among those who fail to achieve viral suppression after the switch to these second-line ART regimens (12). Moreover, if HIV drug resistance ensues in such patients, the efficacy of subsequent drug regimens may be compromised.

Although there are three types of treatment failure, namely clinical, immunological, and virological failure, the WHO recommends viral load (VL) testing as a gold standard technique for assessing treatment failure in the form of virological failure in resource-limited settings (13). In addition, a costly genotype drug resistance test, if available, is

mainly used for guiding the switch to third-line ART regimens for patients failing to achieve viral re-suppression while on second-line ART (13). Since the number of patients experiencing virological failure while on these second-line ART regimens is thought to be increasing, early detection of this treatment complication is critical. Diagnostic timeliness ensures that there is timely initiation of patient-centred interventions that target the achievement and maintenance of virological re-suppression in such patients.

Many studies have attempted to identify predictors of virological failure among patients on either first-line or second-line ART. Specific to second-line ART virological failure, these have been identified as mainly patient socio-demographic, clinical factors, and immunological factors. Despite the interplay of these different factors, ART adherence has singularly been described as a primary predictor of virological, immunological, and clinical treatment outcomes (14,15). Efforts have been made to improve adherence to ART, but many non-adherent patients, while on first-line ART, tend to have poor adherence when switched to second-line ART (15,16). A South African study has recommended the use of health interventions that focus on improving ART adherence to allow patients to continue using second-line ART regimens after initial failure on this treatment (15).

As a way of improving ART adherence in patients failing second-line ART, clinics provide enhanced adherence support through adherence counselling (13). Most of these patients achieve viral re-suppression with undetectable Viral Loads (VL) within 12 months after receiving this adherence support (12,17). Once this is achieved and maintained, these patients continue their treatment on second-line ART regimens with scheduled VL tests after six months, 12 months, and then annually in accordance with the usual standard of care. However, after achieving viral re-suppression, some of these patients experience virological rebound while still on these second-line ART regimens. The actual burden of virological rebound has not been well documented in resource-limited settings, including South Africa. Nevertheless, two studies in South Africa, have reported high proportions of patients (19% and 51%) who experienced virological rebound (18,19).

Although costly third-line drug regimens exist as an alternative for patients failing second-line treatment due to confirmed genotype drug resistance, these drugs are not readily available in resource-limited settings. A recent report estimates the cost of third-line ART to be six times that of second-line ART (20). Hence, only a few countries in these settings can make a provision of this treatment for these patients (1,21). South Africa, amongst the few, offers third-line ART to such patients who fail to achieve and sustain virological re-suppression due to genotype drug resistance despite receiving adherence support (1,22). In short, second-line ART remains the treatment of choice even for patients with confirmed genotype drug resistance and is often the last option available for most of these patients in resource-limited settings. Therefore, maximising the duration of second-line ART regimens is critical in such settings.

Even though most patients on second-line ART achieve virological re-suppression after experiencing virological failure through enhanced adherence support, their risk of virological rebound is unclear. Understanding the burden and associated factors of virological rebound are crucial for the development of targeted patient-centred interventions that can improve treatment outcomes in these patients. This essential information can help to reduce switches to more expensive and limited third-line ART regimens.

1.2 LITERATURE REVIEW

The present study focuses on examining the burden and determinants of virological rebound in adult patients who achieved virological re-suppression after experiencing second-line ART failure. In order to provide a detailed background for this research, the main areas of emphasis in this literature review are the HIV burden and uptake of ART, ART failure, virological failure in second-line ART, and predictors of virological failure in second-line ART. Part of the review attempts to illustrate the basis and relevance of the present study by highlighting some gaps in the literature.

1.2.1 HIV burden and ART uptake among HIV-infected patients

Human Immunodeficiency Virus (HIV) infection has remained a global burden for the past three and a half decades, making it a worldwide health priority. As of 2017, there were approximately 36.7 million people worldwide living with HIV/AIDS, of which 69% were in Africa (23). The HIV/AIDs pandemic still stands as a public health problem and, more so, in low-income and middle-income countries (LMIC), particularly in the sub-Saharan African region, which is the most affected. In 2012, reports indicated that this region contributed an estimated 69% (23.5 million) to the global HIV burden, with about 71% of global new adult HIV infections emanating from this region (24). The same report stated that most of the global deaths (70%) occurred in the same region. South Africa, in particular, has the highest number of HIV-infected individuals in the region and globally (25). In 2017, the country had approximately 7.9 million people living with HIV, and the prevalence rate in adults aged 15-64 years was 20% (26).

The scale-up of antiretroviral therapy (ART) globally, has led to an increased ART coverage in LMIC (4). This initiative has contributed to a global decline in AIDS-related deaths by 48%, from approximately 1.9 million in 2005 to 1 million in 2016 (27). Evidence indicates that HIV/AIDS mortality in sub-Saharan Africa has also declined markedly by 32% from 2005 to 2011 (24). South Africa alone started the ART program in 2004, and this significantly reduced HIV-related deaths over 13 years. From the highest record in 2005 to 2017, there was a 70% and 59% reduction among adult women (15 and older) and adult men, respectively (28). This difference among the two sexes is attributed to the discrepancy in ART coverage, which drastically improved from an overall 2% in 2005 to 58% among women but 52% among men in 2017 (28). Reports indicate that since the introduction of combined ART, there has also been a significant decline in the morbidity and risk of transmission among HIV patients (29). The increased coverage and access has also contributed to an increase in the life expectancy evidenced by an increase in the number of people living with HIV aged 50 years and above (30,31).

1.2.2 ART failure and treatment options among HIV-infected patients

Although first-line ART regimens have been quite effective and successful in the Sub-Saharan Africa region, some patients experience treatment failure due to poor adherence or having a drug-resistant HIV strain (6,15). Annually, about 6% of the HIV-infected patients who experience first-line ART failure are switched to second-line ART regimens in the region (21). This proportion is expected to increase over the years due to drug and routine VL monitoring availability (3,32). Approximately 300 000 patients were receiving second-line ART in 2013 in the region, and these numbers are also projected to increase to 2–4 million by 2030 (3). South Africa, in particular, has an estimated 10% of HIV-infected patients being switched to second-line ART after failing first-line ART (1,21). Due to increasing rates of first-line ART failure, high demand for second-line ART regimens, which are almost twice the cost of first-line ART regimens, is inevitable (33).

South Africa adopted the World Health Organisation (WHO) recommendations for HIV management and offers first-line treatment as a combination of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) with a single non-nucleoside reverse transcriptase inhibitor (NNRTI) (34). Second-line treatment is offered as a combination of two NRTIs different from those in first-line treatment, together with a protease inhibitor (PI). The PI is typically ritonavir-boosted lopinavir (LPV/r) or ritonavir-boosted atazanavir (ATV/r). Studies have demonstrated that viral re-suppression is a common finding in patients using PI-based regimens, even in the absence of the NRTI component (6,35–37). Furthermore, other studies have found that these drug regimens markedly reduce mortality (38), increase life expectancy (39), and decrease the risk of developing drug resistance (40). Third-line ART regimens, which are an alternative to second-line ART regimens exist. However, only a few countries in sub-Saharan Africa and other resource-limited settings have access. These drug regimens are costly and require drug resistance testing facilities before initiation. South Africa is one of the few countries that have limited access to these third-line ART regimens for patients not responding to second-line treatment even after efforts of improving patient ART adherence (41). However, access to these limited drug regimens is only granted to patients with confirmed PI drug resistance by an expert committee.

ART failure takes three forms: clinical, immunological, and virological failure. However, some patients may have a combination of these. All these forms have unique confirmatory diagnostic approaches, which are considered as ART monitoring techniques. Clinical failure is confirmed based on the World Health Organization (WHO) staging for HIV/AIDS and disease progression. Immunological failure is assessed by examining the Cluster Designated 4 (CD4) cell count trends over time. Virological failure confirmation entails measuring the Viral load [i.e., plasma HIV RNA (Human Immunodeficiency Virus Ribonucleic Acid) levels]. Viral load testing compared to the other two ART monitoring approaches is more practical and efficient for early detection of ART failures (42). After all, in 2013, the WHO recommended it as a preferred ART monitoring and treatment failure diagnostic approach (13).

1.2.3 Virological failure among patients on Second-line ART

In parallel with the high numbers switching to second-line ART, treatment failure in these patients after the switch has also emerged as a growing concern. According to a multi-cohort analysis of ART programs from resource-limited settings, the overall incidence rate of second-line ART virological failure was found to be 46% higher than that of first-line ART (43). In addition, a large volume of published studies from the Sub-Saharan African region, have reported varying prevalence estimates for second-line ART virological failure, ranging from 14% to 32% (8–10,15,37,44,45).

The WHO recommends a viral load threshold of 1000 copies/ml for defining virological failure based on two main research findings. Firstly, viral blips or intermittent low viremia between 50-1000 copies/ml can occur but may not be associated with virological failure (46). Secondly, the risk of HIV transmission and disease progression is lower in patients with viral loads below 1000 copies/ml (47).

Researchers have attempted to establish possible causes of persistent second-line ART virological failure in resource-limited settings. Several studies have found that PI resistant

viral mutations develop in patients with persistent virological failure while on second-line ART (48,49). Conversely, several studies in similar settings suggest that virological failure in patients on second-line ART is mainly due to poor adherence and not drug resistance (9,44,50). Again, the literature suggests that the absence of PI resistant viral mutations in patients experiencing virological failure as confirmed by genotype drug resistance tests may be a strong indication of poor adherence (51). However, studies in South Africa have reported low rates of drug resistance in patients on these drug regimens (11,14,51). This evidence shows that improving ART adherence among such patients may extend the time on second-line treatment and may also reduce rates of patients switching to the limited third-line ART.

Due to the high-cost implications, delaying the switch to third-line ART regimens is crucial in resource-limited settings. Hence, enhanced adherence counselling (EAC) is offered to patients who have elevated viral load measurements followed by a repeat viral load test 1-3 months later to check for any virological improvements. In South Africa, patients failing to re-suppress after receiving repeated EAC are permitted to switch to a third-line regimen after a confirmed genotype drug resistance. Genotype drug resistance testing is also used for generating a patient drug resistance profile, which is used for identifying the most appropriate patient-specific third-line drug combination. Research supporting the use of EAC as an intervention impacting on ART adherence and viral suppression during first-line ART is well documented (52–54). Also, previous research has shown that by triggering viral re-suppression in second-line ART patients, this intervention reduces the proportion of patients switching to third-line ART (17). Based on such evidence, the WHO has recommended this intervention to patients failing second-line treatment in resource-limited settings.

Long-term effects on virological outcomes after viral re-suppression in patients failing second-line ART have not been studied extensively. Debate continues on the best strategies for the management of such patients. In South Africa, these patients are offered routine standard care, with virological monitoring being done at six months and then annually if viral suppression is maintained. However, virological rebound is increasingly

being experienced in some of these patients. And, the available literature on this clinical complication is scanty, and there is a need for further research in this regard. So far, two South African longitudinal studies have reported conflicting proportions of patients experiencing virological rebound of 19% (18) and 61.5% (55). In view of this, one may suppose that delayed virological monitoring commonly offered in standard care (at the 6-month, 12-month visit, and every 12-months thereafter) is responsible for interruption of early virological rebound detection. Also, one may argue that virological rebound in these patients may also be attributed to poor adherence practices or genotype drug resistance, but further research is required to explore such associations.

1.2.4 Predictors of virological failure among patients on second-line ART

Factors thought to be influencing second-line ART failure have been explored in several studies. A number of these studies have reported that suboptimal or poor adherence is a primary predictor of second-line failure (6,37,56). However, other studies have attempted to identify other associated factors. Several studies have demonstrated that a high viral load at the start of second-line ART is also a strong independent risk factor (57–59). Equally, high viral loads during first-line ART have also been associated with subsequent treatment failure during second-line ART (60,61). Other researchers have shown that the type of regimen offered as a second-line treatment can determine treatment failure. Evidence suggests that the use of PI-based drug regimens containing ritonavir-boosted lopinavir has been associated with a lower risk of treatment failure (43). In addition, the literature suggests that patients with a previous TB infection or on anti-TB treatment are at higher risk of second-line ART failure (55). Patients on anti-TB treatment more commonly have a reduction in PI plasma concentration by more than 90% if the dose of ritonavir-boosted lopinavir is not adjusted upwards (62–64). As such, other research findings suggest that these patients require high tolerance to high doses of the PIs for successful TB treatment (65–67). In the case of self-reported adverse drug reactions (ADRs), these have also been linked to virological failure in patients on second-line ART (56). Furthermore, low CD4 count at second-line ART initiation has emerged as another significant predictor of second-line ART virological failure (68,69). And, one study in South

Africa has reported that patients on second-line ART with early viral re-suppression, are likely to maintain this virological status compared to those with delayed viral re-suppression (70).

While some studies have reported a higher risk of second-line ART failure among females (15,71), others have suggested that males have a higher risk (72–74). However, a significant number of studies have displayed no significant association with sex (55,56). Additionally, older age has also been considered to be associated with a lower risk of second-line ART failure in both resource-rich and resource-limited settings (37,75). This, together with education, provide a backbone for understanding and valuing the importance of treatment adherence (76). Furthermore, research has also confirmed that unemployment is a predictor of virologic failure in second-line ART (56). Other factors considered as predictors include low Body Mass Index (56) and duration on ART (9).

Evidence suggests that offering EAC during first-line ART initiation reduces the risk of poor adherence and virological failure (77). However, research on the impact of EAC on the long-term virological response on second-line ART is sparse. A recent study by Fox et al. (2016) in South Africa, limited by the absence of a comparison group, found 67% of the patients who failed second-line ART and later received enhanced adherence counselling achieved viral re-suppression (15). In contrast, a study conducted in Swaziland with a comparison group found conflicting results on this association (17). Even though this intervention is thought to have beneficial results on adherence, its effect on long-term virological response may be considered as inconclusive.

No previous study has investigated the predictors of virological rebound among patients on second-line ART who achieved virological re-suppression after experiencing initial failure. However, Le Moing et al. (2002) conducted a longitudinal study in a resource-rich setting to establish the predictors of virological rebound in patients initiated on PI-based drug regimens as a first-line treatment (77). The study found that lower adherence, a baseline CD4 count of <500 $\times 10^6$ cells/l, current lower CD4 count, baseline viral load of

<400 copies/ml, younger age, and longer duration on antiretroviral treatment were significant predictors.

1.3 PROBLEM STATEMENT

Monitoring patients on ART is crucial for successful treatment, establishing poor treatment adherence, and confirming treatment failure. Accordingly, multiple resources are required to ensure successful ART monitoring. As the ART programmes and coverage expand, patient monitoring becomes a challenge in the public health sector in resource-limited settings with insufficient healthcare workforce. With the increasing number of patients on ART against the available health care workforce, monitoring is more likely to be compromised (78,79). This challenge coincides with the reportedly high rates of virological ART failure among patients on second-line ART regimens. In such an environment where monitoring is a challenge, virological rebound experiences are bound to occur.

In South Africa, because third-line ART is costly, this alternative for patients failing second-line ART is only restricted to patients with confirmed genotype drug resistance (41). Such patients need to be reviewed by a third-line ART expert review committee under the National Department of Health in order to be granted access to these drug regimens. With this limited access, patients on second-line ART who experience virological rebound resulting from genotype drug resistance may not be initiated on third-line ART regimens on time. This issue has grown in importance since second-line treatment failure is increasingly becoming common in the country (80). Questions have been raised about what can be done to improve the durability of treatment on second-line ART regimens. Based on WHO recommendations, patients failing second-line ART are offered EAC to improve ART adherence, which prevents further virological rebound and promotes maintenance of a viral suppressed state. With the ongoing enrolment of HIV-infected patients in large ART programmes in such resource-limited settings, the quality of patient-centred care also becomes compromised (81). Thus, adherence support may not be sufficiently offered or offered at all to some patients failing these drug regimens, and this may have implications on long-term treatment outcomes. Even though patients receive EAC, studies in South Africa have reported high proportions of these patients

experiencing virological rebound after viral re-suppression (18,55). For this reason, a focus on pragmatic strategies to sustain patients on these second-line regimen is highly crucial.

1.4 JUSTIFICATION

The literature review reports second-line treatment failure as a growing global problem, especially in the sub-Saharan Africa region, which has the highest HIV burden. In addition, South Africa stands with the highest HIV prevalence globally and has an increasing number of such patients. The high burden of HIV disease in the country is enough to necessitate the search for possible contributing factors to the high rates of treatment failure among HIV-infected patients on the available treatment options.

An increasing concern of poor long-term treatment outcomes in patients failing second-line ART has ensued. One of these outcomes to be considered is virological rebound, particularly among patients who achieved viral re-suppression after experiencing initial virological failure while on this treatment option. However, the literature review has suggested that certain aspects related to the occurrence of virological rebound in patients on second-line ART have not been wholly explained. The determination of its occurrence has been hampered by the lack of research focusing on this aspect. The absence of scientific knowledge in this area, calls for the need to investigate the actual burden of virological rebound and explore predictors of this health challenge in HIV disease management. In addition, the available literature on virological rebound in these patients, particularly in South Africa, is quite scanty. Most of the research available has mainly focused on the occurrence of initial virological failure after being switched from first-line ART. Moreover, the few studies that have reported on the burden of virological rebound have used small sample sizes making their evidence not be representative of the actual burden in the study settings. Such paucities underscore the importance of further research to determine the incidence rates and predictors of virological rebound in such patients.

Overall, this knowledge base is crucial for designing effective preventive strategies of virological rebound and other strategies aimed at improving treatment outcomes of

patients on second-line ART. Also, this information would provide further insights on treatment monitoring which may inform future interventions to help reduce the growing demand for third-line ART regimens.

1.5 STUDY RESEARCH QUESTION, AIM AND OBJECTIVES

1.5.1 Research Question:

This study aimed to address the following research question:

- *What are the incidence rates of, and factors associated with virological rebound among re-suppressed HIV-infected patients aged adults (≥ 18 years) after failing second-line ART in Johannesburg, South Africa?*

1.5.2 Aim

This study aimed to determine the incidence rates and predictors of virological rebound among re-suppressed HIV-infected adult patients after failing second-line ART in Johannesburg, South Africa.

1.5.3 Objectives

Outlined below are the objectives of this study:

1. To describe the demographic and clinical characteristics of adult patients who re-suppressed after failing PI-based second-line ART.

2. To determine the incidence rates of virological rebound among adult HIV-infected patients who re-suppressed after failing PI-based second-line ART.

3. To investigate predictors of virological rebound among adult HIV-infected patients who re-suppressed after failing PI-based second-line ART.

1.6 CONCEPTUAL FRAMEWORK

Implementation of this research study's objectives was guided by a conceptual framework that explicates the interplay of several factors that may influence patient treatment adherence while on second-line ART. In the conceptual framework, ART adherence was hypothetically considered as a potential primary driver of virological rebound among patients who achieved viral re-suppression after their initial treatment failure whilst on second-line ART.

