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HIV treatment failure-a retrospective analysis of patients receiving 2nd line ARVS at the Tshepong wellness clinic

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Abstract

Background: Expansion of the antiretroviral (ARV) programme has led to an increasing number of patients requiring second line ARV therapy. Little is known about the outcomes and factors influencing the success of second line regimens in South Africa, a resource limited setting.

Objectives: To report the treatment outcomes of patients on second line ARVs, and factors predisposing to treatment failure.

Methods: We conducted a retrospective record review of patients receiving Lopinavir/ritonavir (lpv/rtv) containing second line ARVs at a public hospital in Klerksdorp, South Africa.

Results: In the two years to December 2012, 383 potentially eligible patients were included in the study. 160 were excluded due to: switches due to adverse drug reactions (14%) or absence of an HIV viral load (VL) after second line regimen change (26%). Of the 223 included patients, median age was 42 years (IQR, 36 – 49), and 58% were women. Overall, median baseline CD4 count at first ARV initiation was 96 cells/mm^3 (IQR, 36 – 160) and at initiation of lpv/rtv was 179 cells/mm^3 (IQR, 101 – 275). The median VL at second line initiation was 46,838 (IQR, 21,341-151,333). Median follow up was 62 months (IQR, 46 – 83) since first line ARV initiation and 27 months (IQR: 19-42) since second line initiation; 47% of patients had suppressed VL on second line ARVs with a median time to suppression of seven months (IQR, 6 – 12 months) after switch. By Kaplan Meier survival analysis, the

mean time to second line treatment failure from initiation of ARVs was 43.6 months. The median CD4 count and HIV VL at the time of the regimen switch was 148 cells/mm^3 (IQR, 71-221) and 49,000 copies/ml (IQR, 12,000-176,400), respectively. Thirty six (16%) of study patients had genotypic drug resistance studies performed. Of these, 75% had significant NRTI and NNRTI mutations. There were no clinically significant PI mutations. Using univariate and multivariate analysis, predictors of treatment failure were as follows: 1) patients never suppressed on first line therapy (HR 1.798, 95% CI 1.239 – 2.610) and 2) age > 49 years (HR 1.577, 95% CI 1.159 – 2.942).

Conclusion: This study showed that there is an high rate of failure on second line ARVs in this resource-limited setting with increasing age and failure to suppress on first line therapy associated with treatment failure. Adherence is a major patient mediated factor, which fosters the selection of resistance mutations. Based on earnest results, second line ARVs would be effective in all the patients who received resistance testing. Reinforcement of adherence and compliance measures is needed, clinicians require training, and the role of resistance testing in these patients needs to be evaluated.

RESEARCH PROPOSAL WITH EXTENDED LITERATURE REVIEW

Human Immunodeficiency Virus (HIV) was first discovered in 1983, two years after the recognition of Acquired Immunodeficiency Syndrome (AIDS). In the last thirty years, HIV/AIDS has become a global epidemic affecting approximately thirty four million people worldwide. The burden of disease lies mainly in low resource settings such as South Africa, which in 2012 had an estimated HIV prevalence rate of approximately 12.2% (6.4 million people) (1).

Antiretroviral drugs (ARVs) were first introduced in government roll out programmes in South Africa in 2004 (2). As is common in other developing countries, standardised and affordable fixed dose combinations of ARVS including two nucleotide reverse transcriptase inhibitors (NRTIs) and one non-nucleotide reverse transcriptase inhibitor (NNRTI) are recommended for first line treatment (2-4). The extensive use of ARVs and adverse drug effects have resulted in an increasing number of patients requiring regimen two ARVS, which currently consist of a protease inhibitor (PI) and two NRTIs (2-4). The regimen with a PI backbone has been shown to be durable with multiple mutations required to confer resistance to the drugs (5) and is therefore ideal for second line regimens.

South Africa has the largest ARV programmes in the world with approximately 2.5 million people having been initiated on ARVs at the end of 2012 (6, 7). In 2009, it was estimated that 3 – 5% of patients would require second line therapy (8). With the rapid expansion of the ARV programme (9), prolonged exposure to ARVs and improved life expectancy, the proportion and absolute

number of patients on second line therapy is likely to have increased, as was found in Khayelitsha, where one in five patients was on second line therapy after five years on treatment (10).

There are three spheres in which treatment failure can be assessed – clinical, immunological and virological. Clinical failure is defined as HIV progression, development of AIDS, development of new opportunistic infections and death. It is primarily used in low-income countries as a marker of treatment failure where HIV viral load monitoring is not feasible due to cost implications. It has been shown to be a poor marker of treatment failure (11, 12). Immunological failure is the failure to achieve and maintain adequate CD4 responses despite adequate virological suppression – defined by the World Health Organisation (WHO) as a CD4 T-cell measurement ≤ 100/mm^3 at 12 months or CD4 T-cell fall below baseline (13, 14). In the South African HIV guidelines, treatment failure is defined as a confirmed HIV RNA viral load more than one thousand copies/ml in two measurements taken one to three months apart (15).

Current ARV treatment guidelines in South Africa recommend viral resistance studies for patients who have failed second line therapy (4, 15). Third line treatment options require an expert panel evaluation of the patient and although they are available, they not easily accessible to patients outside specialist centres.

Risk factors for virological and treatment failure have been studied since the introduction of antiretroviral drugs in the early 1990s. Non-modifiable risk

factors include age, gender, ethnicity, baseline CD4 count and viral load. Modifiable risk factors include adherence, socioeconomic status, sexual practices, adverse drug reactions and interactions between the patient and health care system (15-26).

Mills et al defined adherence as taking ninety five percent of the prescribed medication (27). Due to the rapid evolution of HIV, adherence is a critical part of achieving virological suppression and ensuring the success of treatment. Poor adherence has consequences which impact the patient and the health system with the development of antiretroviral drug resistance, development of opportunistic infections and progression of disease, all of which are associated with increased hospitalization rates.

There are biological, psychosocial and institutional factors which influence adherence to treatment. Due to the nature of HIV transmission, there is a strong stigma and culture of discrimination associated with the disease despite various efforts to reduce it. The fear of stigma results in delayed health seeking behaviours and may hamper adherence to therapy as patients attempt to hide their illness. Multiple studies have shown an association between non-disclosure of HIV status, lack of a support structure and poor adherence to treatment, ultimately resulting in treatment failure (28, 29). The patient's socioeconomic status also has a bearing on his or her ability to adhere to prescribed treatment. Poverty and unemployment are strongly associated with treatment interruptions and poor adherence as the patient is unable to attend clinic visits and comply with treatment. Poverty is also

associated with food insecurity and inadequate nutrition (30), which is an integral part of holistic management of HIV infected patients. The initiation of antiretroviral drugs is sometimes associated with a worsening of health before improvement, most likely due to immune reconstitution and adverse drug reactions. The first few months of therapy is associated with the highest level of treatment default and the highest risk of death (31). Conversely, the chronic nature of the disease and lack of a cure is associated with treatment failure after a period of virological suppression and immune reconstitution because patients often stop treatment once they perceive themselves to be healthy. Patient's perception of the institution where they receive treatment also has a strong influence on the retention of patients on treatment. Patients who have had negative experiences from health care workers and institutions are more likely to develop treatment failure than those who have had positive experiences.

HIV drug resistance is an important and predictable factor, which drives treatment failure (25). HIV virological resistance is classified into primary resistance and induced resistance. Primary resistance refers to patients who have been infected with HIV strains, which exhibit resistance to a particular antiretroviral agent or drug class and have not been exposed to ARVS (32). Although primary resistance is beyond the scope of this research, literature on primary resistance gives insight about the baseline prevalence of virological resistance in a particular study population. The estimated prevalence of pre-treatment genotypic resistance in Sub-Saharan Africa is estimated as 5.6% (33).