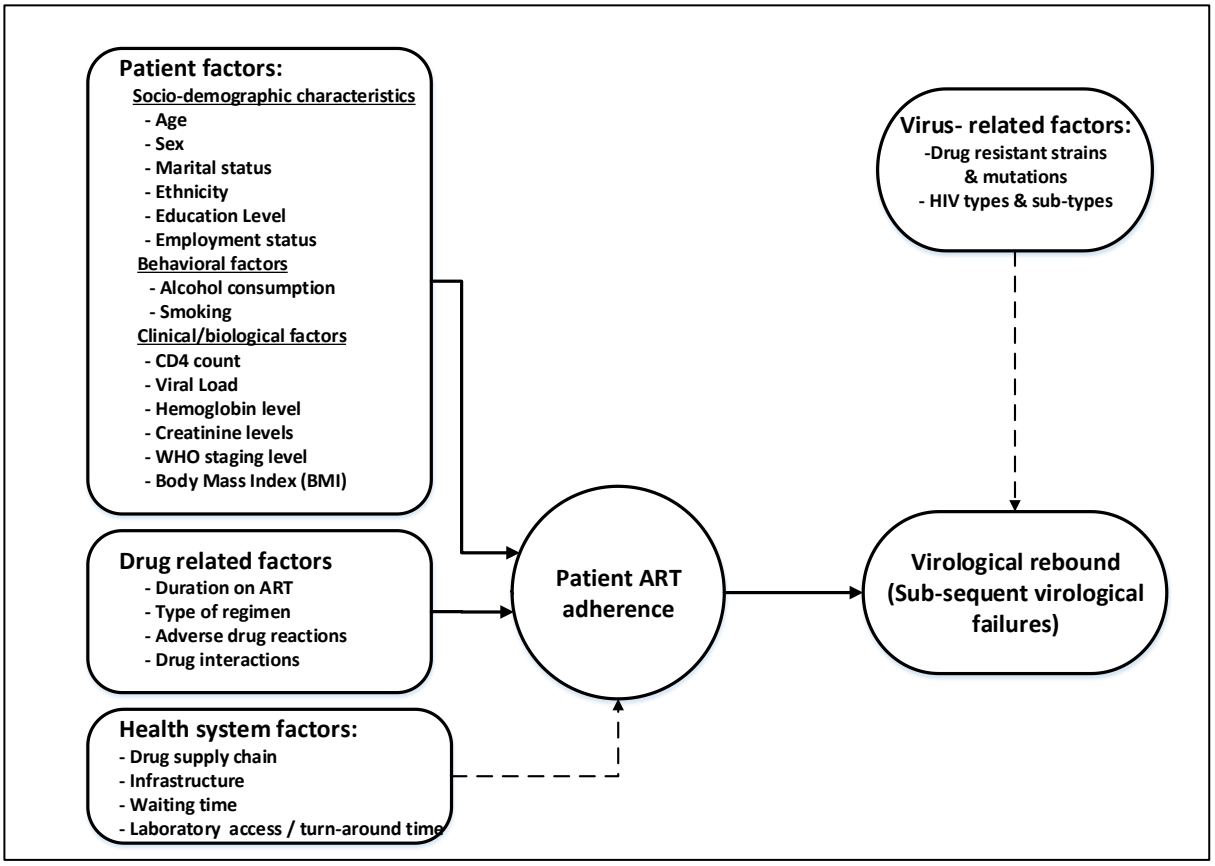


Figure 1 Conceptual framework of factors associated with Virological rebound

Figure 1 illustrates the relationship of these factors in influencing the occurrence of virological rebound in these patients. These factors include patient-related, drug-related, health system, and virus-related factors. However, the present study focused mainly on patient-related and drug-related factors. Patient factors considered were demographic factors (age, sex, marital status and employment status), behavioural factors (alcohol

consumption and smoking history), and clinical factors (CD4 count, viral load, and anaemia). Drug-related factors included duration on ART, type of regimen combination, and adverse drug reactions. For this study, the assumption made was that patient-related and drug-related factors directly influence treatment adherence based on the literature review. The hypothesis considered for this study is that poor performance in these factors may be associated with poor ART adherence, which has been well documented as a primary predictor of virological failure. This principle was applied to this study, where the outcome considered was virological rebound.

In contrast to most previous studies, the outcome in the present study was measured among re-suppressed adult patients who had experienced initial virological failure while on second-line regimens. However, most studies have suggested that these factors may be considered as common predictors of the initial virological failure. For that reason, another hypothesis considered is that these factors may also be primary drivers of virological rebound.

CHAPTER 2 - METHODS

2.1 INTRODUCTION

This chapter presents the methodology used in data collection, data management, and analysis. It begins by providing a brief description of the study design, study setting, study population, and sampling methods used. Then data collection methods are described, followed by a presentation of measurement variables and data management methods used in this study. Next, a step by step description of the analysis methods that were applied to address the study objectives is reported. The chapter ends by reporting all the ethical considerations of this study.

2.2 STUDY DESIGN

The study used a retrospective cohort study design. The design was employed since it was suitable for estimating the incidence of virological rebound. Also, this was appropriate for exploring factors predicting the risk of virological rebound over time in this context patient demographics, immunological, and clinical characteristics. Also, considering that the study used existing prospectively collected patient data of a subset of patients from a parent clinic cohort, this study design was suitable for measuring causal association since the temporal sequence of the exposure factors, and virological rebound was explicit.

2.3 STUDY SETTING

The study used routinely collected patient data from the Themba Lethu Clinic (TLC). TLC is one of South Africa's largest urban-based clinics, situated at Helen Joseph hospital in Johannesburg. Figure 2 shows the geolocation of this study site. TLC has been operational since its establishment in 2004, and its patient services are guided by South Africa's National HIV Treatment Guidelines, which are based on the WHO treatment recommendations. It operates under the support of a South African non-governmental organisation (NGO), Right to Care. The Themba Lethu Clinical Cohort, which has a detailed profile (82), has been followed-up since the clinic became operational; and is

estimated to have about 40,000 patients receiving first-line ART and over 3000 who are on second-line ART (12). The clinic offers genotype drug resistance testing, and third-line ART regimens have been available to qualifying patients failing second-line since 2012 (82). Patients who fail second-line ART and achieve viral re-suppression (< 400 copies/mL) after receiving EAC are offered routine standard monitoring of ART. According to the guidelines, such patients have viral load tests after six and then at 12 months, followed by annual tests if viral suppression is maintained (83).

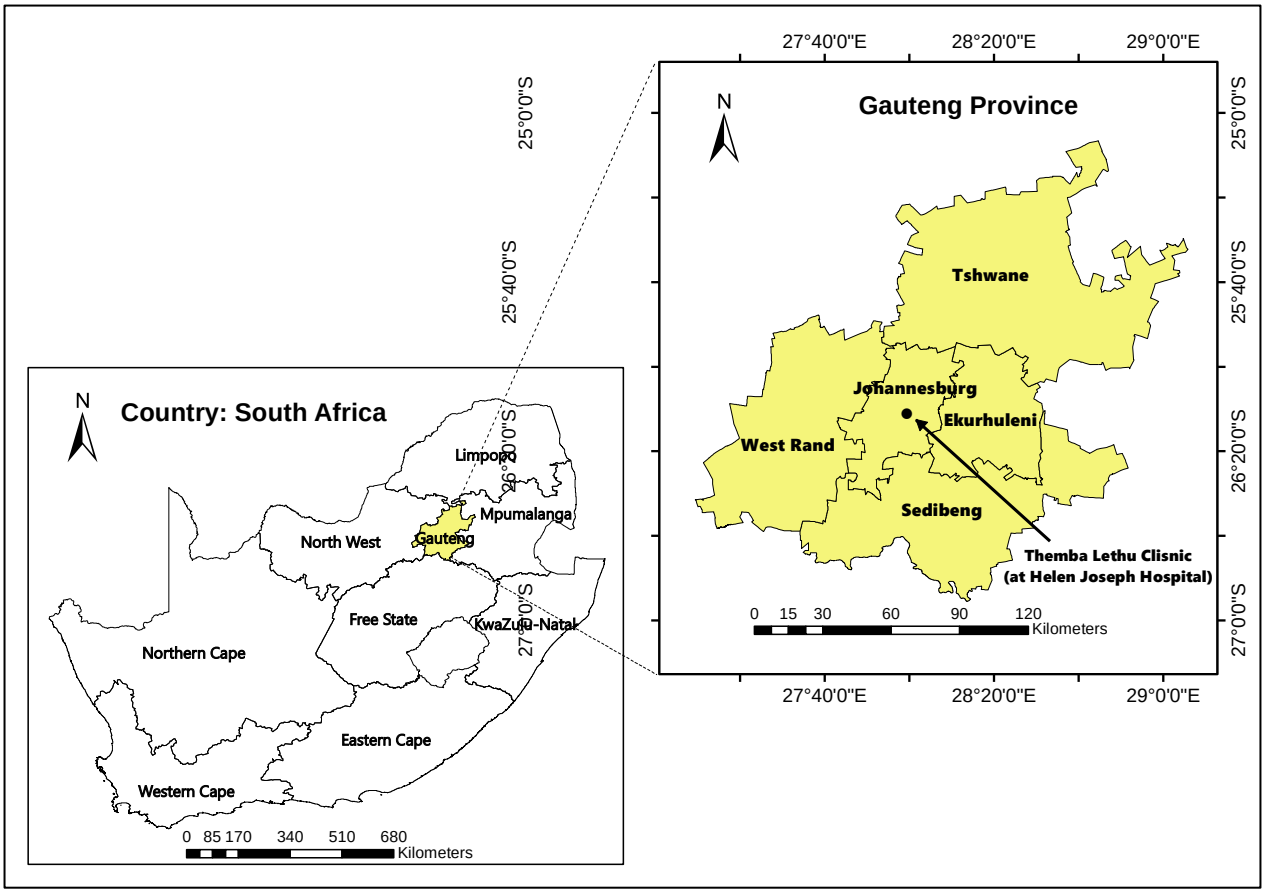


Figure 2 Map of the study site

2.4 STUDY POPULATION

This study included HIV-infected adult patients who achieved viral re-suppression within 12 months after failing a PI-based second-line ART regimen under TLC care within the

period between 1st January 2012 to 31st December 2016, in South Africa. These patients formed a sub-set of patients from the parent Themba Lethu Clinical Cohort, that met a set of inclusion and exclusion criteria. These are stated below:

2.4.1 Exclusion and inclusion criteria:

This secondary data analysis was restricted to adult patients (≥ 18 years) who achieved viral re-suppression after failing a PI-based second-line ART, precisely those who had their virological response monitored after viral re-suppression at TLC. Patients who failed second-line ART but never achieved viral re-suppression within 12-months and pregnant women were not included in the analysis. Also excluded were patients who dropped out of the TLC cohort within 3-months after second-line ART initiation, initial virological failure, and viral re-suppression after the initial virological failure.

2.5 SAMPLING

The longitudinally collected patient data at Themba Lethu clinic is captured on an electronic patient management system known as Therapy Edge-HIV™ (TE). Data for this sub-set of interest was extracted based on the set inclusion and exclusion criteria. The subset was followed up for 24-months to establish the occurrence of the outcome of interest (virological rebound) from the date of confirmed viral re-suppression (baseline). Censoring occurred at the time of loss to follow-up (LTFU), death, transfer-out, change to a non-PI based regimen, or at the end of the 24-months follow-up. In this analysis, type I right censoring, as displayed in Figure 3, was used since the outcome of interest was observed before or at the end of this prespecified follow-up time. Patients who maintained viral suppression throughout the follow-up period were censored at the last VL test or last clinic visit. Closure of the dataset was set as 31st December 2018 to allow for a maximum 24-month follow-up time.

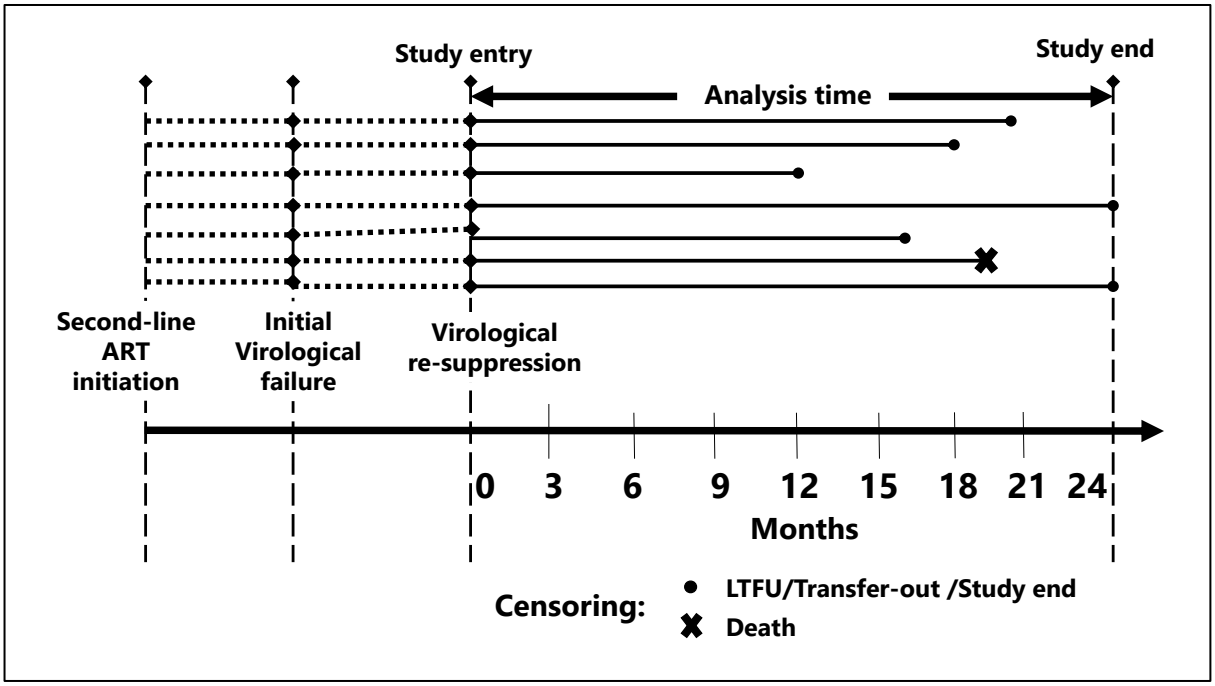


Figure 3 Study participant flow through the study

2.6 ESTIMATED SAMPLE SIZE

According to studies conducted in South Africa, the proportion of study participants on second-line therapy that experienced virological rebound after failing second-line ART on the repeat viral load test after receiving EAC was 19-61.5% (17,55). With reference to these figures, it was assumed that 40% of these patients under standard conditions would experience virological rebound, and that this proportion would increase to 50%. With this assumption, a sample size of 191 was estimated. This sample size was considered statistically sufficient to detect such an increase as being statistically significant at the 5% level of significance with an 80% power.

2.7 DATA COLLECTION AND EXTRACTION

Demographic data was captured at the time of entry into the parent TLC cohort, which for most patients was the time of ART initiation or transfer from another HIV treatment site. Patient clinical and anthropometric characteristics measured over time were entered in TE at each clinic visit. This information was directly captured into TE and updated when

necessary. Clinical laboratory tests were processed and analysed at the National Health Laboratory Service (NHLS) branch within Helen Joseph hospital. An integrated system enabled new laboratory results to be updated directly in TE. Data for the present study was extracted from TE as a subset of the parent TLC cohort. This subset of interest was identified, guided by the inclusion and exclusion criteria. All data were de-identified and anonymised prior to analysis.

2.8 STUDY VARIABLES

2.8.1 Outcome variable

The outcome variable of interest, virological rebound, was measured as a binary variable. Two definitions were considered in this study. Virological rebound was defined as an elevated VL of ≥1000copies/ml at least three months later from baseline. Two consecutive VLs, which were 3-months apart with the first VL as 400-999 copies/ml followed by a VL ≥400 copies/ml, 3-months from baseline were also considered as virological rebound. Patients who maintained viral re-suppression, i.e., Low VL of <400 copies/ml, including undetectable levels (<50 copies/ml), were considered not to have experienced virological rebound in or at the end of the follow-up period.

2.8.2 Exposure variables

Baseline socio-demographic characteristics included age, sex, nationality, employment status; and baseline lifestyle factors considered were smoking status and alcohol use. Age was considered as both a categorical and continuous variable. The categorical variable was categorised into three categories <30 years, 30-49 years, or ≥50 years. Sex was a dichotomous variable with two categories, male or female. South African nationality, employment status, smoking history, and alcohol use history were also binary variables with categories defined as yes or no.

Baseline Body Mass Index (BMI) was another exposure variable included in this analysis. This was considered as an ordinal categorical variable categorised using the conventional

WHO classification (84) : underweight (<18.5 kg/m^2), normal BMI (18.5-24.9 kg/m^2), Overweight (25-29.9 kg/m^2) or obese (≥30 kg/m^2).

Baseline clinical characteristics included CD4 count, VL, anaemia, TB co-infection history, and ART adherence at the time of viral re-suppression. CD4 count was categorised into three categories as an ordinal variable <350, 350-499, or ≥500 cells/µl. Viral load was categorised into two categories as a binary variable with <200 or 201-399 copies/ml. Anaemia was defined based on the WHO classification (85), as any haemoglobin below 11.5g/dl for females and <13.0 g/dl for males. WHO staging was considered as an ordinal categorical variable with four categories based on the WHO HIV clinical staging criteria (stage 1, 2, 3 or 4) (86). Furthermore, TB co-infection history a dichotomous variable (yes or no) was any record of TB diagnosis before and at baseline. ART adherence at baseline was self-reported based on the pills taken in the previous week. This binary variable was defined as ≥90% (all pills) and <90% (not all pills).

Other variables considered in the analysis were duration on ART, duration on second-line ART, adverse drug reactions (ADRs), frequency of EAC, and type of second-line ART Regimen. Duration on ART was categorised into four ordinal categories, <1year, 1-2 years, 3-4 years, or ≥five years. Duration on second-line ART was dichotomised into two categories <2 years or 2-5 years. ADRs were diagnoses that occurred during the study's follow-up duration, and these include dermatitis, gastroenteritis, gynaecomastia, hepatitis, lactic acidosis, lipodystrophy, muscle/joint pain, or neuropathy. The ADRs covariate was a binary variable (yes or no) defined based on this list. The frequency of EAC was defined as the number of EAC sessions a patient received from baseline, and this was categorised into a binary variable (<5 or ≥5 sessions). A nominal variable for the type of ART regimen was defined using four categories, AZT+3TC+LPVr, 3TC+LPVr+TDF, d4T+3TC+LPVr, or other ART regimen combinations. The latter category comprised of non-standard PI-based (LPVr or ATV) regimen combinations.

In the descriptive analyses, some of these exposure variables were also measured as continuous variables. However, all variables were considered as categorical variables in the inferential analysis.

2.9 DATA MANAGEMENT

Extracted data from TE was converted to Stata format, and all patient identifiers were removed from the data. Data preparation for analysis involved pooling data from different clinic departments with reference to patient identifiers, viral load test dates, ART regimen start and end dates. Data for all patients not meeting the inclusion criteria were excluded. Data cleaning involved data consistency checks, checking for outliers, and missing data. Some of the inconsistencies were corrected using other existing data variables in the dataset. All duplicate records were excluded, and all uncorrected outliers were set to missing in the final dataset. Besides the existing exposure variables in the dataset, new variables were generated based on the existing ones. All continuous exposure variables were categorised and coded to form new categorical variables using a pre-defined categorisation for the measure of interest. All missing data formed a missing category for the categorical variables. After defining the outcome and exposure variables, patients meeting the inclusion criteria were tracked for 24 months and available viral load test results at each clinic visit were used to identify those that experienced virological rebound. Data preparation and analysis scripts with the final data file were all stored in a password protected storage device during the entire research period. A detailed summary of the data management and analysis dataset preparation steps are provided in Appendix 4.

2.10 STATISTICAL ANALYSES

To achieve the objectives of the study, both descriptive and inferential statistical analyses were performed using Stata version 15.1 (StataCorp, College Station, TX) as presented below:

2.10.1 Descriptive analyses:

In the descriptive analyses, all patient characteristics were stratified by sex. Medians and interquartile ranges were used to summarise continuous variables because of the non-normality of the data. Additionally, frequencies and percentages were used to summarise all categorical variables.

The person-years (PY) that each participant contributed to the study were calculated from baseline to the time they experienced virological rebound or the time they were censored in the follow-up period. Since the estimated upper limit of the survival function in this study did not fall below 50% of the survival probability, the mean survival time with its 95% confidence interval was estimated. Even though it is common practice to report the median survival time, it tends to be right-skewed as a result the mean survival time was the appropriate measure to estimate the location of the survival distribution in the present study (87).

Overall cumulative incidence and incidence rates of virological rebound (per 100 PY) were estimated at 6, 12, 18, and 24 months of follow-up. Kaplan Meier (KM) curves were used to compare the probability of survival of virological rebound stratified by sex, CD4 count, BMI and frequency of EAC. The log-rank test was used to statistically test the equality of the survival functions based on these stratifications for each exposure variable.