Induced resistance refers to drug resistance acquired in patients who have been exposed to ARVS. HIV infection is characterized by a state of high viral replication and turnover rate. During seroconversion, there is an initial rapid increase in viral load and decline in CD4 count. In the chronic phase of the infection, the viral load reflects the balance between the host defence mechanisms and the rate of viral replication. The enzyme, Reverse Transcriptase, is particularly prone to error and has been found to have approximately five to ten errors per HIV-1 genome per round of replication in vivo (34).

Wild type virus is usually the fittest form of the virus. Virus mutations can be advantageous or detrimental to the host. Advantageous mutations result in a virus strain, which is too weak to replicate or survive. Detrimental virus strains result in partial or complete resistance to ARVS. In the presence of viral quasi-species that confer drug resistance, initiation of ARVS results in the suppression of the wild type virus and persistence of the mutant virus. Once ARVS are withdrawn, the wild type virus dominates after a period of time.

The absence of a cure for HIV has necessitated lifelong treatment and treatment adherence is a major component of most ARV rollout programs. The development of drug resistance requires on-going viral replication in the presence of antiretroviral drugs. Even brief exposure to a drug can result in the development of drug resistance as was found in patients who were exposed to single agent nevirapine as part of prevention-of-mother-to-child transmission strategies (35, 36).

Partial suppression with residual replication under drug selective pressure results in virological resistance to a particular drug (37, 38). With on going drug selective pressure and the development of mutations, the virus develops detrimental mutations, which confer resistance not only to the drugs to which the patient is exposed, but also the entire drug class. The relationship between adherence and treatment is bell shaped, indicating that very high and very low levels of adherence posed little or no threat to the development of drug resistance. It is the 'in-between' levels of adherence, which carry a higher risk of the development of drug resistance (39).

Virological resistance can be assessed phenotypically and genotypically. Genotypic tests assess the viral genome for the presence of specific mutations, which are known to cause resistance to specific drugs. Using PCR, viral genes are amplified and examined for mutations. Sequencing assays identify all mutations in the viral genome. Point assays assess specific mutations, which are associated with resistance to a particular drug. For genotypic tests to be accurate, the patient must have a detectable viral load of at least 1000 copies/ml. Phenotypic tests directly measure the sensitivity of a laboratory-cultured virus to specific ARVS by measuring the minimum inhibitory concentration of the drug required to stop the virus from replicating. Phenotypic resistance is a research tool, and is very rarely used. For accuracy, the patient must be taking ARVS at the time of testing. A third type of test is a virtual phenotype in which a genotypic test is conducted and

the identified mutations are run through a database of known mutations in which genotypes and phenotypes are linked.

It is common practice in first world countries to conduct virological resistance tests before treatment initiation and at treatment failure. Due to the cost of the tests, this principle is impractical in the South African setting. However, the South African HIV Clinicians Society released guidelines for HIV viral resistance testing in November 2012, which highlighted the need for resistance testing in the South African context as well as the need for further research on the subject.

AIMS AND OBJECTIVES

Aim

To investigate the failure rate of patients receiving second line (PI based) ARVS at the Tshepong Wellness unit and identify factors associated with failure

Objectives

Primary Objectives:

- Determine the proportion of patients with treatment failure on second line ARVS at the Tshepong Wellness Unit
- Determine predictors of treatment failure in patients on second line ARVS at the Tshepong Wellness Unit
- Determine the proportion of clinically significant HIV resistant mutations in the patients who have had viral resistance tests done

Secondary Objectives

- Determine the time to treatment failure
- Determine the predictors of time to treatment failure

METHODS

Study Site

The retrospective record review will be conducted at the Tshepong Hospital Wellness Clinic (THWC). This HIV clinic serves as the main referral site in the Kenneth Kaunda district in the North West and serves a population of approximately 696 500 people. At the end of 2012, the clinic had initiated approximately 26 000 patients on ARVs, some of them collecting treatment at the local clinics. On 31 December 2012, there were approximately 1800 patients on PI based ARVS in the district, of which 300 were collecting them at THWC. All viral resistance tests from the Tshepong Wellness Clinic are conducted at the Charlotte Maxeke Johannesburg Academic Hospital NHLS laboratory. The laboratory uses an in house genotypic assay, which has been validated against commercially available assays, to identify mutations in the HIV RNA.