2.10.2 Inferential analysis

2.10.2.1 Model building:

To help identify predictors of virological rebound, bivariate and multivariable Cox proportional hazards regression models were estimated. All covariates that displayed marginal statistical significance (i.e. p-value <0.2) in the bivariate analysis, were included in the multivariable modelling. However, some exposure variables were considered for inclusion in the multivariate analysis a priori based on knowledge of previous literature and not statistical significance. In addition, to ensure adjustment for age or sex-related confounding, age and sex were included in all the model building steps. These two

covariates were included in the final model as fixed variables on the grounds of this biological plausibility, regardless of statistical significance. However, the inclusion of other exposure variables in the final model was solely based on statistical significance. Additionally, interaction terms were introduced in the models and only those that were statistically significant were considered for inclusion in the final adjusted model. The crude and adjusted hazard ratios (HRs) with 95% confidence intervals were estimated. A two-sided p-value < 0.05 was considered to indicate statistical significance. In the final model, all statistically significant covariates in the multivariable analysis were considered as predictors of virological rebound.

2.10.2.2 Model Diagnostics:

The Cox proportional hazard regression is constrained to follow the assumption that the hazard ratio is constant over time, and it is important to check this assumption. This proportional hazards assumption was checked by using the Grambsch-Therneu test on Schoenfeld residuals (88). This test checked for a non-zero slope in a linear regression of scaled Schoenfeld residuals with time for individual covariates and globally for all covariates of the final multivariate model. The null hypothesis was that the scaled Schoenfeld residuals follow a zero slope, which was equivalent to testing if the log hazard-ratio function was constant over time. Rejection of the null hypothesis (P<0.05) indicated a violation of the Cox proportion hazard assumption. The scaled Schoenfeld residuals were estimated using a mathematical formula as defined below using the Grambsch-Therneu notation:

$$r_{kj} = (x_{kj} - \hat{\bar{x}}_{w_j k}) = x_{kj} - \frac{\sum_{i \in R_j} X_{KI} exp(X_i \hat{\beta}_x)}{\sum_{i \in R_j} exp(X_i \hat{\beta}_x)} \quad (87)$$

Where the Schoenfeld residual (r_{kj}) was the difference between the k^{th} covariate value for patient j and the weighted mean ($\hat{\bar{x}}_{w_i k}$) of covariate values for patients at risk of experiencing virological rebound when the j^{th} patient experienced this event.

The overall goodness of fit test of the final adjusted model was assessed using the Cox-Snell residuals (89). The Nelson-Aalen cumulative hazard function was plotted in a graph

against the Cox-Snell residuals. Cox-Snell residuals for the j^{th} patient were defined as below:

$$CSr_j = \hat{H}_0(t_j)exp(x_j\hat{\beta}_x) \quad (87)$$

where $\hat{H}_o$ was the baseline cumulative hazard function for the j^{th} patient at time t and $\hat{\beta}_x$ was the predicted β estimate of x^{th} covariate value for the j^{th} patient derived from the fitted final cox regression model. A model fitting the data was expected to have a cumulative hazard function with a hazard rate of 1 in an exponential distribution, and the plotted Cox-Snell residuals at this hazard rate would follow a straight 45-degree line(87). Any significant deviation from the 45-degree reference line in this analysis was considered as an indication of a model not fitting the data well.

In addition to the diagnostic tests mentioned, a link test was used to check the correct model specification. Models were considered correctly specified if the coefficient for the squared linear predictor of the model was not statistically significant.

Sensitivity analyses were also conducted to evaluate the performance of the final adjusted model. In order to do this, another model was fitted, in which all “missing” categories for categorical variables with missing data assumed missing values. A complete case analysis strategy was applied to this new model, and its estimates were compared with those in the main analysis model. The goal was to assess any changes in the direction of the outcome-covariate relationship in the new model. In addition, the assessment also checked for any variations in the magnitude of the regression model estimates.

2.11 ETHICS CONSIDERATIONS

Prior to commencing the study, permission to use secondary data from TLC was sought from the Health Economics and Epidemiology Research Office (HE^2RO). HE2RO obtained ethical approval from the University of the Witwatersrand’s Human Research and Ethics Committee-medical (HREC-medical) to conduct retrospective epidemiological analyses of anonymised TLC’s routinely collected patient data from the Therapy Edge-

HIV ™ (TE) database (Ref. no. M140201). In addition, the Chief Executive Officer (CEO) of Helen Joseph Hospital (HJH) under which TLC operates, also provided authorisation to access the data before ethical clearance was granted by HREC-medical (see Appendix 2:). Following this, the study was granted ethical clearance under HREC-medical (Ref. No. M181004) (see Appendix 3: HREC (Medical) Ethics clearance certificate). All data were de-identified and anonymised before analysis. Password-secure access to all research data and analysis scripts were only granted to the researcher and supervisors throughout the research project.

CHAPTER 3 - RESULTS

3.1 INTRODUCTION

This chapter provides details of the study findings with reference to the study objectives and analysis plan. These aspects have been described in the previous chapters. The current chapter begins by describing the overall baseline characteristics of the study cohort based on sex stratification. The chapter then presents the burden of the virological rebound in the cohort, followed by a comparison of the time to virological rebound and probability of survival stratified by several exposure variables of interest. It then concludes by reporting findings on the predictors of virological rebound while comparing these results with those of the sensitivity analyses.

3.2 STUDY PARTICIPANTS

Of the 2371 patients who were switched to PI-based second-line ART between 1st January 2012 and 31st December 2016, a total of 242 eligible patients met the inclusion criteria and were included in the analysis. Figure 2 shows the study flow diagram, illustrating how patients were included and excluded in the analysis. A total of 315 patients out of the 2371 were excluded since they dropped out of the TLC cohort within 3-months after the switch to the PI-based second-line ARTs regimens. Out of the 315 patients, a total of 137 were LTFU; 99 were transferred out to another HIV treatment site, and 79 patients died. The remaining 2056 patients were followed up, and 860 patients recorded initial virological failure. As such, a total of 1196 patients that maintained viral suppressed (Viral load <400 copies/ml) while on second-line ART were excluded. In addition, a total of 164 patients among those who experienced initial virological failure were excluded since they maintained a high viral load despite receiving EAC support. Similarly, 24 patients were excluded at this stage because of LTFU (19), transfer-outs (4), and death (1). Notably, a total of 672 patients among the 860 patients who experienced initial virological failure were able to achieve virological re-suppression. Of these 672 patients, four patients aged below 18 years, and two patients that were pregnant women were excluded. Other patients excluded were those that dropped out of the TLC cohort within three months after

achieving re-suppression. LTFU, transfer-outs to other treatment sites, and deaths accounted for 64, 22, and 2 patients, respectively. This whole step-by-step exclusion process identified 242 patients that were included in the analysis, after meeting the study's set of inclusion and exclusion criteria.

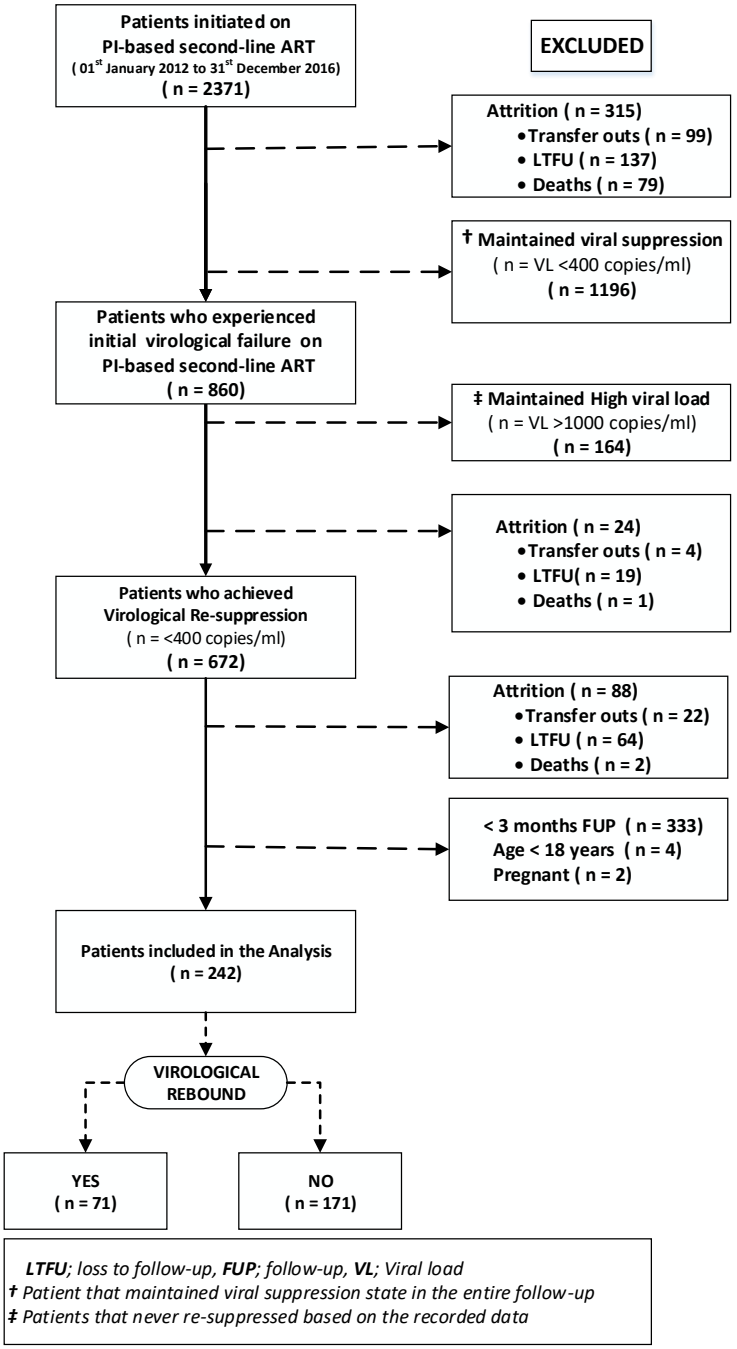


Figure 4 Study Flow Chart showing the study sample selection process

This sample size for the study (242 patients) accounted for an estimated power of 93.91% at a 5% level of significance. Out of these patients, 71 (29.34%) patients experienced virological rebound during the follow-up period. As such, the observed change in the proportion of patients experiencing virological rebound to 29.34% from the expected 50% in the sample size estimation, as mentioned in the previous chapter.

During the study's follow up period, a total of 10 (4.13%) among the 242 patients were transferred to other HIV treatment sites, 121 (50%) were LTFU, and 1 (0.41%) patient died. A total of 39 (16.12%) patients completed the 24-month follow-up period.

3.3 BASELINE SOCIO-DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

Table 1 presents the baseline characteristics of the 242 study participants at the time they achieved viral re-suppression stratified according to sex. The majority were females (57.44%; 139/242), with males accounting for 42.56% (103/242). Most of the patients were of South African nationality (85.12%; 206/242) and had a median age of 41.5 (IQR: 36-47) years, with most of them (75.21%; 182/242) falling within the 30-49 years age category (not shown). Most of the patients reported being employed (55.37%; 134/242), with no alcohol use history (77.69%; 188/242) and no smoking history (73.97%; 179/242) at the time of first-line ART initiation. Normal BMI (18.5-24.9 kg/m^2) was a finding in 33.47% (81/242) of the patients, while 13.64% (33/242), 32.64% (79/242) and 17.77% (43/242) of them were underweight, overweight and obese respectively. However, a minority of the patients (2.48%; 6/242) had a missing BMI.

A high proportion (42.98%; 104/242) of these patients were on an AZT+ 3TC + LPVr regimen; whilst 28.10 % (68/242) and 7.02% (17/242) were on TDF+ 3TC+ LPVr and d4T+3TC+LPVr regimens, respectively. Patients on other non-standard PI-based second-line ART regimen combinations accounted for 21.90 % (53/242). A total of 89 (36.78% ; 89/242) patients were initiated on a PI-based second-line ART regimen in the year 2012; whilst (26.03%; 63/242), (18.60%; 45/242), (15.29%, 37/242) and (3.31%; 8/242) were initiated in years 2013, 2014, 2015 and 2016 respectively. The overall median duration on

ART was 3.5 (IQR: 2-6) years, while the median duration on second-line ART was 1.3 years (IQR: 0.8-2.11 years) (not shown). Most of the patients (95.5%; 232/242) had been on ART for over one year from the time they were initiated on first-line ART. On the other hand, 4.5% (11/242) patients were initiated on first-line ART, switched to second-line, experienced initial failure on ART, and later achieved viral re-suppression within one year.

At baseline, the median (IQR) CD4 count was 403 (223-603) cells/µl and the median (IQR) viral load was 73.50 (49-185) copies/ml (not shown). The mean baseline haemoglobin was 13.38 (SD±2.07) g/dl (not shown), and anaemia was reported in 29 (11.98%; 29/242) patients, which were mostly females (58.62%, 17/29). A tuberculosis co-infection history at baseline was recorded in 19 patients (7.9 %). The median time to viral re-suppression after initial virological failure while on second-line ART was 3.70 (IQR: 2.80-4.60) months, with the median time to the initial virological failure from second-line ART initiation being 11.70 (IQR: 6.00-21.40) months.

Table 1 Demographic and clinical characteristics of re-suppressed patients after the initial failure of PI-based second-line antiretroviral therapy between 2012 and 2016

Characteristic	Male (%)	Female (%)	Total (%)
	N = 103 (42.56%)	N = 139 (57.44%)	N = 242 (100%)
Age (years) at baseline			
Median age (IQR)	43.0 (39.0 - 48.0)	39.0 (34.0 - 45.0)	41.50 (36.0 - 47.0)
South African nationality			
No	17 (16.50%)	19 (13.67%)	36 (14.88%)
Yes	86 (83.50%)	120 (86.33%)	206 (85.12%)
Employment status at ART initiation			
No	43 (41.75%)	60 (43.17%)	103 (42.56%)
Yes	60 (58.25%)	74 (53.24%)	134 (55.37%)
Missing	0 (0.00%)	5 (3.60%)	5 (2.07%)
Alcohol use at ART initiation			
No	77 (74.76%)	111 (79.86%)	188 (77.69%)
Yes	12 (11.65%)	6 (4.32%)	18 (7.44%)
Missing	14 (13.59%)	22 (15.83%)	36 (14.88%)
Smoking status at ART initiation			
No	67 (65.05%)	112 (80.58%)	179 (73.97%)

Yes	24 (23.30%)	5 (3.60%)	29 (11.98%)
Missing	12 (11.65%)	22 (15.83%)	34 (14.05%)
BMI categorised (kg/m^2) at baseline			
<18.5 (underweight)	11(10.68%)	22 (15.83%)	33 (13.64%)
18.5-24.9 (Normal range)	42 (40.78%)	39 (28.06%)	81 (33.47%)
25-29.9 (Overweight)	35 (33.98%)	44 (31.65%)	79 (32.64%)
≥30 (Obese)	12 (11.65%)	31 (22.30%)	43 (17.77%)
Not measured/Missing	3 (2.91%)	3 (2.16%)	6 (2.48%)
CD4 count (cells/µl) at baseline			
<350	21 (20.39%)	16 (11.51%)	37 (15.29%)
350-499	22 (21.36%)	12 (8.63%)	34 (14.05%)
≥500	13 (12.62%)	19 (13.67%)	32 (13.22%)
Not indicated	47 (45.63%)	92 (66.19%)	139 (57.44%)
Viral load (copies/ml) at baseline			
<200	71 (68.93%)	114 (82.01%)	185 (76.45%)
201-399	32 (31.07%)	25 (18.99%)	57 (23.55%)
Anaemia at baseline			
No	39 (37.86%)	45 (32.37%)	84 (34.71%)
Yes	12 (11.65%)	17 (12.23%)	29 (11.98%)
Not measured	52 (50.49%)	77 (55.40%)	129 (53.31%)
Regimen at baseline			
AZT+3TC+LPVr	53 (51.46%)	51 (36.69%)	104 (42.98%)
3TC+LPVr+TDF	27 (26.21%)	41 (29.50%)	68 (28.10%)
d4T+3TC+LPVr	4 (3.88%)	13 (9.35%)	17 (7.02%)
Other regimens [a]	19 (18.45%)	34 (24.46%)	53 (21.90%)
ART adherence at baseline			
≥ 90%	79 (76.70%)	113 (81.3%)	192 (79.34%)
<90%	5 (4.85%)	5 (3.6%)	10 (4.13%)
Missing	19 (18.45%)	21 (15.1%)	40 (16.53%)
Duration on ART (Years)			
<1year	4 (3.88%)	7 (5.04%)	11 (4.55%)
1-2 years	32 (31.07%)	45 (32.37%)	77 (31.82%)
3-4 years	25 (24.27%)	40 (28.78%)	65 (26.86%)
≥5 years	42 (40.78%)	47 (33.81%)	89 (36.78%)
Duration on second-line ART (years)			
<2 years	79 (76.70%)	99 (71.22%)	178 (73.55%)

2-5 years	24 (23.30%)	40 (28.78%)	64 (26.45%)
Year of second-line ART initiation			
2012	29 (28.16%)	60 (43.17%)	89 (36.78%)
2013	27 (26.21%)	36 (25.90%)	63 (26.03%)
2014	23 (22.33%)	22 (15.83%)	45 (18.60%)
2015	20 (19.42%)	17 (12.23%)	37 (15.29%)
2016	4 (3.88%)	4 (2.88%)	8 (3.31%)
TB co-infection history at baseline [b]			
No	90 (87.4%)	133 (95.68%)	223 (92.15%)
Yes	13 (12.6%)	6 (4.32%)	19 (7.85%)
Time to initial virological failure on second-line ART			
3-5 months	31 (30.10%)	30 (21.58%)	30 (25.21%)
6-8 months	18 (17.48%)	22 (15.83%)	22 (16.53%)
≥9 months	54 (52.43%)	87 (62.59%)	87 (58.26%)
Time to viral re-suppression after initial virological failure on second-line ART			
0-3 months	40 (38.83%)	58 (41.73%)	98 (40.50%)
4-6 months	63 (61.17%)	81 (58.27%)	(9.50%)

***ART**: Antiretroviral therapy; BMI: Body mass Index; **TB:** tuberculosis; **IQR:** Interquartile range;* **3TC:** Lamivudine; **AZT:** Zidovudine; **d4T**: Stavudine; **LPVr**: Lopinavir; **TDF:** Tenofovir

[a] *represent other non-standard PI-based regimen combinations*

[b] *TB co-infection history based on the past and recent records*

3.4 CLINICAL CHARACTERISTICS MEASURED DURING FOLLOW-UP

Table 2 provides a profile of the patients' clinical characteristics measured overtime during the 24-month follow-up. These included ART interruptions, ADRs, and the frequency of EAC sessions they received during the follow-up period. Most patients (94.21%; 228/242) reported no treatment interruption during follow-up. Even though 79.34 % (192/242) of the patients reported high treatment adherence at baseline (see Table 1), EAC was still offered to all patients as per ART guidelines. However, approximately an equal proportion of patients (50.41%, 122/242) received at least five adherence counselling sessions during the follow-up period. For ADRs, these were reported in a high proportion of patients (24.38 %; 59/242) patients.

Table 2 Clinical characteristics during follow-up

Characteristic [a]	Male (%)	Female (%)	Total (%)
	N = 103 (42.6%)	N = 139 (57.4%)	N = 242 (100%)
Frequency of EAC sessions after baseline			
<5	55 (53.40%)	67 (48.20%)	122 (50.41%)
≥5	48 (46.60%)	72 (51.80%)	120 (49.59%)
ART interruption after baseline			
No	98 (95.1%)	130 (93.53%)	228 (94.21%)
Yes	5 (4.85%)	9 (6.47%)	14 (5.79%)
ADRs after baseline			
No	74 (71.84%)	109 (78.42%)	183 (75.62%)
Yes	29 (28.16%)	30 (21.58%)	59 (24.38%)

EAC: Enhanced adherence counselling; **ART**: Anti-retroviral Therapy; **ADRs:** Adverse drug reactions

[a]All patient characteristics were measured from baseline to the time the patients were censored or experienced virological rebound

3.5 INCIDENCE AND RISK OF VIROLOGICAL REBOUND

The 242 patients forming the analysis subset contributed a total of 3676.6 person-months of follow-up to the analysis, with the minimum patient follow-up time being 3.70 months. Overall, 71 patients experienced virological rebound during the follow-up period, accounting for a prevalence of 29.34% (71/242) at the time of administrative censoring. A total of 132 (54.55%) patients were censored before completing the entire 24-months follow-up time. The median follow-up time for these censored patients was 14.2 (IQR: 11.70-17.55) months; and they contributed 1900 person-months at risk in this analysis. On the other hand, 39 (16.12%) patients were followed to the end, and they contributed 889.2 person-months at risk in the analysis.

The overall incidence rate of virological rebound was 23.20 (95% CI: 18.38-29.27) per 100 PYs. Overall incidence rates varied by sex, with males having a higher incidence rate [24.40 (95% CI: 17.16-34,69) per 100PYs] compared to females [22.35 (95% CI :16.39-30.47) per 100 PYs]. Table 3 provides the overall incidence rates at 4-time points (6, 12, 18, and 24 months) in the analysis. The greatest risk of experiencing virological rebound occurred between 18 to 24 months of follow-up with an incidence rate of 71.35 (95 % CI: 43.71-116.47) per 100 PYs. The incidence rate was the lowest in the first six months [10.06 (95 % CI: 5.71-17.71) per 100 PYs] and increased overtime during the follow-up period.

Table 3 Incidence rates of virological rebound among re-suppressed patients on second-line ART within a 24-month follow-up time

Months from Baseline	Person-months	Number of failures	Incidence rate (per 100 person-years)	95% CI
0-6	1432.20	12	10.06	5.71-17.71
6-12	1235.90	23	22.34	14.85-33.62
12-18	737.60	20	32.59	21.03-50.52
18-24	270.90	16	71.35	43.71-116.47
Overall	**3676.60**	**71**	**23.20**	**18.38-29.27**

Considering loss to follow-up, transfer-outs, and deaths as competing risks, Figure 5 provides a comparison of the estimated cumulative incidence over the 24-month follow-up according to sex. Overall, the cumulative incidence was not different between males and females. A small disparity was observed after 12-13 months, with males having a slightly higher cumulative incidence; however, based on the Pepe and Mori test (p-value =0.499) (90), there was no statistically significant difference.

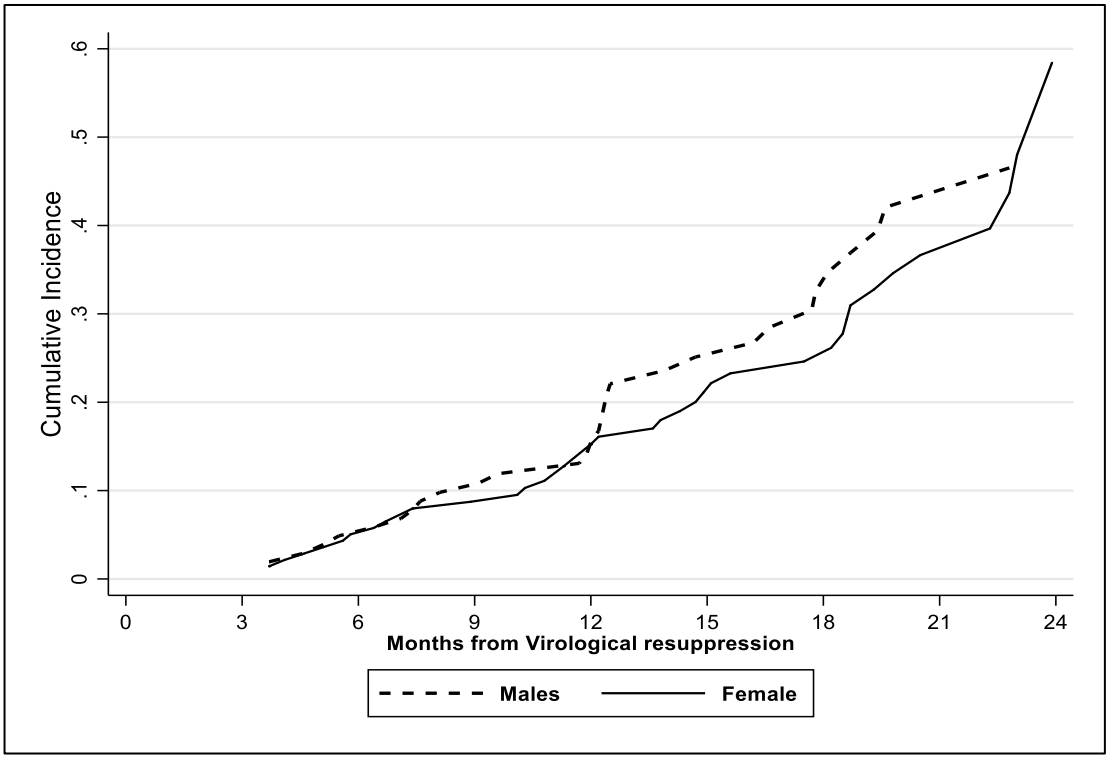


Figure 5 Cumulative incidence of virological rebound by sex

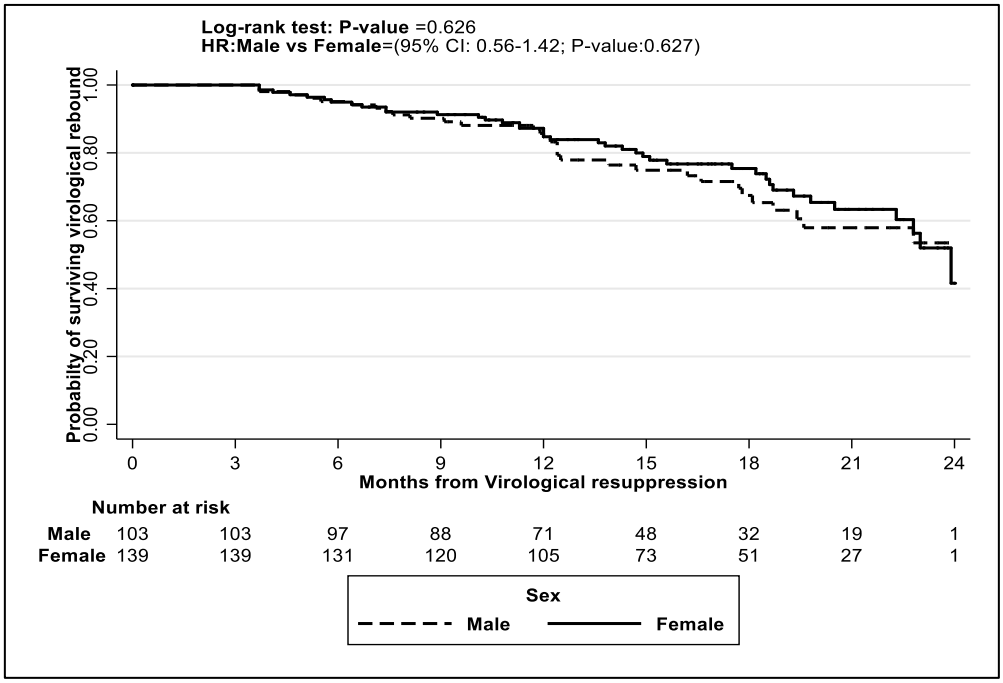
3.6 SURVIVAL ANALYSIS ESTIMATES FOR VIROLOGICAL REBOUND

The estimated restricted mean survival time was 19.74 (95% CI:18.90-20.58) months. More than 50% of the patients were censored, the upper limit of the 95% confidence interval for median survival time could not be estimated [median survival time = 23.95 (95% CI: 22.80, .) months] as stated in the previous chapter. As such, the mean survival time was considered as an appropriate measure.

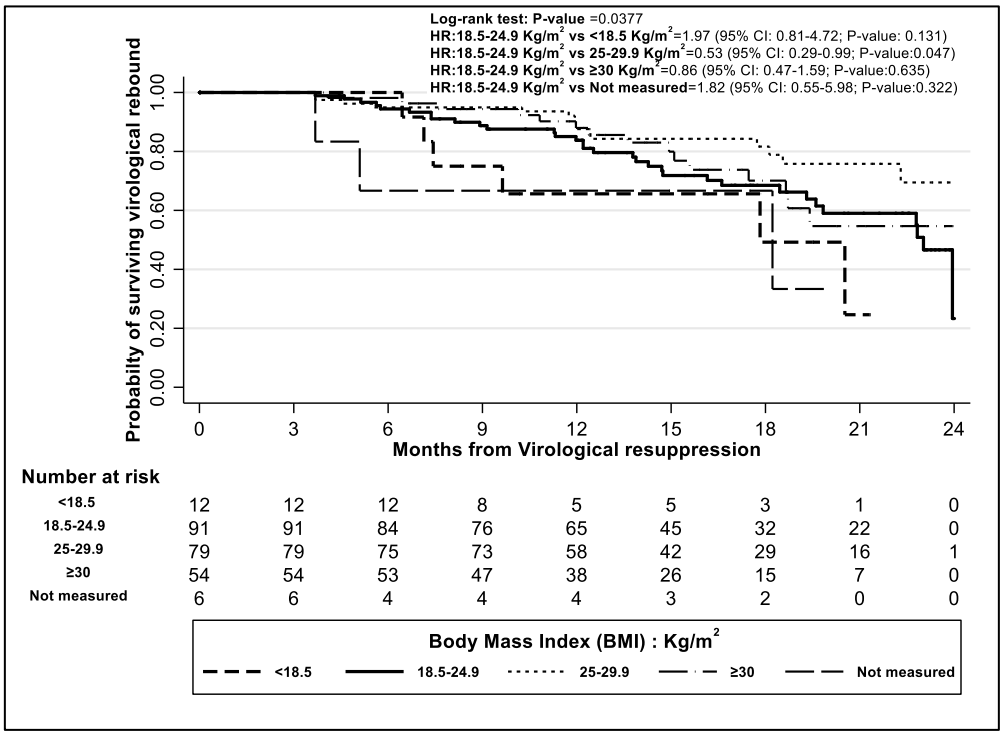
Figure 5 compares the survival functions across selected covariate stratifications using Kaplan-Meir estimates. The probability of surviving virological rebound was significantly higher for patients who received at least five EAC sessions compared to those who received less than five (log-rank test: p-value <0.0001). In addition, according to the Kaplan-Meir survival curve (C), underweight patients had a lower probability of survival compared to those with a normal BMI between 3-9 months, and then there was no marked difference up to the end of follow-up. However, there was an overall statistical difference in the survival functions between each of the BMI categories (log-rank test: p-value

=0.038). Notably, there was no statistically significant difference in the survival functions by sex (log-rank test: p-value =0.626), and ART adherence at baseline (log-rank test: p-value =0.257).

(A)



(B)



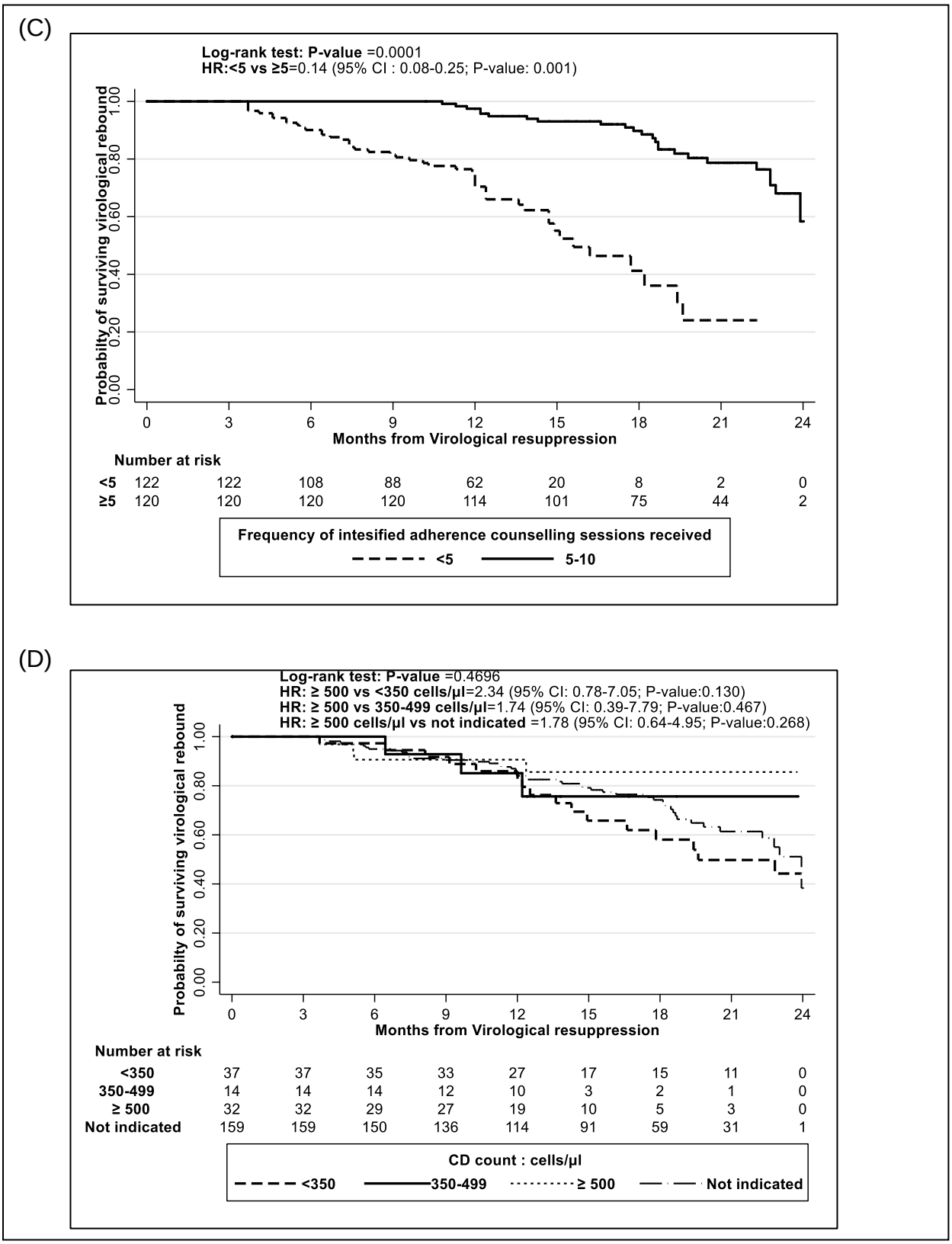


Figure 6 Kaplan Meir curves of virological rebound survival

3.7 PREDICTORS OF VIROLOGICAL REBOUND

Results from the bivariate and multivariate Cox regression models for the occurrence of the virological rebound are presented in Table 4. Covariates included in the final adjusted cox proportional hazard regression model formed the most parsimonious model. The addition of other covariates and interaction terms did not improve the model as guided by the likelihood ratio (LR) tests.

Table 4 Unadjusted and adjusted HR estimates of virological rebound among viral re-suppressed patients who experienced initial virological failure on PI-based second-line antiretroviral therapy between 2012 and 2016 at Themba Lethu Clinic, Johannesburg

Factor	Level	HR (95% CI)	aHR (95% CI)	LR test P-value
Sex	Male	**Reference**	**Reference**	0.414
	Female	0.89 (0.56-1.42)	1.25 (0.73-2.12)	
Age [a]	<30	**Reference**	**Reference**	0.741
	30-49	0.83 (0.37-1.84)	1.01 (0.37-2.72)	
	≥50	1.01 (0.40-2.51)	1.29 (0.43-3.87)	
BMI (kg/m2) [a]	<18.5 (underweight)	1.97 (0.84-4.58)	3.09* (1.17-8.16)	0.041
	18.5-24.9 (Normal)	**Reference**	**Reference**	
	25-29.9 (Overweight)	0.54* (0.29-0.99)	0.64 (0.34-1.22)	
	≥30 (Obese)	0.86 (0.47-1.57)	0.81 (0.42-1.59)	
	Missing/Not measured	1.82 (0.51-6.56)	0.75 (0.21-2.73)	
CD4 count (cells/µl) [a]	<350	2.34 (0.75-7.29)	3.68* (1.10-12.33)	0.033
	350-499	1.74 (0.37-8.29)	2.37 (0.49-11.33)	
	≥500	**Reference**	**Reference**	
	Not indicated	1.78 (0.62-5.14)	2.68 (0.86-8.38)	
Viral load (copies/ml) [a]	<50	Reference	Reference	0.251
	50-199	1.32 (0.76-2.31)	1.59 (0.86-2.92)	
	200-399	1.66 (0.90-3.09)	1.58 (0.82-3.06)	
Adverse drug reactions (ADRs)	No	**Reference**	-	
	Yes	0.72 (0.41-1.28)	-	
Anaemia [a]	No	**Reference**	**Reference**	0.688

	Yes	0.98 (0.46-2.09)	1.42 (0.65-3.11)	
	Not measured/Missing	0.93 (0.56-1.55)	1.05 (0.56-1.97)	
Regimen [a]	AZT+3TC+LPVr	**Reference**	**Reference**	0.711
	3TC+LPVr+TDF	0.81 (0.44-1.49)	1.10 (0.58-2.08)	
	d4T+3TC+LPVr	1.49 (0.68-3.24)	1.46 (0.57-3.73)	
	Other[b]	1.26 (0.71-2.24)	1.38 (0.73-2.61)	
ART adherence [a]	≥ 90%	**Reference**	-	
	<90%	2.31 (0.88-6.09)	-	
	Not measured/Missing	1.07 (0.55-2.05)	-	
Frequency of EAC sessions after baseline	<5	**Reference**	**Reference**	0.0001
	≥5	0.14*** (0.08-0.25)	0.11*** (0.06-0.20)	
Duration on ART (Years) [a]	<1year	**Reference**	-	
	1-2 years	0.71 (0.23-2.19)	-	
	3-4 years	0.64 (0.20-1.99)	-	
	≥5 years	0.73 (0.24-2.24)	-	
Duration on second-line ART (years) [a]	<2 years	**Reference**	**Reference**	
	2-5 years	1.08 (0.63-1.88)	1.34 (0.70-2.58)	0.380
Time to initial virological failure [c]	3-5 months	**Reference**	-	
	6-8 months	0.44* (0.21-0.94)	-	
	≥9 months	0.61 (0.37-1.01)	-	
Time to re-suppression [d]	0-3 months	**Reference**	-	
	4-6 months	0.88 (0.55-1.40)	-	

95% CI: 95 % confidence interval; ***HR:*** Crude Hazard ratio; ***aHR:*** Adjusted hazard ratio; ***BMI:*** Body mass index; ***EAC:*** Enhanced adherence counselling; **3TC:** Lamivudine; **AZT:** Zidovudine; **d4T**: Stavudine; **LPVr**: Lopinavir; **TDF:** Tenofovir

[a] Covariates measured at baseline (Virally re-suppressed status)

[b] *Represents other non-standard PI-based regimen combinations*

[c] Measured as the duration between initial virological failure date and second-line ART initiation date.