Case Identification

Study population

The Tshepong Wellness Unit currently has approximately 300 patients on PI-based ARVS. Patient names will be collected from the Wellness Unit pharmacy and records of all patients on second line ARVS will be assessed.

Study Definitions

Treatment failure: two HIV VL measurements > 1000 RNA copies/ml, with adherence and other issues addressed in the interval in a patient who has been on second line ARVs for more than 6 months (14).

Virological suppression: HIV viral load < 1000copies/ml

The study design is a case series of all patients on second line ARVS from January 2011 – December 2012

Eligibility criteria

- Patients > 18 years age
- Receiving second line ARVS from the Tshepong Wellness Unit
- Good clinical records which must indicate the following: the date of second line treatment initiation, the sequence and dates of ARV switches, CD4 count and HIV VL results after the regimen change, and dates and durations of treatment interruptions)
- Patients on second line ARVS for reasons other than treatment failure (ie. adverse drug reactions on regimen 1 ARVS).

Case entry and data collection

The following patient characteristics will be recorded (Addendum 1):

- Age
- Sex
- Date of ARV initiation

- ARV history (initial regimen/regimen changes/current regimen)
- History of treatment default
- Baseline CD4 count and HIV viral load
- Latest CD4 count and HIV viral load
- CD4 count and HIV viral load at point of viral resistance test (if done)
- Duration of ART with detectable VL prior to change from initial regimen to PI based/number of detectable viral load prior to change
- Exposure to PMTCT
- Comorbidities
- Co-therapies
- Opportunistic infections
- History of treatment default

Statistics

- Characteristics of patients will be presented as mean and standard deviation or median and range for continuous variables if normally distributed or not normally distributed respectively
- Categorical variables will be presented as frequencies and percentages with 95% confidence interval. CD 4 counts will be log transformed.
- Multiple logistic regression will be used to predict virological failure.
- The mean time to the development of treatment failure will be determined using a Kaplan Meier product-limit method

- The predictors of time to virological failure will be assessed using a Cox Proportional Hazards Regression analysis

Ethics

The proposal of this study will be submitted to the Postgraduate Committee of the University of the Witwatersrand and the Human Research Ethics committee (HREC) of the University of the Witwatersrand. All the information will be obtained from the patient files. Data will be taken collected by myself from the patient records. Written permission will be obtained from the Shaping Hospital CEO, Medical Manager, Head of Internal Medicine and Unit Manager of the Wellness Clinic.

During data collection, the patient's details will be documented using a numbering system. The biological resistance test results are available in a digital format from the laboratory and all documents will be kept confidential.

The study will be conducted in adherence to the principles of the Declaration of Helsinki (40).

Potential Problems

Quality of patient records

The biological resistance test results have patient's names only. In the absence of file numbers, there may be difficulty in obtaining patient files from the archives and as a result the data may be incomplete.

Data Collection

Data will need to be collected from the Tshepong Wellness Clinic during working hours. Over a period of 3 months, the researcher will liaise with 2 sisters from the Tshepong Wellness Unit who will retrieve files in order for the researcher to extract the required data.

ADDENDUM A: DATA COLLECTION SHEET

Patient Demographics

Patient Number		0001	
Age			
Race	Black	White	Coloured
Income source	Employed	Unemployed	Disability Grant

Clinical Information

WHO STAGING			
PMTCT			
If yes, regimen		**YES**	**NO**

ARV History
Initiation (baseline)

Date		CD4	VL
Regimen			

Regimen Change 1

Date		CD4	VL
Regimen			
Indication			

Regimen Change 2

Date		CD4	VL
Regimen			
Indication			

Regimen Change 3

Date		CD4	VL
Regimen			
Indication			

Viral Resistance Test Results yes/no

Phenotypic Resistance (ARVS available in the state sector)

History of treatment default

DATE	DURATION	REASON

Opportunistic Infections

DATE	TREATMENT	ADJUSTMENT OF ARVS

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RESEARCH REPORT (“submissable” publication format)

HIV TREATMENT FAILURE – A RETROSPECTIVE ANALYSIS OF PATIENTS RECEIVING 2ND LINE ARVS AT THE TSHEPONG WELLNESS CLINIC

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Background

The burden of HIV-infection is primarily in low resource settings in sub-Saharan Africa - such as South Africa, which in 2014 had an estimated 6.8 million people living with HIV (1) and an estimated 3m receiving antiretroviral (ARV) therapy.

As is in other developing countries, standardised and affordable combinations of ARVs are recommended; first line includes two nucleoside reverse transcriptase inhibitors (NRTIs) – Stavudine (D4T), Tenofovir (TDF), Zidovudine (AZT), Lamivudine (3TC), and Abacavir (ABC); and a non-nucleoside reverse transcriptase inhibitor (NNRTI) – nevirapine (NVP) or efavirenz (EFV). Second line therapy includes a protease inhibitor (PI), namely lopinavir/ritonavir and two NRTIs (2-5). From 2003 until 2010, patients received D4T + 3TC + EFV (or NVP for pregnant women). Thereafter, all patients received TDF + 3TC + EFV for first line therapy. The regimen with a PI backbone has been shown to be durable with multiple mutations required to confer resistance to the drugs (6) and as a result forms the backbone of second line therapy.

South Africa has the largest ARV roll out programmes globally, with over 2.5 million people having being initiated on ARVS at the end of 2012 (7, 8). In 2009, it was estimated that 3-5% of patients would require second line therapy (9). With the rapid expansion of the ARV programme (10), prolonged exposure to ARVs and improved life expectancy, the proportion and absolute

number of patients on second line therapy has increased; in Khayelitsha one in five patients was on second line therapy after five years of treatment (11). Treatment failure is measured using clinical, immunological and virological definitions; clinical failure is HIV progression, development of AIDS, development of new opportunistic infections and death. In the South African HIV guidelines, treatment failure (virological failure) is HIV RNA viral load of >1000 copies/ml on two separate specimens, taken 1-3 months apart (13). According to the guidelines, viral resistance testing is recommended after failure of second line treatment.

To assess the rates of virological failure on Lpv/rtv based second line ARVs and identify factors, which may influence response to treatment, including the presence of genotypic viral resistance, we abstracted data from clinical records of patients attending a large HIV clinic in South Africa.

METHODS

Study Site

A retrospective record review was conducted at a regional hospital's HIV clinic in Klerksdorp, South Africa - the Tshepong Hospital Wellness Clinic (THWC), the primary HIV referral site for the Dr Kenneth Kaunda district in the North West Province, serving a population of 700,000 people. The clinic receives patients from the following areas: Jouberton, Alabama and Klerksdorp CBD which were defined as close (<15km or <2 taxis needed to get to the clinic); and Tigane, Kanana and Khuma (>15 kms and/or > 2 taxis needed to get to the clinic). At the end of 2012, the clinic had initiated approximately 26,000

patients on ARVs in the district. On 31 December 2012, there were approximately 1,800 patients on PI-based ARV treatment in the Dr Kenneth Kaunda Health District - 383 patients were collecting them at THWC and the rest were collecting them from the local public sector primary care clinics and general practitioners.