[d] Measured as the duration between initial virological failure date and re-suppression date while on second-line ART.

p-value: * $p < 0.05$, *** $p < 0.001$

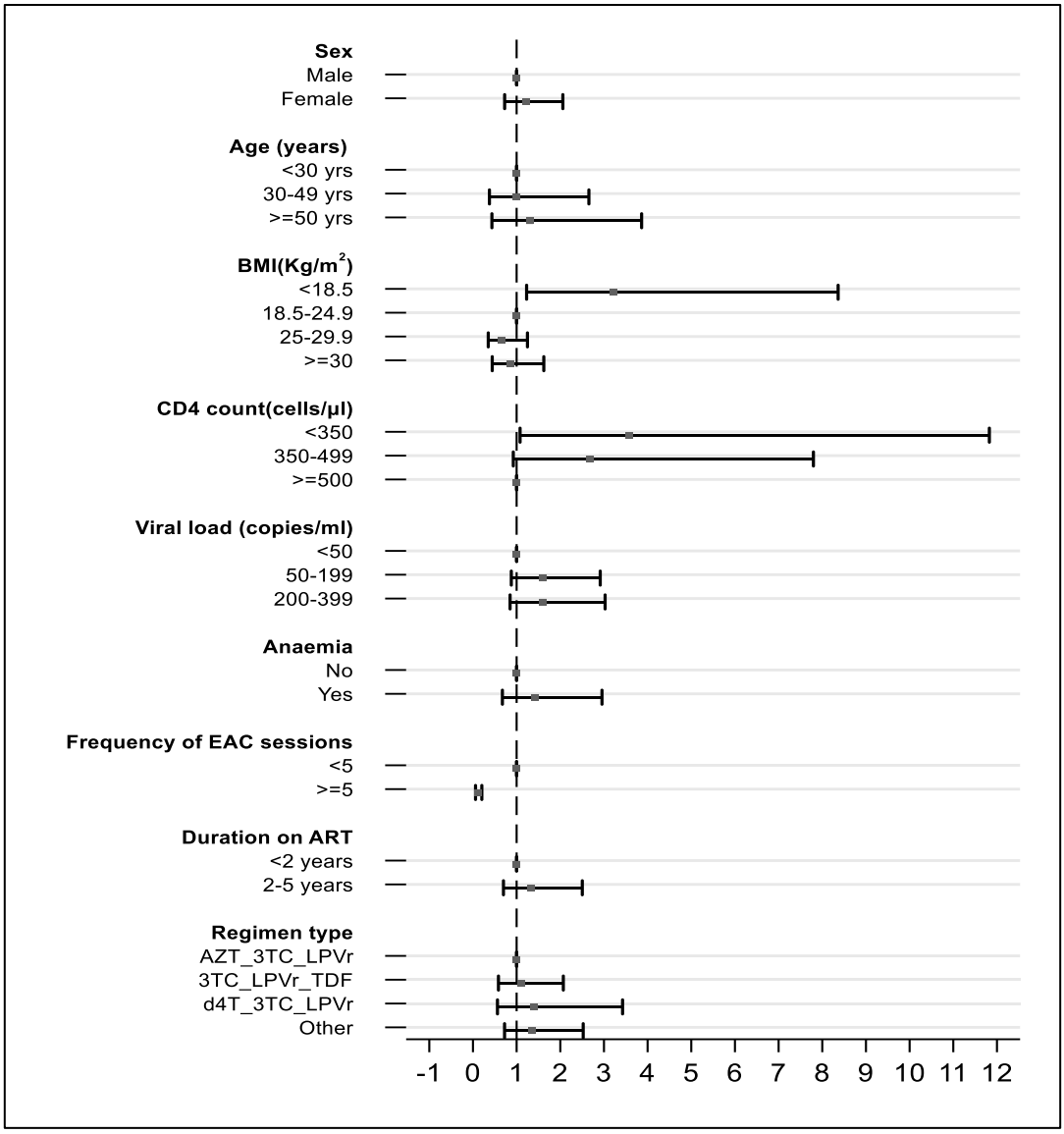


Figure 7 Adjusted HR estimates of virological rebound

3.8 INTERPRETATION OF THE UNADJUSTED AND ADJUSTED MODELS

In the final adjusted model as displayed in Table 4 and Figure 7, covariates included were sex, age, CD4 count, viral load, anaemia, regimen type, frequency of adherence counselling and duration on second-line ART. In the bivariate analysis, BMI at baseline was found to be statistically significant (P-value<0.001). Overweight patients (BMI: 25.5-29.9 Kg/m^2) seemed to have 46% (HR:0.54; 95% CI:0.29-0.99) lower hazards of experiencing virological rebound compared to those with normal BMI (BMI: 18.5-24.9 kg/m^2). Overall, BMI maintained statistical significance after adjusting for the other

covariates with virological rebound (LR test p-value: 0.041). From the adjusted model, patients with low BMI (BMI<18.5 kg/m^2) had 3.09 times higher hazards of experiencing virological rebound as compared to those with normal BMI while keeping the other covariates constant (aHR=3.09; 95%CI 1.17-8.16; P-value=0.023). CD4 count at baseline in the bivariate analysis did not reach statistical significance. However, in the multivariate analysis, there was an association between CD4 count and virological rebound (LR test p-value: 0.033) after adjusting for the other covariates. Patients with a low CD4 count of <350 cells/µl at baseline had 3.68 times higher risk of virological rebound compared to those with a CD4 count of >500 cells/µl (aHR=3.68; 95% CI 1.10-12.33; P-value=0.035). Another key finding was the reduced risk of virological rebound among patients who received a higher number of EAC sessions. Both in the bivariate and multivariate analysis, this factor remained highly significant. In the bivariate analysis, patients who received more than 5 EAC sessions had 86% lower risk of virological rebound (HR=0.14; 95% CI 0.08-0.25; P-value<0.0001). This risk was lower in the adjusted model, which showed that these patients compared to those who received less than 5 EAC sessions, had 89% lower risk of virological rebound by (HR=0.11; 95% CI 0.06-0.20; P-value<0.0001). Even though other baseline covariates were included in the final model (age, sex, viral load, anaemia, regimen type, and duration on second-line ART), none of these covariates approached statistical significance. Variables such as ART adherence, duration on ART, time to initial virological failure, and time to viral re-suppression showed no statistical significance in the bivariate analysis and were not included in the multivariate modelling since their inclusion did not improve the model.

3.8.1 Proportional-hazards assumption

Based on the Grambsch-Therneu test on Schoenfeld residuals, the overall adjusted model did not violate the proportional hazard assumption. (Global test: χ^2=15.48; P-value= 0.6917). Also, the test for each covariate in the adjusted model showed no statistical significance under this diagnostic test confirming non-deviation from the proportional hazard assumption. Figure 8 shows the graphical representation of these individual tests. All curves present roughly a zero slope. As such, the null hypothesis that indicates that there is no violation of the proportional hazards assumption is not rejected for each covariate and the entire model.

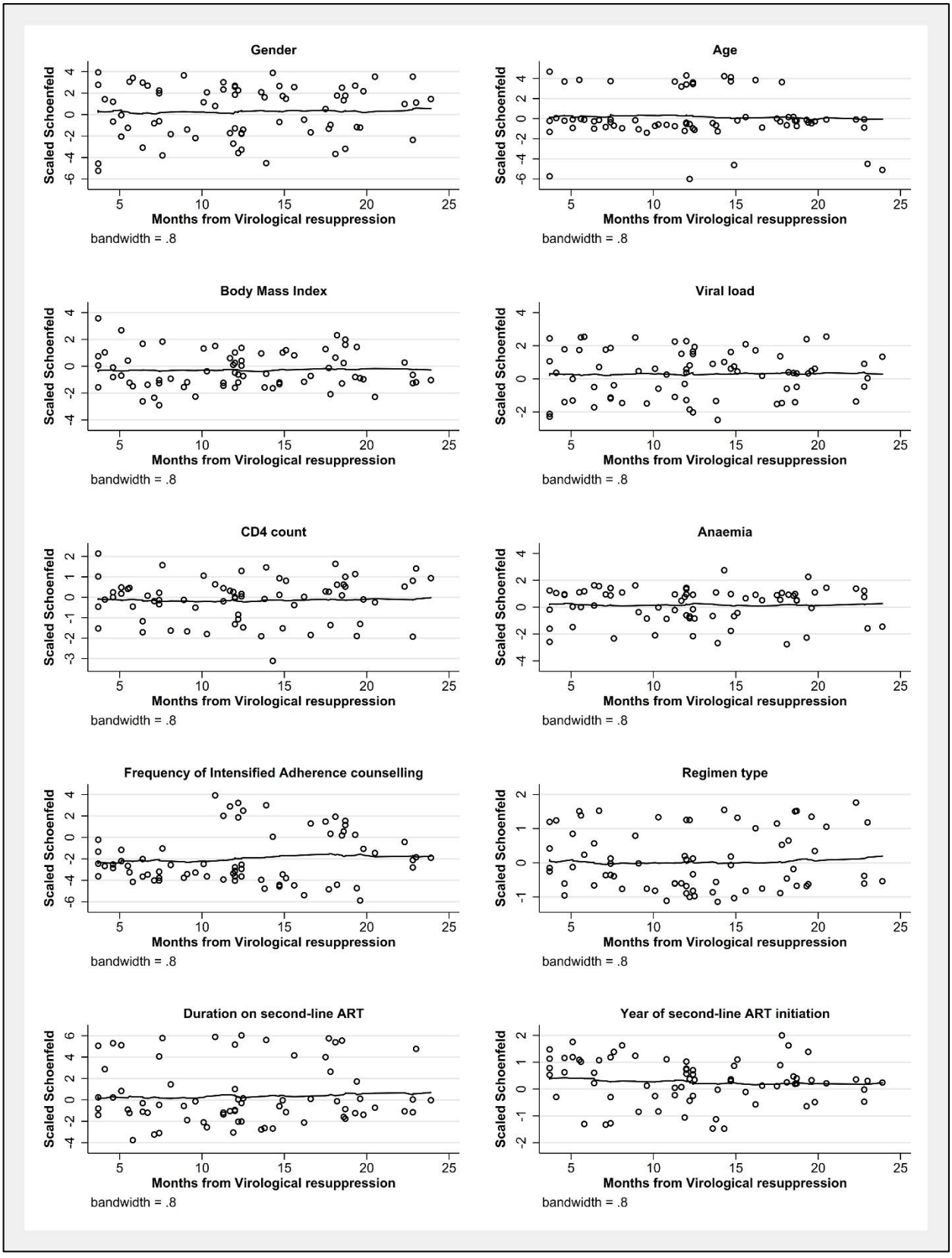


Figure 8 Proportional-hazards assumption test based on Schoenfeld residuals for each covariate in the adjusted model

3.8.2 The goodness of fit of the model and Model specification

Figure 9 presents the results of an evaluation of the goodness of fit of the model using the Cox-Snell residuals. The graphed Nelson-Aalen cumulative hazard function roughly follows the 45-degree diagonal line, which indicates that it has approximately an exponential distribution with a hazard rate of one. This finding confirms that the final adjusted model is a good fit.

In addition, a link test was used to evaluate the correct specification of the model. The coefficient for the squared linear predictor of the model was not statistically significant (p-value = 0.482). This finding confirms that the final adjusted model was correctly specified and that adding more covariates to the model would add minimal power.

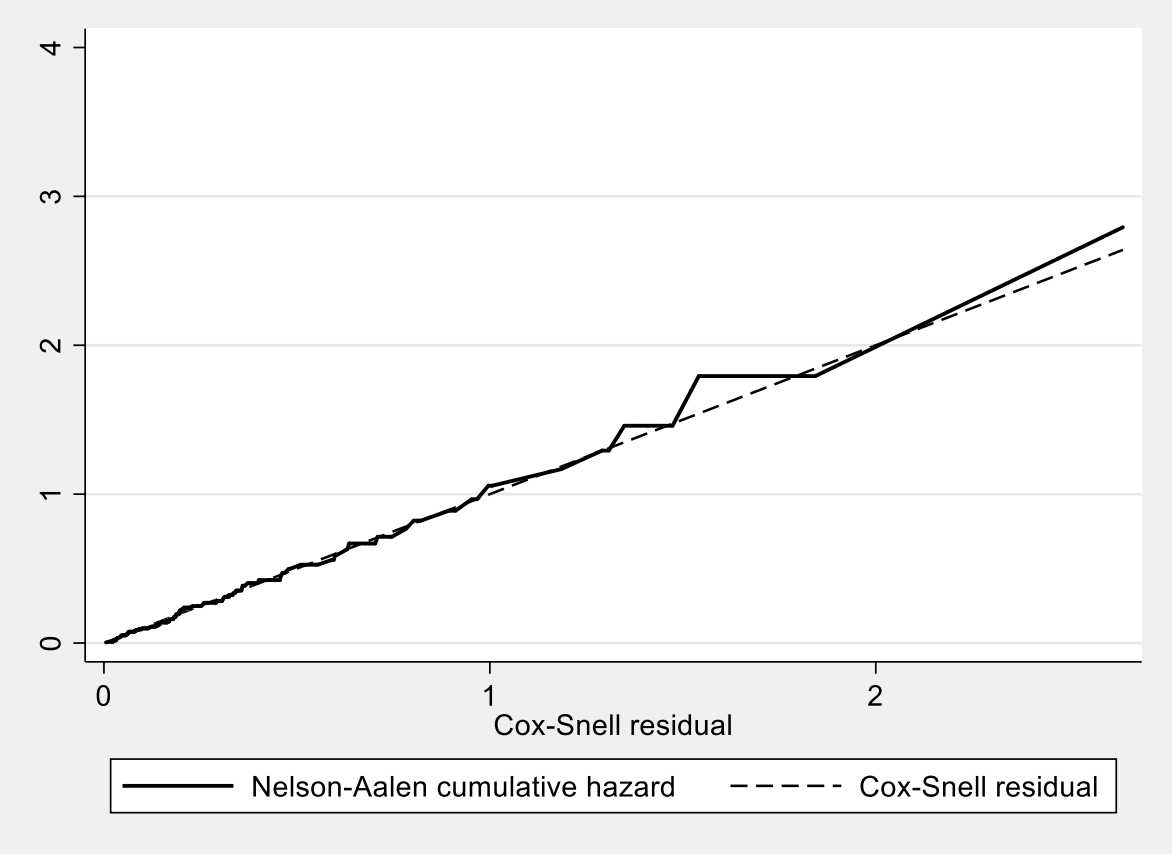


Figure 9 Goodness of fit of the Adjusted Cox proportional hazard model using Cox-Snell residuals.

3.9 SENSITIVITY ANALYSIS

As is typical with routine clinic data, some covariates had missing values in the analysis dataset. The sensitivity analysis compared the final model and a model were all missing, and non-measured values were considered to have been measured. All patients with missing values or non-measured BMI values assumed a normal BMI while missing CD4 count values assumed a CD4 count of >500 cells/µl. Also, all patients with unmeasured anaemia status assumed a non-anaemic state. Table 5 shows the re-modelled estimates compared with those of the model from the main analysis. The results show minimal deviation (<10%) of the estimates from those modelled in the main analysis model. In addition, none of the estimates in the new model displayed a change in the direction of the outcome-covariate relationship as observed in the main analysis.

Table 5 A summary of Sensitivity analysis of adjusted HR estimates of virological rebound among virally re-suppressed patients who experienced initial virological failure on PI-based second-line ART between 2012 and 2016 at Themba Lethu Clinic, Johannesburg

Factor	Level	Main analysis aHR (95% CI)	Missing / non-measured values excluded aHR (95% CI)
Sex	Male	**Reference**	**Reference**
	Female	1.25 (0.73-2.12)	1.22 (0.73,2.06)
Age[a]	<30	**Reference**	**Reference**
	30-49	1.01 (0.37-2.72)	1.00 (0.38,2.66)
	≥50	1.29 (0.43-3.87)	1.30 (0.43,3.86)
BMI (kg/m2) [a]	<18.5	3.09* (1.17-8.16)	3.21* (1.23,8.36)
	18.5-24.9	**Reference**	**Reference**
	25-29.9	0.64 (0.34-1.22)	0.66 (0.35,1.25)
	≥30	0.81 (0.42-1.59)	0.85 (0.44,1.62)
	Missing/Not measured	0.75 (0.21-2.73)	-
CD4 count (cells/µl) [a]	<350	3.68* (1.10-12.33)	3.57* (1.08,11.83)
	350-499	2.37 (0.49-11.33)	2.68 (0.92,7.80)
	≥500	**Reference**	**Reference**
	Not indicated	2.68 (0.86-8.38)	-
Viral load (copies/ml) [a]	<50	**Reference**	**Reference**
	50-199	1.59 (0.86-2.92)	1.60 (0.88,2.91)

	200-399	1.58 (0.82-3.06)	1.60 (0.85,3.03)
Anaemia [a]	No	**Reference**	**Reference**
	Yes	1.42 (0.65-3.11)	1.41 (0.67,2.96)
	Missing/Not measured	1.05 (0.56-1.97)	
Regimen [a]	AZT-3TC-LPVr	**Reference**	**Reference**
	3TC-LPVr-TDF	1.10 (0.58-2.08)	1.10 (0.58,2.07)
	d4T-3TC-LPVr	1.46 (0.57-3.73)	1.39 (0.56,3.43)
	Other	1.38 (0.73-2.61)	1.35 (0.72,2.52)
Frequency of EAC sessions after baseline	<5	Reference	Reference
	≥5	0.11*** (0.06-0.20)	0.11*** (0.06,0.20)
Duration on second-line ART (years) [a]	<2 years	**Reference**	**Reference**
	2-5 years	1.34 (0.70-2.58)	1.32 (0.70,2.50)

95% CI: 95 % confidence interval; ***aHR:*** Adjusted hazard ratio; ***BMI:*** Body mass index; ***EAC:*** Enhanced adherence counselling; **3TC:** Lamivudine; **AZT:** Zidovudine; **d4T**: Stavudine; **LPVr**: Lopinavir; **TDF:** Tenofovir

[a] Covariates measured at baseline (virally re-suppressed status)

[b] Measured as the duration between initial virological failure date and second-line ART initiation date.

[c] Measured as the duration between initial virological failure date and re-suppression date while on second-line ART.

p-value: * $p < 0.05$, *** $p < 0.001$

CHAPTER 4 - DISCUSSION

4.1 INTRODUCTION:

This chapter provides an in-depth analysis, interpretation, and synthesis of the study findings under specific themes with reference to the existing body of knowledge. In addition, it provides new insights into the existing literature and elaborates on the impact of the study findings on health policy. The chapter further highlights the study's limitations and provides recommendations for future research.

Other studies from resource-limited settings have demonstrated that PI-based second-line ART successfully achieves viral suppression in resource-limited settings (91,92). The present study was set out with the aim of determining the incidence rates and predictors of virological rebound among HIV-infected adult patients who achieved viral re-suppression after failing second-line ART in Johannesburg, South Africa. In addition, it is the first study to report on this outcome in a cohort of patients on second-line ART being managed in a resource-limited setting. Specifically, this chapter reviewed three aspects of the study findings, which include the baseline characteristics of the study participants, the burden of virological rebound, and finally, the predictors of virological rebound.

4.2 BASELINE CHARACTERISTICS

An initial objective of the present study was to describe the baseline characteristics of the virally re-suppressed patients who had failed a PI-based second-line ART regimen in the Themba Lethu Clinical Cohort.

It is interesting to note that most of these patients were female. These findings match those observed in earlier studies conducted at TLC and those reported in the cohort profiles (56,82,93). This further supports the fact that females have a higher ART uptake through HIV testing and ART initiation services which are readily available during pregnancy and postpartum periods. Another explanation for this is the disproportionately

high HIV prevalence among females in many regions, including the sub-Saharan region, particularly in South Africa.

Most patients were employed which is not consistent with an earlier report on the Themba Lethu cohort profile (82). These differences can be explained by the fact that patients included in the analysis were able to achieve viral re-suppression, probably due to a higher socio-economic status which made them maintain optimal ART adherence. Again, most of the patients were middle-aged, which may correspond with the higher employment rates observed.

Normal BMI and overweight patients (66.11%) made the highest proportion in this sub-cohort. A similar finding was found in other studies conducted at the same study site (56,93). However, a significant number of patients (13.4%) were underweight, which may mostly be explained by poor nutrition and advanced HIV disease.

Most of the patients were on an AZT+ 3TC + LPV regimen (42.98%) and TDF+3TC+ LPVr (28.10%), in accordance with the national ART guidelines. However, a significant proportion of patients (21.90%) were on non-standard PI-based regimen combinations. This finding may have been due to the unavailability of the standard ART regimens. Another possible explanation could be that most prescribing clinicians were not adhering to the national ART guidelines.