Study Patients

Patients were identified from the THWC Pharmacy treatment dispensing records. Their files were then extracted from the clinic filing room, and evaluated. They were included in the study if they were 18 years or older and were receiving lpv/rtv based combination ARVs as second line treatment due to treatment failure on first line ARVs between January 2010 and December 2012. Additionally, they had to have adequate clinical records which at a minimum had to indicate: the date of second line treatment initiation, the sequence and dates of ARV switches, CD4 count and VL results after the switch to second line, and dates and durations of treatment interruptions. Patients were excluded from the study if they were changed to second line ARVs due to an adverse drug reaction on regimen one.

The study definition of treatment failure was two consecutive HIV viral load measurements ≥1000 RNA copies/ml taken at least three months apart (13), with adherence and other issues addressed in that interval, in a patient prescribed ARVs for at least 6 months. At the time of diagnosis of treatment failure, the routine procedure at the clinic was as follows: failing patients were referred to an HIV counsellor to reinforce adherence and compliance, and

patients with suboptimal social circumstances were referred to a social worker for assistance and if required given a letter for application for a disability grant.

In this study, adherence to treatment was treated as a dichotomous variable, determined from either pill counts or a history of treatment default in the clinical records. Health care worker (doctor, nurse or counsellor) notes of poor adherence, sexual risk behaviours on ARVs (ie. unprotected sexual intercourse, multiple sexual partners) and barriers to seeking treatment or taking medication were also abstracted - the latter to assess the risk of secondary HIV infections. We used the township where the patient lived as a proxy for distance from the THWC.

Where they were available, we abstracted viral resistance results assayed at the National Health laboratory Service (NHLS), on an in-house genotypic assay cross-referenced with the Stanford database.

Tests of association were assessed by chi-square analysis while medians were compared non-parametrically using the Kruskall-Wallis test. Time to failure by gender, age group and CD4 were assessed by the Kaplan-Meier test and logrank p-values. Cox proportional hazards regression was used to assess predictors of failure. Hazard rates and their 95% confidence intervals were determined at the univariate level. Using knowledge about the field and backward regression procedure, the final multivariate regression was developed. All statistical analysis was performed by SAS Enterprise Guide 5.3 (SAS Institute, Cary NC).

Approval was obtained from the Human Research Ethics Committee (HREC) of the University of the Witwatersrand and from Research Committees of the North West Department of Health.

Results

Three hundred and eighty three patients were registered as collecting lpv/rtv based combination ARVs at the THWC pharmacy between January 2010 and December 2012. Of these, 23 were excluded because regimen changes were due to adverse drug reactions (lactic acidosis (17), pure red cell aplasia (2) and psychosis (4). Twenty-nine of the remaining files had inadequate records with scanty data – most did not have adequate drug history histories; 42 files were excluded because they did not have an HIV VL available after the regimen switch; and 66 files could not be found, leaving 223 (58%) subjects eligible for analysis (figure 1).

Forty-four percent of the patients lived in Jouberton and the remaining were from surrounding townships, namely Kanana (21%), Khuma (14%), Tigane (5%), Alabama (10%) and Klerksdorp CBD (1%). Twenty seven percent of the patients were unemployed and 31% dependent on disability and other government grants (pension and child support grant). Virtually all (95%) of the included patients were black African and the majority (58%) were women (figure 2). Overall, the median age was 42 years (IQR, 36 – 49 years) (table 1). There were no significant differences in the demographic and socioeconomic statuses of patients with virological failure and virological

suppression, however 22% of patients with virological failure were receiving disability grants compared to 15% of patients virologically suppressed (p = 0.721)(table1).