As mentioned in the literature review, poor ART adherence is considered as the primary predictor of second-line ART virological failure. It has also been considered as one of the main predictors of HIV disease progression and HIV-related deaths after a poor immunological response to ART (94,95). Patients on ART tend not to be consistently adherent to treatment and may be at risk of experiencing virological failure even after they have achieved viral suppression. In the present study, at baseline, most of the patients displayed near-perfect ART adherence of >90%. Although these patients were virally suppressed, a few patients had a low ART adherence of less than 90%. This finding could be suggestive of inconsistent ART adherence among these patients despite receiving

EAC after experiencing the initial second-line ART failure. Some of the reasons for being non-adherent to ART reported in earlier studies include drug stock-outs at a treatment site, lack of adherence ART follow-ups, forgetfulness, low motivation levels, multiple drug prescriptions, adverse drug reactions, being in good health, high transportation costs, and stigma linked to ART (96–101). Pill count was the method used to assess adherence in the present study's context. However, it has been considered as a poor marker of ART adherence since there is no way of verifying treatment compliance. And, considering that high ART adherence was reported at baseline in most patients, it was not included in the inferential analysis.

The risk of HIV transmission is lower in patients with low viral loads (102). And, achieving and maintaining viral suppression is essential for good treatment outcomes, which lead to improved quality of life (103). Although all patients included in this analysis achieved viral re-suppression (VL <400 copies/ml) at baseline, most of them (59.92%) failed to achieve undetectable viral loads of <50 copies/ml. There could be a number of factors contributing to these observed sub-optimal viral suppression states. Some of the explanations that may be considered may include comorbidities that may affect ART adherence, reduced efficacy due to exposure to suboptimal ART regimens, drug interruptions, drug interactions with concomitant medications and/or drug adverse drug reactions.

Surprisingly, the median (IQR) CD4 count 403 (223-603) cells/µl is suggestive of low immunological response at baseline in most (>50%) of these patients. This finding indicates that most of these patients showed discordant immunological and virological responses. Some studies have suggested that a discordant relationship where low viremia does not reflect good immunological response may be due to delays in ART initiation (104). However, there seems not to be substantial literature on the pathogenesis of these discordant responses, which may be dependent on the interaction of viral, host, and treatment-related factors.

A Tanzanian study found a longer duration on ART to be a risk factor of non-adherence to first-line ART (105). In the present study, the overall median duration on ART was 3.5

(IQR: 2-6) years at the study's baseline which is a considerable duration on treatment. This also accords with the fact some of these patients had poor adherence during first-line ART; and following the switch to second-line ART, they may have also been non-adherent to the treatment and eventually experienced early second-line ART virological failure. Another important finding was the short median duration on second-line ART of 1.3 years (IQR:0.8-2.11 years) at baseline. Studies have shown that patients on ART for longer periods have high toleration to second-line ART (9). Interestingly, most patients achieved viral re-suppression in less than four months after experiencing their initial virological failure on this treatment option. This finding may reflect the impact of EAC offered to all patients after experiencing their initial virological failure.

Most patients in the present study were non-anaemic at baseline. However, a higher anaemia prevalence (<50%) was reported before the initial second-line ART virological failure in a previous study at TLC (93). Since this sub-cohort already experienced the initial failure and had received some other clinical services, this may have impacted on this observed disparity. Additionally, TB-coinfection was observed in a low proportion of patients (7.9%) in the present study, which was a similar finding in an earlier study focusing on initial virological failure at the same study site (12).

4.3 INCIDENCE OF VIROLOGICAL REBOUND

The second objective of the present study was to determine the incidence rate of virological rebound among virally re-suppressed patients after the initial failure of second-line ART.

This is the first study to investigate the burden of this long-term outcome in second-line ART. The findings indicate the overall incidence rate of virological rebound was 23.20 per 100 person-years, and the prevalence was 29.34%. Compared to incidences rates of the initial second-line ART virological failure, the present study reports quite similar high incidence rates of virological rebound. Studies in similar settings have documented incidence rates of the first second-line ART virological failures between 19.1-28.3 per 100 person-years (8,12,15,69,93). This similar finding suggests the rates of virological

rebound are comparably high. However, since virological rebound in this study was observed after the initial failure, comparisons of the burden of virological failure at different occurrences need to be interpreted with caution. Also, comparisons of these rates across different studies and countries may be difficult to compare due to the differences in the duration on ART, study designs, virological failure definitions, sample size, and follow-up periods. However, these results emphasise the fact that adult patients who achieve viral re-suppression after experiencing virological failure during second-line ART remain at risk of developing virological rebound within two years after registering the first episode.

Interestingly, the incidence rates were highest between 18-24 months of follow-up from baseline. However, the rates were lower before 12 months of follow-up, with the rate increasing over the follow-up period. In contrast, a meta-analysis of studies in resource-limited settings focusing on the virological failure rates after the second-line ART switch found contrasting findings (106). The pooled virological failure rates in this meta-analysis showed a decreasing pattern over the follow-up period, which is contrary to what was observed in the present study. Furthermore, the results of the present study show that the most incident cases of virological rebound occur between 18-24 months of follow-up, which is suggestive of a relatively poor virological response over time within the follow-up period.

Another additional finding was the comparable cumulative incidence of virological rebound among males and females. Though slightly higher among males, the cumulative incidence was statistically similar. However, the incidence rates were higher among males compared to females. A similar record can be seen in studies that have reported on the incidence rates of the initial failure while receiving second-line ART (55). This finding suggests that incidence rates of virological rebound differ according to sex and are much higher in males.

With the present study's findings on the incidence rates of virological rebound, it is worth noting that patients who achieve viral suppression after experiencing their initial virological failure during second-line ART, remain at risk of experiencing virological rebound later in

the course of this treatment. This finding points to the fact that poor management of patients on second-line ART may eventually have long-term consequences even in patients who have achieved viral re-suppression. It is important to have standardised clinical guidance, that targets this group of patients.

4.4 PREDICTORS OF VIROLOGICAL REBOUND

The final objective of the present study focused on investigating the predictors of virological rebound in patients who achieved viral re-suppression after experiencing their initial virological failure on second-line ART. In the adjusted analysis, several factors were found to be associated with virological rebound in these patients and may be considered as predictors.

Available evidence indicates that PI-based second-line ART regimens are highly potent (6,36–40) and they have low rates of drug-resistance (9,44,50). It is evident that poor ART adherence is an important predictor of second-line ART failure as stated in the literature review. As such, patients need to receive EAC uninterruptedly for the achievement and maintenance of viral suppression. In the present study, patients received EAC even though all the patients had achieved viral re-suppression at baseline as per National ART guidelines. The most interesting finding was that patients who received a higher number of EAC sessions had a lower risk of experiencing virological rebound. To explain this finding, it is worth noting that the overall high ART adherence at baseline in most of the patients may have been maintained after they had received more EAC sessions. This finding provides evidence on the dose-response relationship between frequency of EAC and the risk of experiencing virological rebound. Even though there is substantial evidence suggesting that EAC has an impact in reducing the risk of virological failure in patients on second-line ART (17), there is a paucity of knowledge on the optimal number of EAC sessions patients they need to receive to achieve this. The present study found that patients who received at least five EAC sessions had a lower risk of experiencing virological rebound in the 24-month follow-up period. Further studies need to be conducted to provide substantial evidence on the optimal number of EAC sessions that

patients need to receive to achieve viral re-suppression in such a period. However, the findings suggest that it is essential for virally suppressed patients with high ART adherence to continuously receive EAC to ensure that they maintain good virological response to these PI-based second-line ART regimens.

Wasting has been considered as a clinical complication of HIV infection since early in the HIV epidemic (107), and its presence eventually leads to low BMI. The present study found that patients with low BMI (underweight) had a higher risk of experiencing virological rebound compared to those with a normal BMI. However, no statistical difference was found between patients that were overweight or obese and those who had a normal BMI. A Malawian study has found that patients failing second-line ART are less likely to be overweight or obese (108). Likewise, a study at TLC has reported low BMI as a predictor of virological failure in patients on second-line ART (56). The data reported here appear to support the idea that patients with low BMI having a higher risk of virological rebound. A reasonable explanation for this is that weight loss and low BMI have been indexed as predictors of HIV disease progression and malnutrition (109), and this may result in the poor immunological response to treatment. This immuno-compromised state may have impeded them from maintaining the achieved viral re-suppression state and increased the risk of experiencing virological rebound.

Another key clinical finding in support of the previous discussion was that patients with low immunological status, as defined by a CD4 count < 350 cells/µl, had a higher risk of virological rebound. The explanation here is that poor immunological response may have eventually led to a poor virological response in the form of virological rebound. This finding accords with previous studies and emphasises that poor immunity predisposes patients on ART to virological failure while on second-line ART regimens (68,69). As such discordant immunological and virological response in these patients may be considered as a marker of risk of virological rebound.

Surprisingly, there was no statistically significant association between viral load at baseline and virological rebound. This suggests that even having an undetectable viral

load in these patients does not reduce the risk of virological rebound in such patients. However, it is quite certain that maintaining a high virological response is crucial for successful second-line ART. Since all patients were virally suppressed at baseline, it is adequate to say that other factors play a more significant role in reducing or increasing the risk of virological rebound.

Furthermore, age and gender were also not statistically associated with the outcome. However, studies have suggested that females (15,71) and others have suggested that males (72–74) have a higher risk (55,56) of experiencing the initial virological failure of second-line ART. And, other studies have found that older-aged patients have a lower risk (37,75). Quite important is the fact that these two factors were adjusted for in the final model, in case they confounded the relationship between the virological rebound and the other measured factors.

The duration on ART, time to the initial virological failure while on second-line ART, and time to viral re-suppression were not included in the adjusted analysis since they failed to reach statistical significance. Though studies have reported that patients with longer duration on ART have a high tolerance to second-line ART as earlier stated (9), this study did not find similar findings. Other factors, such as regimen type, anaemia, and duration on second ART, were also not associated with the outcome in the final model. These factors were kept in the final model solely based on knowledge of previous literature and may not necessarily be considered as predictors of virological rebound. However, such factors and others not investigated in the present study need further research in future studies.

The sensitivity analysis provided more insights on the validity of the results and reaffirmed the present study's findings. This step of the analysis provided a means of showcasing how these findings were not biased, although missing or non-measured categories for some categorical exposure variables were included in the final analysis.

CHAPTER 5 - LIMITATIONS

5.1 INTRODUCTION:

The study findings should be considered in light of some limitations. This chapter presents the limitations of the present study and discusses how these may have restricted the generalizability of the presented findings.

5.2 LIMITATIONS OF THE STUDY

Firstly, being a retrospective cohort study that used routinely collected clinic data, missing information is inevitable. Due to the limited data available on ART adherence, WHO staging, and alcohol use history, these covariates were not included in the inferential analysis. The lack of data on these covariates meant that they could not be linked to virological rebound. Hence, other additional potential explanations and risk factors of virological rebound may not have been adequately addressed.

Secondly, the exclusion of patients who had a high viral load within three months from the time they became virally re-suppressed from the analysis may have introduced some survivorship bias. It was assumed that there was a high likelihood that high viral loads in these patients might have been viral blips other than virological failures. However, patients who registered a high viral load after these three months may have been overall healthier patients. Restricting the analysis to this group of patients may have underestimated the burden of virological rebound. In addition, due to the sparsity of previous literature on the topic, the comparison of this study's findings on the risk of virological rebound among virally re-suppressed patients after the initial virological failure with those of studies that focussed on the risk of the initial virological failure may have also introduced survivor bias in most of the discussions. Patients who were able to survive the initial virological failure and achieve viral re-suppression were more likely to have more favourable outcomes. As such, the potential impact of survivorship bias in both of these scenarios needs to be considered when interpreting the study findings.

Thirdly, the outcome was subject to misclassification. Based on the outcome definition, some viral blips may have been considered as virological rebound and vice versa. Even though all high viral loads within the first three months after viral re-suppression were considered as viral blips, some patients may have experienced a viral blip outside this period which may have been misclassified as virological rebound. High viral loads may have also been misclassified as virological rebounds during the follow-up period since not all viral load tests were done at scheduled times as per standard guidelines. The former scenario may have overestimated, and the latter underestimated the incidence rates of virological rebound. A pragmatic definition of virological rebound, as defined by a single VL >1000 copies/ml, was used in the present study. This is based on the WHO recommendation on the threshold of 1000 copies/ml for defining virological failure, as mentioned in the literature review. In addition, two consecutive viral loads between 400-999 copies/ml that were three months apart were also considered as virological rebound in the present study. There is a chance that the latter definition may have misclassified some patients to have experienced virological rebound and may represent a more stringent monitoring policy. This limitation may have particularly, overestimated the actual burden of virological rebound. As such, this limitation, together with the previous two scenarios, should be considered when making inferences with the presented results.

The fourth limitation to be considered is the study's relatively small sample size, which may have affected the precision of the reported estimates. However, the sample size is larger than that of the few studies that have attempted to report on virological rebound burden. Also, this is comparable with sample sizes of previous studies on initial second-line ART failure in similar settings. In addition, the high statistical power of the sample size sufficiently supports and validates the results of this study as being statistically plausible.

The fifth limitation of the present study is that it did not investigate the incidence of drug resistance across the different second-line ART regimen types. Drug resistance testing would have also provided information to rule out the possibility of the reported virological rebound cases reported in this study being due to drug resistance and not poor ART

adherence. Even though high rates of virological rebound have been reported, it is difficult to be certain that all the patients that experienced virological rebound were required to switch to third-line regimens since drug resistance testing is not readily available to all patients in this setting to confirm this. Although previous studies have reported that most patients fail second-line ART mainly due to poor adherence (51), the present study does not exclude the idea that some of the virological rebound cases reported may be due to drug resistance. Furthermore, this study was limited to patients with documented viral re-suppression after initial virological failure who mostly had good ART adherence at baseline. However, this study was unable to comment on subsequent ART adherence behaviours to rule out drug resistance. However, some studies have suggested that patients with persistent virological failure on PI-based second-line ART may show some traces of PI genotype resistance (48,49). This further demonstrates that this aspect warrants further investigation.

A sixth limitation focuses on the generalisability of the study findings. The diversity of patients accessing services at TLC by nationality and race is already a substantial limitation towards the generalisability of the present study's results to similar patients from all HIV treatment sites in South Africa. Again, TLC being an urban HIV treatment site, the patients accessing health services have different characteristics in comparison to those accessing these services at rural-based HIV treatment sites. Besides, the standard of care may differ in rural-based and urban-based HIV treatment sites, which also adds to the poor generalisability of the study findings. Also, TLC is one of the accredited Comprehensive Care, Management, and Treatment (CCMT) sites in South Africa (82). As such, the patients included in this study may not be truly reflective of those in the general HIV population. The intense monitoring and EAC support offered to these patients may have a positive impact on their attitude towards health. In comparison with the general HIV population, such patients are likely to have more successful treatment outcomes. Therefore, considering the study's setting and patient characteristics, these study findings may not be generalizable to other populations having different patient and health system characteristics.

Lastly, the most common limitation with any cohort study design is LTFU, and in the present study, this seemed to be high during the study's follow-up period, as presented earlier. Viral load test results for patients that were LTFU, transferred to other treatment sites, or died close to the study's baseline were not available. As such, these patients were excluded from the analysis. The lack of follow-up data for these patients reduced the sample size included in this analysis, which may have lowered the power of the study sample size. This effect on sample size may have also impacted on the incidence rate of virological rebound observed in this study. Furthermore, patients that were censored at the time they exited the cohort may have experienced virological rebound outside the treatment site during the follow-up period. Many settings consider LTFU patients as ART defaulters (110) since they have a high likelihood of dying within one year of treatment discontinuation. Studies in South Africa reported deaths between 27%- 48% among such patients who were traced (111,112). It seems reasonable to censor patients at the time they were LTFU, but it does not entirely rule out the chances of these patients experiencing virological rebound outside the cohort within the study's follow-up period. As such, it is possible that the censoring of these LTFU patients may have underestimated the reported rates of virological rebound. However, it suffices to say that LTFU is common in cohort studies that use clinic data, and in this study, this provides a true reflection of patient characteristics that need to be considered in HIV management.

5.3 STRENGTHS OF THE STUDY

Although the study may be considered to have had some limitations, it is the first study to investigate virological rebound in this subgroup of patients and it forms a foundation for future studies. Most importantly, is the fact that the results of the present study provide important information for health care providers, particularly on the importance of closely monitoring patients who achieved viral re-suppression after experiencing their initial virological failure during second-line ART. As mentioned earlier, this study had a substantially large sample size, and the duration of follow-up of patients was longer as compared to other previous studies on second-line ART treatment outcomes (6,8). Also, the study used routinely collected clinic data, which gives a true reflection of second-line ART management in a resource-limited setting. In addition, the study partially used a

standard definition of virological failure to define virological rebound in accordance with WHO guidelines, which enabled comparison with other study findings. In general, the data used in this analysis was considered robust and reliable, which allowed the application of survival analysis methods. Lastly, to ensure standard reporting quality, it is worth mentioning that the study has been reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (113).

CHAPTER 6 - RECOMMENDATIONS AND CONCLUSION

6.1 INTRODUCTION:

This chapter presents the recommendations and conclusion of the present research study. It begins by highlighting the recommendations that emanate from the findings of the study while referring to earlier key discussions. It ends by providing a detailed conclusion for the study findings.

6.2 RECOMMENDATIONS:

With the high rates of virological failure being reported in studies from resource-limited settings focussing on second-line ART, novel approaches to improve second-line ART are still urgently needed as more patients are being switched from first-line ART to second-line ART. For patients showing early good outcomes, new innovative interventions need to be established to ensure that these patients maintain a good response to second-line ART.

An important observation from this study was the high incidence rate of virological rebound. Although several factors may play a role, ranging from patient to health system factors, the evidence underscores the need for HIV programs to establish continued active follow-up mechanisms for patients even after achieving viral suppression. Notably, for patients who achieve viral re-suppression after initial virological failure while on second-line ART, where resources are available, more vigilant ART monitoring through viral load testing is recommended beyond the current WHO recommendations for early detection of virological rebound.

In addition, further research is needed to understand the profile of these patients in resource-limited settings and to investigate if traces of drug resistance exist. This would provide further evidence to help reduce the burden of virological rebound and forecast the need for third-line regimens are not readily available in resource-limited settings.

Following an improvement in ART adherence through EAC, patients who experience virological failure are expected to have raised CD4 counts if ART immune recovery starts. However, according to this study in some patients, failure of CD4 recovery is common, and for some this immuno-suppressed state can persist. As such, patients who have a low CD4 count after achieving viral suppression following an initial virological failure whilst on second-line ART, may be at risk of experiencing virological rebound. Furthermore, this should alert health care providers of a risk of virological rebound in these patients. More importantly, more attention should be given to these patients since they have proven to have a higher risk of long-term non-response to second-line ART.

With the growing number of patients failing second-line ART in resource-limited settings, prolonging the effectiveness of this treatment and patient survival relies on maintenance of good treatment adherence. To ensure that good virological response is maintained in patients after failing second-line ART, intensifying EAC is essential even though viral suppression is achieved. From this study's findings patients receiving a minimum of five adherence counselling sessions had a lower risk of experiencing virological rebound over a 24-months follow-up. However, to achieve optimum long-term treatment outcomes, the recommendation is that clinics should offer EAC to patients at each visit and irrespective of virological state if resource permitting. This intervention will ensure that health workers or adherence counsellors provide a substantial number of EAC sessions, which are a necessity for the maintenance of good treatment adherence and extend the patients' time on second-line treatment by reducing the risk of virological rebounding. Further investigation is needed to assess the effectiveness of adherence interventions, particularly, EAC. Also, future studies should establish the optimum number of EAC sessions that patients should receive within a year to maintain good treatment adherence.

The importance of measuring BMI in HIV-infected patients as a marker of treatment failure needs to be championed. An important observation in the present study is that low BMI in patients who failed second-line ART may be considered as a marker of future virological rebound. Such patients need intensive nutritional support to maintain the viral suppressed state. In addition, closer monitoring through regular BMI measurements is advised in these patients to maintain or achieve normal BMI.

As more patients continue to fail second-line ART, HIV management of these patients should not ignore the fact that not all virological failures in these patients are due to poor treatment adherence. As such, a growing need for third-line regimens is likely to ensue in these patients. Hence, ongoing studies focusing on drug resistance testing among this group of patients should be conducted to measure the proportion of virological rebounds, not due to treatment non-adherence but rather drug resistance. This knowledge may help to quantify the need for third-line regimens in future HIV management policies.

Finally, the high patient LTFU noted in this study is a strong indication of the need for clinics from RLS, including TLC, to conduct more active follow-up to ensure that such patients have prolonged second-line ART virological response. Also, the presence of missing information in routine clinic data, as observed in this study, shows the importance of continuous training, supportive supervision, and performance monitoring of health workers at HIV treatment sites in RLS. This recommendation will help to improve recording and completion of that clinical information in Electronic Medical Record (EMR) systems.

6.3 CONCLUSION:

In conclusion, this study shows that virological rebound is highly incident among HIV-infected patients on PI-based second-line ART who virally re-suppress after experiencing initial virological failure. This finding emphasises the importance of more frequent and intensive monitoring approaches among these patients, comparable to those offered to patients who fail to achieve viral re-suppression. Attention should be paid to markers of virological rebound in these patients in resource-limited settings. These include low CD4 count and low BMI, which can be used to identify patients who are at higher risk of experiencing virological rebound. Offering ongoing adherence support through enhanced adherence counselling may reduce this risk and promote long-term good virological response. Hence, enhancing patient-centred interventions that promote good ART adherence, immune recovery, and normalised BMI; are essential for the reduction of switches to more expensive and limited third-line regimens

REFERENCES

1. Fox MP, Cutsem G Van, Giddy J, Maskew M, Keiser O, Prozesky H, et al. Rates and predictors of failure of first-line antiretroviral therapy and switch to second-line ART in South Africa. J Acquir Immune Defic Syndr. 2012;60(4):428.
2. Boulle A, Van Cutsem G, Hilderbrand K, Cragg C, Abrahams M, Mathee S, et al. Seven-year experience of a primary care antiretroviral treatment programme in Khayelitsha, South Africa. AIDS. 2010;24(4):563–72.
3. Estill J, Ford N, Salazar-Vizcaya L, Haas AD, Blaser N, Habiyambere V, et al. The need for second-line antiretroviral therapy in adults in sub-Saharan Africa up to 2030: A mathematical modelling study. Lancet HIV. 2016;3(3):132–9.
4. World Health Organization. Access to antiretroviral drugs in low-and middle-income countries: technical report July 2014 [Internet]. 2014 [cited 2018 Jul 5]. Available from: http://apps.who.int/medicinedocs/documents/s21630en/s21630en.pdf
5. World Bank. World development indicators 2014 [Internet]. 2014 [cited 2019 Jul 7]. Available from: https://openknowledge.worldbank.org/bitstream/handle/10986/18237/9781464801631.pdf
6. Hosseinipour MC, Kumwenda JJ, Weigel R, Brown LB, Mzinganjira D, Mhango B, et al. Second-line treatment in the Malawi antiretroviral programme: high early mortality, but good outcomes in survivors, despite extensive drug resistance at baseline. HIV Med. 2010;11(8):510–8.
7. Murphy RA, Sunpath H, Lu Z, Chelin N, Losina E, Gordon M, et al. Outcomes after virologic failure of first-line ART in South Africa. AIDS. 2010;24(7):1007.
8. Fox MP, Ive P, Long L, Maskew M, Sanne I. High rates of survival, immune reconstitution, and virologic suppression on second-line antiretroviral therapy in South Africa. J Acquir Immune Defic Syndr. 2010;53(4):500–6.
9. Ajose O, Mookerjee S, Mills EJ, Boulle A, Ford N. Treatment outcomes of patients on second-line antiretroviral therapy in resource-limited settings. AIDS. 2012;
10. Levison JH, Orrell C, Losina E, Lu Z, Freedberg KA, Wood R. Early outcomes and the virological effect of delayed treatment switching to second-line therapy in an

antiretroviral roll-out programme in South Africa. Antivir Ther. 2011;16(6):853–61.

11. El-khatib Z, Ekström AM, Ledwaba J, Mohapi L, Laher F, Karstaedt A, et al. Viremia and drug resistance among HIV-1 patients on antiretroviral treatment - a cross-sectional study in Soweto, South Africa. Aids. 2011;24(11):1679–87.
12. Evans D, Hirasen K, Berhanu R, Malete G, Ive P, Spencer D, et al. Predictors of switch to and early outcomes on third-line antiretroviral therapy at a large public-sector clinic in Johannesburg, South Africa. AIDS Res Ther. 2018;15(1):10.
13. World Health Organisation. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. 2013.
14. Levison JH, Orrell C, Gallien S, Kuritzkes DR, Fu N, Losina E, et al. Virologic failure of protease inhibitor-based second-line antiretroviral therapy without resistance in a large HIV treatment program in South Africa. PLoS One. 2012;7(3):e32144.
15. Murphy RA, Sunpath H, Castilla C, Ebrahim S, Court R, Nguyen H, et al. Second-line antiretroviral therapy: long-term outcomes in South Africa. J Acquir Immune Defic Syndr JAIDS. 2012;61(2):158–63.
16. Ramadhani HO, Bartlett JA, Thielman NM, Pence BW, Kimani SM, Maro VP, et al. Association of first-line and second-line antiretroviral therapy adherence. Open Forum Infect Dis. 2014;1(2):79.
17. Fox MP, Berhanu R, Steegen K, Firnhaber C, Ive P, Spencer D, et al. Intensive adherence counselling for HIV-infected individuals failing second-line antiretroviral therapy in Johannesburg, South Africa. Trop Med Int Heal. 2016;21(9):1131–7.
18. Garone DB, Conradie K, Patten G, Cornell M, Goemaere W, Kunene J, et al. High rate of virological re-suppression among patients failing second-line antiretroviral therapy following enhanced adherence support: A model of care in Khayelitsha, South Africa. South Afr J HIV Med. 2013;14(4):170–5.
19. Jobanputra K, Parker LA, Azih C, Okello V, Maphalala G, Kershberger B, et al. Factors associated with virological failure and suppression after enhanced adherence counselling, in children, adolescents and adults on antiretroviral therapy for HIV in Swaziland. PLoS One. 2015;10(2).
20. Medecins Sans Frontieres. Untangling the web of antiretroviral price reductions.

Medecins Sans Frontieres. 2011.
21. UNAIDS. GLOBAL REPORT: UNAIDS report on the global AIDS epidemic 2013. Unaids. 2013.
22. Health ND of. National consolidated guidelines for the prevention of mother-to-child transmission of HIV (PMTCT) and the management of HIV in children, adolescents and adults. National Department of Health. 2014.
23. UNAIDS. Fact sheet - Latest statistics on the status of the AIDS epidemic [Internet]. UNAIDS. 2017 [cited 2019 Jul 5]. Available from: http://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf
24. UNAIDS/WHO. UNAIDS report on the global AIDS epidemic. Programme on HIV/AIDS (UNAIDS) and World Health. 2012.
25. UNAIDS. Ending Aids Progress Towards the 90-90-90 Targets. UNAIDS. 2017.
26. Human Sciences Research Council (HSRC). The Fifth South African National HIV Prevalence, Incidence, Behaviour and Communication Survey, 2017: HIV Impact Assessment Summary Report [Internet]. HSRC Press. Cape Town; 2018 [cited 2019 Aug 22]. Available from: http://www.hsrc.ac.za/uploads/pageContent/9237/SABSSMV_Impact_Assessment_Summary_ZA_ADS_cleared_PDFA4.pdf
27. World Health Organization, Unicef, Unaids. Global update on HIV treatment 2013: Results, impact and opportunities [Internet]. World Health Organization. 2013 [cited 2019 Jul 8]. Available from: http://www.who.int/iris/bitstream/10665/85326/1/9789241505734_eng.pdf?ua=1
28. UNAIDS. Fact Sheet on HIV among men in South Africa [Internet]. UNAIDS. 2017 [cited 2018 Jul 8]. Available from: http://www.unaids.org/en/file/114691/download?token=tx9wfqZ_
29. UNAIDS. Global AIDS Update 2016 [Internet]. 2016. Available from: http://www.unaids.org/en/resources/documents/2016/Global-AIDS-update-2016
30. Mahy M, Autenrieth CS, Stanecki K, Wynd S. Increasing trends in HIV prevalence among people aged 50 years and older: evidence from estimates and survey data. AIDS. 2014;28(4):S453.
31. Negin J, Gregson S, Eaton JW, Schur N, Takaruza A, Mason P, et al. Rising levels

of HIV infection in older adults in eastern Zimbabwe. PLoS One. 2016;11(11):e0162967.

32. Haas AD, Keiser O, Balestre E, Brown S, Bissagnene E, Chimbetete C, et al. Monitoring and switching of first-line antiretroviral therapy in adult treatment cohorts in sub-Saharan Africa: Collaborative analysis. Lancet HIV. 2015;2(7):e271-8.
33. Long L, Fox M, Sanne I, Rosen S. The high cost of second-line antiretroviral therapy for HIV/AIDS in South Africa. AIDS. 2010;24(6):915–9.
34. World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach-2010 revision. World Health Organization. 2010.
35. Patel D, Desai M, Shah a N, Dikshit RK. Early outcome of second line antiretroviral therapy in treatment-experienced human immunodeficiency virus positive patients. Perspect Clin Res. 2013;4(4):215.
36. Ferradini L, Ouk V, Segeral O, Nouhin J, Dulioust A, Hak C, et al. High efficacy of lopinavir/r-based second-line antiretroviral treatment after 24 months of follow up at ESTHER/Calmette Hospital in Phnom Penh, Cambodia. J Int AIDS Soc. 2011;14(1):14.
37. Sigaloff KCE, Hamers RL, Wallis CL, Kityo C, Siwale M, Ive P, et al. Second-line antiretroviral treatment successfully resuppresses drug-resistant hiv-1 after first-line failure: Prospective cohort in sub-saharan africa. J Infect Dis. 2012;205(11):1739–44.
38. Gsponer T, Petersen M, Egger M, Phiri S, Maathuis MH, Boulle A, et al. The causal effect of switching to second-line ART in programmes without access to routine viral load monitoring. AIDS. 2012;26(1):57.
39. Kimmel AD, Weinstein MC, Anglaret X, Goldie SJ, Losina E, Yazdanpanah Y, et al. Laboratory monitoring to guide switching antiretroviral therapy in resource-limited settings: Clinical benefits and cost-effectiveness. J Acquir Immune Defic Syndr. 2010;54(3):258.
40. Nichols BE, Sigaloff KCE, Kityo C, Hamers RL, Baltussen R, Bertagnolio S, et al. Increasing the use of second-line therapy is a cost-effective approach to prevent the spread of drug-resistant HIV: A mathematical modelling study. J Int AIDS Soc.

2014;17(1):19164.

41. Department of Health Republic of South Africa. National consolidated guidelines for the prevention of mother-to-child transmission of HIV (PMTCT) and the management of HIV in children, adolescents and adults. Dep Heal Repub South Africa. 2014;
42. Harrigan R. Measuring Viral Load in the Clinical Settings. JAIDS J Acquir Immune Defic Syndr. 1995;10:S34-40.
43. Pujades-Rodríguez M, Balkan S, Arnould L, Brinkhof MAW, Calmy A. Treatment failure and mortality factors in patients receiving second-line HIV therapy in resource-limited countries. JAMA. 2010;304(3):303–12.
44. Johnston V, Cohen K, Wiesner L, Morris L, Ledwaba J, Fielding KL, et al. Viral suppression following switch to second-line antiretroviral therapy: Associations with nucleoside reverse transcriptase inhibitor resistance and subtherapeutic drug concentrations prior to switch. J Infect Dis. 2014;209(5):711–20.
45. Schoffelen AF, Wensing AMJ, Tempelman HA, Geelen SPM, Hoepelman AIM, Barth RE. Sustained Virological Response on Second-Line Antiretroviral Therapy following Virological Failure in HIV-Infected Patients in Rural South Africa. PLoS One. 2013;8(3):e58526.
46. Havlir D V., Bassett R, Levitan D, Gilbert P, Tebas P, Collier AC, et al. Prevalence and predictive value of intermittent viremia with combination HIV therapy. J Am Med Assoc. 2001;286(2):171–9.
47. Loutfy MR, Wu W, Letchumanan M, Bondy L, Antoniou T, Margolese S, et al. Systematic Review of HIV Transmission between Heterosexual Serodiscordant Couples where the HIV-Positive Partner Is Fully Suppressed on Antiretroviral Therapy. PLoS One. 2013;8(2):e55747.
48. Magambo B, Nazziwa J, Bbosa N, Gupta RK, Kaleebu P, Parry CM. The arrival of untreatable multidrug-resistant HIV-1 in sub-Saharan Africa. AIDS. 2014;28(9):1373–4.
49. Rawizza HE, Chaplin B, Meloni ST, Darin KM, Olaitan O, Scarsi KK, et al. Accumulation of Protease Mutations among Patients Failing Second-Line Antiretroviral Therapy and Response to Salvage Therapy in Nigeria. PLoS One.

2013;8(9):e73582.

50. Boyd MA, Moore CL, Molina JM, Wood R, Madero JS, Wolff M, et al. Baseline HIV-1 resistance, virological outcomes, and emergent resistance in the SECOND-LINE trial: An exploratory analysis. Lancet HIV. 2015;2(2):e42-51.
51. Wallis CL, Mellors JW, Venter WDF, Sanne I, Stevens W. Protease inhibitor resistance is uncommon in HIV-1 subtype C infected patients on failing second-line lopinavir/r-containing antiretroviral therapy in South Africa. AIDS Res Treat. 2011;2011.
52. Beach MC, Roter DL, Saha S, Korthuis PT, Eggly S, Cohn J, et al. Impact of a brief patient and provider intervention to improve the quality of communication about medication adherence among HIV patients. Patient Educ Couns. 2015;98(9):1078–83.
53. Golin CE, Earp JA, Tien HC, Stewart P, Porter C, Howie L. A 2-arm, randomized, controlled trial of a motivational interviewing-based intervention to improve adherence to antiretroviral therapy (ART) among patients failing or initiating ART. J Acquir Immune Defic Syndr. 2006;42(1):42.
54. Navarro J, Pérez M, Curran A, Burgos J, Feijoo M, Torrella A, et al. Impact of an Adherence Program to Antiretroviral Treatment on Virologic Response in a Cohort of Multitreated and Poorly Adherent HIV-Infected Patients in Spain. AIDS Patient Care STDS. 2014;28(10):537–42.
55. Collier D, Iwuji C, Derache A, De Oliveira T, Okesola N, Calmy A, et al. Virological outcomes of second-line protease inhibitor-based treatment for human immunodeficiency virus type 1 in a high-prevalence Rural South African setting: A competing-risks prospective cohort analysis. Clin Infect Dis. 2017;64(8):1006–16.
56. Onoya D, Nattey C, Budgell E, van den Berg L, Maskew M, Evans D, et al. Predicting the Need for Third-Line Antiretroviral Therapy by Identifying Patients at High Risk for Failing Second-Line Antiretroviral Therapy in South Africa. AIDS Patient Care STDS. 2017;31(5):205–12.
57. Amin J, Boyd MA, Kumarasamy N, Moore CL, Losso MH, Nwizu CA, et al. Raltegravir non-inferior to nucleoside based regimens in second-line therapy with lopinavir/ritonavir over 96 weeks: A randomised open label study for the treatment

of HIV-1 infection. PLoS One. 2015;10(2):e0118228.

58. Boettiger DC, Nguyen VK, Durier N, Bui H V., Heng Sim BL, Azwa I, et al. Efficacy of second-line antiretroviral therapy among people living with HIV/AIDS in Asia: Results from the TREAT Asia HIV observational database. J Acquir Immune Defic Syndr. 2015;1(68):186.
59. Napravnik S, Eron JJ, Sterling TR, Juday T, Uy J, Moore RD. Outcomes of Second Combination Antiretroviral Therapy Regimens Among HIV-Infected Persons in Clinical Care: A Multicenter Cohort Study. AIDS Res Hum Retroviruses. 2013;29(3):574–80.
60. Koulla-Shiro S, Ciaffi L, Le Moing V, Ndour C, Sawadogo A, Eymard-Duvernay, S Aghokeng A, et al. Randomized Comparison of Three Second Line ART Regimens in Africa: the 2 Lady Study. In: Conference on Retroviruses and Opportunistic Infections [Internet]. Boston; 2014 [cited 2019 Mar 9]. Available from: http://www.croiconference.org/sites/all/abstracts/541LB.pdf
61. Waters L, Bansi L, Asboe D, Pozniak A, Smit E, Orkin C, et al. Second-line protease inhibitor-based antiretroviral therapy after non-nucleoside reverse transcriptase inhibitor failure: The effect of a nucleoside backbone. Antivir Ther. 2013;18(3):213–9.
62. Burger DM, Agarwala S, Child M, Been-Tiktak A, Wang Y, Bertz R. Effect of rifampin on steady-state pharmacokinetics of atazanavir with ritonavir in healthy volunteers. Antimicrob Agents Chemother. 2006;50(10):3336–42.
63. Gardner EM, Sharma S, Peng G, Hullsiek KH, Burman WJ, MacArthur RD, et al. Differential adherence to combination antiretroviral therapy is associated with virological failure with resistance. AIDS. 2008;22(1):75.
64. Burman W, Gallicano K, Peloquin C. Therapeutic implications of drug interactions in the treatment of human immunodefciency virus-related tuberculosis. Clin Infect Dis. 1999;Mar(1):419–29.
65. Murphy RA, Marconi VC, Gandhi RT, Kuritzkes DR, Sunpath H. Coadministration of Lopinavir/Ritonavir and Rifampicin in HIV and Tuberculosis Co-Infected Adults in South Africa. PLoS One. 2012;7(9):e44793.
66. Nijland HMJ, L'Homme RFA, Rongen GA, Van Uden P, Van Crevel R, Boeree MJ,

et al. High incidence of adverse events in healthy volunteers receiving rifampicin and adjusted doses of lopinavir/ritonavir tablets. AIDS. 2008;22(8):931–5.

67. Sunpath H, Winternheimer P, Cohen S, Tennant I, Chelin N, Gandhi RT, et al. Double-dose lopinavir-ritonavir in combination with rifampicin-based anti-tuberculosis treatment in South Africa. Int J Tuberc Lung Dis. 2014;18(6):689–93.
68. Chakravarty J, Sundar S, Chourasia A, Singh PN, Kurle S, Tripathy SP, et al. Outcome of patients on second line antiretroviral therapy under programmatic condition in India. BMC Infect Dis. 2015;15(1):517.
69. Johnston V, Fielding K, Charalambous S, Mampho M, Churchyard G, Phillips A, et al. Second-line antiretroviral therapy in a workplace and community-based treatment programme in South Africa: Determinants of virological outcome. PLoS One. 2012;7(5):e36997.
70. V C. Addressing treatment failure amongst adults on second line antiretroviral therapy in Khayelitsha. In: 7th South African AIDS Conference. Durban, South Africa; 2015.
71. Cesar C, Shepherd BE, Jenkins CA, Ghidinelli M, Castro JL, Veloso VG, et al. Use of third line antiretroviral therapy in Latin America. PLoS One. 2014;9(9):e106887.
72. Charurat M, Oyegunle M, Benjamin R, Habib A, Eze E, Ele P, et al. Patient retention and adherence to antiretrovirals in a large antiretroviral therapy program in Nigeria: A longitudinal analysis for risk factors. PLoS One. 2010;5(5):e10584.
73. Cornell M, Myer L, Kaplan R, Bekker LG, Wood R. The impact of gender and income on survival and retention in a south african antiretroviral therapy programme. Trop Med Int Heal. 2009;14(7):722–31.
74. Kranzer K, Lewis JJ, Ford N, Zeinecker J, Orrell C, Lawn SD, et al. Treatment interruption in a primary care antiretroviral therapy program in South Africa: cohort analysis of trends and risk factors. J Acquir Immune Defic Syndr. 2010;55(3):e17.
75. Waters L, Patterson B, Scourfield A, Hughes A, de Silva S, Gazzard B, et al. A dedicated clinic for HIV-positive individuals over 50 years of age: A multidisciplinary experience. Int J STD AIDS. 2012;23(8):546–52.
76. Silverberg MJ, Leyden W, Horberg MA, DeLorenze GN, Klein D, Quesenberry CP. Older age and the response to and tolerability of antiretroviral therapy. Arch Intern

Med. 2007;167(7):684–91.

77. Le Moing V, Chêne G, Carrieri MP, Alioum A, Brun-Vézinet F, Piroth L, et al. Predictors of virological rebound in HIV-1-infected patients initiating a protease inhibitor-containing regimen. AIDS. 2002;16(1):21–9.
78. Van Damme W, Kober K, Kegels G. Scaling-up antiretroviral treatment in Southern African countries with human resource shortage: How will health systems adapt? Soc Sci Med. 2008;66(10):2108–21.
79. Bärnighausen T, Bloom DE, Humair S. Human resources for treating HIV/AIDS: needs, capacities, and gaps. AIDS Patient Care STDS. 2007;21(11):799–812.
80. Heestermans T, Browne JL, Aitken SC, Vervoort SC, Klipstein-Grobusch K. Determinants of adherence to antiretroviral therapy among HIV-positive adults in sub-Saharan Africa: a systematic review. BMJ Glob Heal. 2016;1(4):e000125.
81. Coetzee D, Boulle A, Hildebrand K, Asselman V, Van Cutsem G, Goemaere E. Promoting adherence to antiretroviral therapy: the experience from a primary care setting in Khayelitsha, South Africa. AIDS. 2004;18:S27-31.
82. Fox MP, Maskew M, Patrick MacPhail A, Long L, Brennan AT, Westreich D, et al. Cohort profile: The themba lethu clinical Cohort, johannesburg, South Africa. Int J Epidemiol. 2013;42(2):430–9.
83. Department of Health Republic of South Africa. The South African Antiretroviral Treatment Guideline 2013. 2013.
84. Organisation WH. Obesity: preventing and managing the global epidemic-results of a WHO consultation on obesity. 1997.
85. World Health Organization. Nutritional anaemias: Report of a WHO scientific group. World Health Organization - Technical Report Series. 1968.
86. World Health Organization. Interm WHO Clinical Staging Of HIV/AIDS and HIV/AIDS Case Definition For Surveillance. 2005.
87. Cleves M, Gould W, Gutierrez R, Marchenko Y. An introduction to survival analysis using Stata. Second. Stata press; 2008.
88. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. Biometrika. 1994;81(3):515–26.
89. Cox DR, Snell EJ. A General Definition of Residuals. J R Stat Soc Ser B.

1968;30(2):248–65.

90. Pepe MS, Mori M. Kaplan—meier, marginal or conditional probability curves in summarizing competing risks failure time data? Stat Med. 1993;12(8):737–51.
91. Bunupuradah T, Chetchotisakd P, Ananworanich J, Munsakul W, Jirajariyavej S, Kantipong P, et al. A randomized comparison of second-line lopinavir/ritonavir monotherapy versus tenofovir/lamivudine/lopinavir/ritonavir in patients failing NNRTI regimens: The HIV STAR study. Antivir Ther. 2012;17(7):1351–61.
92. Boyd M. Ritonavir-boosted lopinavir plus nucleoside or nucleotide reverse transcriptase inhibitors versus ritonavir-boosted lopinavir plus raltegravir for treatment of HIV-1 infection in adults with virological failure of a standard first-line ART regimen. Lancet. 2013;381(9883):2091–9.
93. Shearer K, Evans D, Moyo F, Rohr JK, Berhanu R, Van Den Berg L, et al. Treatment outcomes of over 1000 patients on second-line, protease inhibitor-based antiretroviral therapy from four public-sector HIV treatment facilities across Johannesburg, South Africa. Trop Med Int Heal. 2017;22(2):221–31.
94. Mills EJ, Bakanda C, Birungi J, Chan K, Ford N, Cooper CL, et al. Life expectancy of persons receiving combination antiretroviral therapy in low-income countries: A cohort analysis from Uganda. Ann Intern Med. 2011;155(4):209–16.
95. Hogg RS, Heath K, Bangsberg D, Yip B, Press N, O'Shaughnessy M V., et al. Intermittent use of triple-combination therapy is predictive of mortality at baseline and after 1 year of follow-up. AIDS. 2002;16(7):1051–8.
96. Sanjobo N, Frich JC, Fretheim A. Barriers and facilitators to patients' adherence to antiretroviral treatment in Zambia: A qualitative study. Sahara J. 2008;5(3):136–43.
97. Bhat VG, Ramburuth M, Singh M, Titi O, Antony AP, Chiya L, et al. Factors associated with poor adherence to anti-retroviral therapy in patients attending a rural health centre in South Africa. Eur J Clin Microbiol Infect Dis. 2010;29(8):947–53.
98. Joglekar N, Paranjape R, Jain R, Rahane G, Potdar R, Reddy KS, et al. Barriers to ART adherence & follow ups among patients attending ART centres in Maharashtra, India. Indian J Med Res. 2011;134(6):954.
99. Merten S, Kenter E, McKenzie O, Musheke M, Ntalasha H, Martin-Hilber A. Patient-

reported barriers and drivers of adherence to antiretrovirals in sub-Saharan Africa: A meta-ethnography. Trop Med Int Heal. 2010;15:16–33.

100. Gilbert L, Walker L. 'My biggest fear was that people would reject me once they knew my status…': stigma as experienced by patients in an HIV/AIDS clinic in Johannesburg, South Africa. Health Soc Care Community. 2010;18(2):139–46.
101. Tuller DM, Bangsberg DR, Senkungu J, Ware NC, Emenyonu N, Weiser SD. Transportation costs impede sustained adherence and access to HAART in a clinic population in Southwestern Uganda: A qualitative study. AIDS Behav. 2010;14(4):778–84.
102. Quinn TC, Wawer MJ, Sewankambo N, Serwadda D, Li C, Wabwire-Mangen F, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. N Engl J Med. 2000;342(13):921–9.
103. Burgoyne RW, Tan DHS. Prolongation and quality of life for HIV-infected adults treated with highly active antiretroviral therapy (HAART): A balancing act. J Antimicrob Chemother. 2008;61(3):469–73.
104. Casotti JAS, Passos LN, Oliveira FJP, Cerutti C. Factors associated with paradoxical immune response to antiretroviral therapy in HIV infected patients: A case control study. BMC Infect Dis. 2011;11(1):306.
105. Muya AN, Geldsetzer P, Hertzmark E, Ezeamama AE, Kawawa H, Hawkins C, et al. Predictors of nonadherence to antiretroviral therapy among HIV-Infected adults in Dar es Salaam, Tanzania. J Int Assoc Provid AIDS Care. 2015;14(2):163–71.
106. Edessa D, Sisay M, Asefa F. Second-line HIV treatment failure in sub-Saharan Africa: A systematic review and meta-analysis. PLoS One. 2019;14(7).
107. Mhiri C, Bélec L, Di Costanzo B, Georges A, Gherardi R. The slim disease in african patients with aids. Trans R Soc Trop Med Hyg. 1992;86(3):303–6.
108. Ongubo DM, Lim R, Tweya H, Stanley CC, Tembo P, Broadhurst R, et al. A cross-sectional study to evaluate second line virological failure and elevated bilirubin as a surrogate for adherence to atazanavir/ritonavir in two urban HIV clinics in Lilongwe, Malawi. BMC Infect Dis. 2017;17(1):461.
109. Van Der Sande MAB, Shim Van Der Loeff MF, Aveika AA, Sabally S, Togun T, Sarge-Njie R, et al. Body mass index at time of HIV diagnosis: A strong and

independent predictor of survival. J Acquir Immune Defic Syndr. 2004;37(2):1288–94.

110. Rosen S, Fox MP, Gill CJ. Patient retention in antiretroviral therapy programs in sub-Saharan Africa: A systematic review. PLoS Med. 2007;4(10):e298.
111. Maskew M, MacPhail P, Menezes C, Rubel D. Lost to follow up - Contributing factors and challenges in South African patients on antiretroviral therapy. South African Med J. 2007;97(9):853–7.
112. Dalal RP, MacPhail C, Mqhayi M, Wing J, Feldman C, Chersich MF, et al. Characteristics and outcomes of adult patients lost to follow-up at an antiretroviral treatment clinic in Johannesburg, South Africa. J Acquir Immune Defic Syndr. 2008;47(1):101–7.
113. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: Guidelines for reporting observational studies. Int J Surg. 2014;147(8):573–7.

APPENDICES

Appendix 1: Plagiarism Declaration

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I **Khumbo Shumba** (Student number: **1708223**) am a student registered for the degree of **Master of Science in Epidemiology in the field of Epidemiology and Biostatistics** in the academic year **2018.**

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: _____10th__February 2020_____

Appendix 2: Research Study permission letter

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

Gauteng Department of Health
Helen Joseph Hospital
Enquiries: Dr. M.R. Billa
Chief Executive Officer
Tel :(011) 489-0306/1087
Fax :(011) 726-5425
E mail: Raymond.Billa@gauteng.gov.za
Date: 21 November 2018

Dr.M.R.Billa
Chief Executive Officer
Helen Joseph Hospital

Dear Dr. M.R Billa

STUDY: Virological rebound among re-suppressed patients on HIV therapy in Johannesburg South Africa

RESEARCHERS: Khumbo Shumba

Above the study was discussed at the Research Committee meeting. We recommend that permission be granted for Helen Joseph Hospital to be used as a site for the above research, However , since this is individual /Patients.
Upon completion of the study, copy thereof should be submitted to Helen Joseph Hospital.
It is duty of the researcher to collect the data to the relevant department after the Research Committee approved the study.

Thank you

Dr. Murimisi Mukansi
CHAIRPERSON
DATE:

Approved

Dr. M.R. BILLA
CHIEF EXECUTIVE OFFICER
DATE: 22-11-2018

Appendix 3: HREC (Medical) Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr K Shumba

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M181004

NAME: (Principal Investigator)	Dr K Shumba
DEPARTMENT:	School of Public Health Division of Epidemiology and Biostatistics Medical School University
PROJECT TITLE:	Virological rebound among re-suppressed patients after failing second-line HIV therapy in Johannesburg, South Africa
DATE CONSIDERED:	26/10/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr D Onoya and Mr C Nattey
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	21/12/2018

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 Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

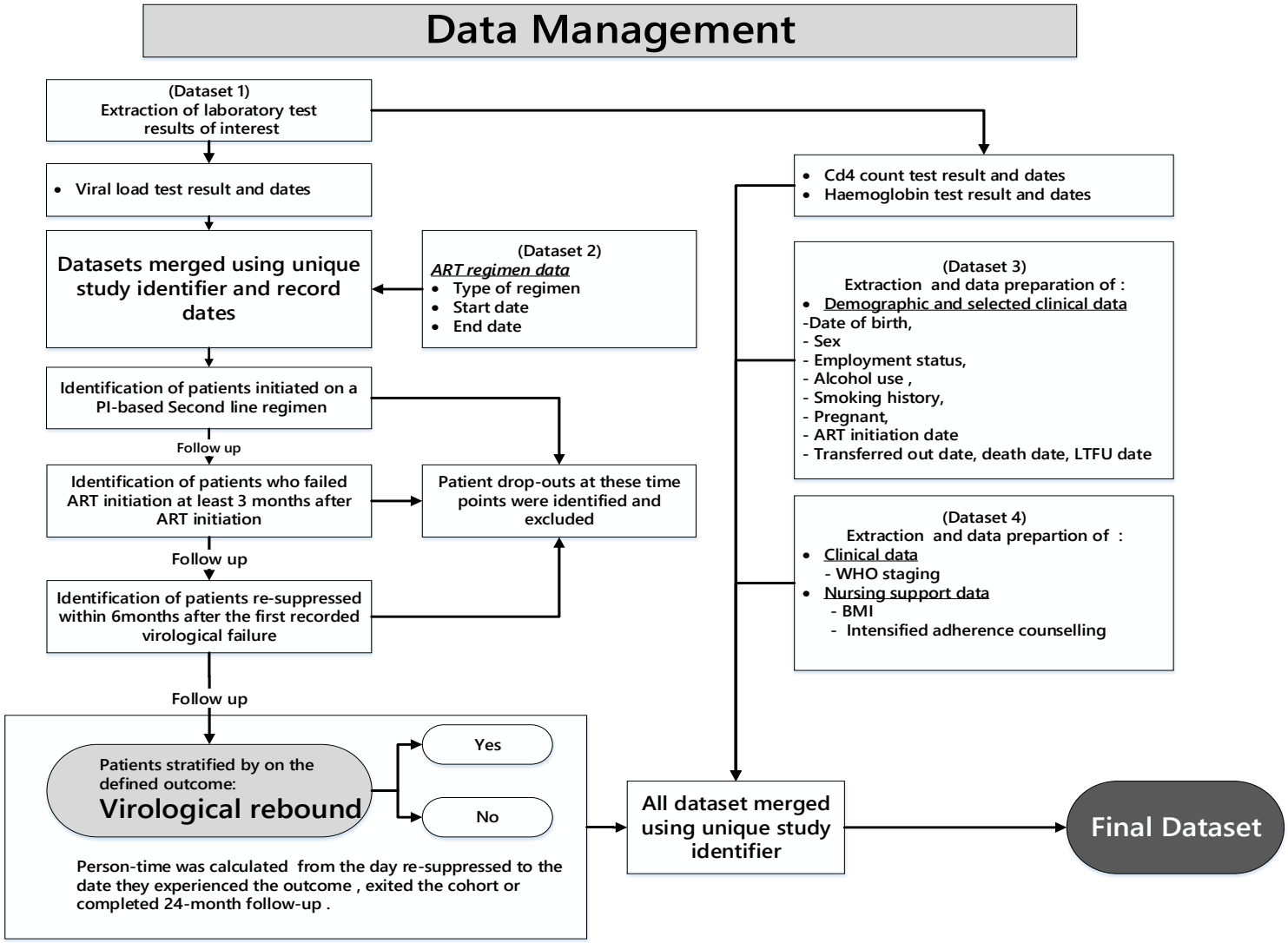
I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. I agree to submit a yearly progress report. When a funder requires annual re-certification, the application date will be one year after the date of the meeting when the study was initially reviewed. In this case, the study was initially reviewed in October and will therefore reports and re-certification will be due early in the month of October each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

04-01-2019
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 4: Data management and analysis dataset preparation



Appendix 5: Definitions for all measurement variables

Variable	Definition	Time of data collection	Variable type	Variable coding
Socio-demographic variables				
Age	The patient's age. A continuous variable measured in years.	At the study's baseline	Exposure variable	N/A
Sex	The patient's sex. A binary categorical variable categorised as male and female.	At the study's baseline	Exposure variable	Male = 1 Female = 2
Employment status	The employment status of the patient. A binary categorical variable categorised as unemployed and employed.	At ART initiation	Exposure variable	Un-employed = 0 Employed =1
Alcohol use	The patient's alcohol use history. A binary categorical variable categorised as yes and no.	At ART initiation	Exposure variable	No = 0 Yes =1
Smoking status	The patient's smoking history. A binary categorical variable categorised as yes and no.	At ART initiation	Exposure variable	No = 0 Yes =1
Clinical variables				
Virologic rebound	A binary variable defined as a high viral load (VL) of >1000 copies/ml after virological re-suppression following initial virological failure on second-line ART. Also considered as virological rebound 3-months from the study's baseline was two consecutive VLs, which were	During follow-up	Outcome variable	No = 0 Yes = 1

	3-months apart with the first VL as 400-999 copies/ml followed by a VL ≥400 copies/ml. This variable was defined as yes (virological rebound) and no (maintained virological re-suppression).			
Enhanced adherence counselling (EAC)	A binary variable categorised based on the number of EAC sessions received from the study's baseline: < 5 and ≥ 5 sessions	During follow-up	Exposure variable	< 5 sessions= 0 ≥5 sessions =1
WHO clinical staging	An ordinal categorical variable categorised into four categories for stages 1, 2, 3, and 4 of the WHO clinical staging criteria	At the study's baseline	Exposure variable	WHO clinical stage 1 = 0 WHO clinical stage 2 = 1 WHO clinical stage 3 = 2 WHO clinical stage 4 = 3 Missing =4
CD4 count	An ordinal categorical variable of the CD4 count result of the patient at the study's baseline categorised as ≥ 500 cells/µl, 350 – 499 cells/µl, and < 350 cells/ µl.	At the study's baseline	Exposure variable	< 350 cells/µl = 0 350 – 499 cells/µl = 1 ≥ 500cells/µl = 2
Viral load	A binary variable defined as the HIV load (copies per millilitre) of the patient at the study's baseline, categorised as <200 copies/ml and 201-399 copies/ml.	At the study's baseline	Exposure variable	<200 copies/ml=0 201-399 copies/ml=1

ART adherence	ART adherence of the patient. A binary variable for self-reported Adherence at the study's baseline. Categorised as ≥ 90% (all pills), <90% (few to most pills).	At the study's baseline	Exposure variable	<90% = 0 ≥ 90% =1
Duration on second-line ART (years)	A binary variable of the patient's overall duration on second-line ART at the study's baseline, categorised as <2 years and 2-5years	At the study's baseline	Exposure variable	0–12 = 0 13–24 = 1 ≥ 25 = 2
Duration on ART (years)	The patient's overall duration on ART (first-line ART and second-line ART) at the study's baseline. An ordinal categorical variable categorised into three categories: <1, 1-2, 3-4, and ≥ 5 years.	At the study's baseline	Exposure variable	<1 = 0 1–2= 1 3-4 = 2 ≥ 5 = 3
Type of second-line ART Regimen	A categorical variable of the type of second-line ART regimen at the study's baseline, categorised into three: • AZT +3TC+LPVr • 3TC+LPVr+ TDF • d4T+3TC+LPVr • Other	At the study's baseline	Exposure variable	TDF +3TC/FTC+LPV = 1 AZT +3TC+LPVr = 2 Other = 3
TB co-infection history	Defined as having TB co-infection history at the study's baseline. TB diagnosed through Ziehl-Neelsen stain (ZN), GeneXpert TB test, and	At the study's baseline	Exposure variable	No = 0 Yes =1

	chest X-rays. Binary categorical variable categorised as yes (TB co-infection) and no (No TB co-infection).			
Anaemia	The patient's anaemia status at the study's baseline. A binary categorical variable categorised as yes (Haemoglobin result <11.5 g/dL for females and <13.0 g/dL for males.), No (>11.5 g/dL for females and >13.0 g/dL for males 8g/dl) and not measured.	At the study's baseline	Exposure variable	No = 0 Yes = 1 Not measure=2
Adverse drug reactions (ADRs)	A binary variable capturing the history of ADRs during the study's follow-up. Categorised as yes (ADRs present) and no (No ADRs). The following diagnosed conditions were possible ADRs: dermatitis, gastroenteritis, gynaecomastia, hepatitis, lactic acidosis, lipodystrophy, muscle/joint pain, or neuropathy.	During the study's follow-up period	Exposure variable	No = 0 Yes =1
Time to initial virological failure on second-line ART (months)	An ordinal categorical variable referring to the duration of the first second-line ART failure from the time of the switch from the first-line ART categorised as 3-5, 6-8, and ≥9 months.	Before the study's baseline	Exposure variable	3-5 months=1 6-8 months=2 ≥9 months=3

Time to virological re-suppression after initial virological failure on second-line ART (months)	A binary variable referring to the time from the initial virological failure while on second-line ART to the time when virological re-suppression was achieved. Categorised as 0-3 and 4-6 months.	Before the study's baseline	Exposure variable	
Anthropometric Variables				
Body Mass index (BMI)	The patient's BMI at the study's baseline. An ordinal categorical variable categorized according to the WHO classification as ≥30kg/m2 (obese), 25 – 29.9 kg/m2 (overweight), 18.5 – 24.9 kg/m2 (normal) and <18.5kg/m2 (underweight).	At the study's baseline	Exposure variable	Underweight = 0 Normal = 1 Overweight = 2 Obese = 3

3TC: *lamivudine;* ***ADRs:*** *Adverse drug reactions*; ***ART:*** *antiretroviral therapy;* ***AZT****: zidovudine;* ***BMI:*** *body mass index;* ***LPV/r****: ritonavir-boosted lopinavir;* ***TDF:*** *tenofovir;* ***WHO:*** *World Health Organisation*