The median total time from initiation of first line ARVs to abstraction of data was 62 months (IQR, 46 – 83 months), and the median time since second line initiation 27 months (IQR: 19-42) . At first initiation of ARVs, the median CD4 count was 96 cells/mm^3 (IQR, 36 – 160) and 69 (32%) of the patients had CD4 counts below 50 cells/mm^3 (figure 3). Patients with virological failure had a lower baseline CD4 count (88 cells/$mm^{3)}$ compared to those with virological suppression (106 cells/mm^3) ($p<0.0001$)(table 1). The median time for switching to second line therapy (lpv/rtv based) from the date of the second unsuppressed VL was 10 months (IQR, 6 – 22 months) for patients who were virologically suppressed on second line and 12.5 months (IQR, 7 – 21 months) for patients with virological failure (p=0.4093); indeed only 35 (18%) of patients were switched within the guideline recommended 3 – 7 months.

Evidence of poor adherence was documented in 98% of patients' records with virological failure and 54% of those with virological suppression ($p < 0.0001$); inappropriate pill counts (61%) and missed appointments (43%) were most frequent. Work related problems (35%), social issues (23%), lack of funds (14%), and use of alternative medicines (7%) were commonly recorded as reasons for defaulting treatment. 63% of the patients from Khuma, 60% of the patients from Tigane and 52% of the patients from Kanana failing treatment; compared to Jouberton with 45% failure rate.

185 (84%) of the patients received D4T + 3TC + NVP/EFV as first line therapy for a median duration of 29 months (IQR, 20 – 44) - 60% of these patients had virological failure on second line ARVs. Twenty eight (12%) received TDF + 3TC + NVP/EFV at ARV initiation for a median duration of 18 months (IQR, 13 – 23.5) - 25% of these patients had virological failure; 6 (1%) received AZT + 3TC + NVP/EFV, all of whom failed second line treatment. The median duration on first line ARVs was 27 months (IQR, 16.1- 42.2) for patients with virological suppression, and 25 months (IQR, 19.1 - 42.2) for patients with virological failure (p=0.6378). Forty percent of the patients had previously suppressed on first line treatment and these patients were more likely to be suppressed on second line treatment than those who were not suppressed on first line regimen (p=0.0057).

Fifty two percent of patients received TDF + 3TC + Lpv/rtv, 29% received AZT + DDI + Lpv/rtv and 18% received AZT + 3TC + Lpv/rtv, for 2nd line therapy. Seventy percent of patients (n = 78) with virological failure on second line ARVs, and 60 (66%) of patients who were virologically suppressed switched regimens after seven months of the second elevated VL. The median time to second line failure was 13 months (IQR, 9 – 23).

At the point of switching to second line treatment, the median CD4 count and HIV VL were 183 cells/mm^3 (IQR, 117 - 266 cells/mm$^{3)}$ and 24,546 copies/ml (IQR, 5100 - 70600 copies/ml) respectively, in patients who were virologically suppressed (figure 4). Patients with virological failure had median CD4 count

and HIV VL of 168 cells/mm^3 (IQR, 97 – 263 cells/mm^3) and 49000 copies/ml (120000 – 176400 copies/ml) respectively.

Fifty three percent (n = 118) of patients on second line treatment, failed treatment at a median time from initiation of first line ARVs of 43.5 months (figure 5). The median CD4 count and HIV VL in patients with virological failure after six months on second line therapy was 169 cells/mm^3 and 49000 copies/ml respectively. Patients who were suppressed on second line treatment after six months on treatment had a median CD4 count of 212 cells/mm^3 (figure 4). Patients who received a D4T based first line regimen were more likely to fail second line therapy than those who received TDF (p = 0.0051).

Overall, there were 36 patients (16%) with a genotypic drug resistance result that coincided with their second line treatment failure. Of those, 9 (25%) had no clinically significant mutations. The most common NNRTI mutation in the remainder was K103N (58%); NRTI mutations were as follows: M184V (62%), Thymidine analogue mutations (18%) and K65R (15%). Of those with resistance, 66% had high-level resistance to NNRTIs. Intermediate or high level resistance to the NRTIs were as follows: 3TC, n=22 (64%); AZT , n= 4 (20%); TDF, n=6 (17%); D4T, n=7 (23%); DDI, n=11 (31%) and ABC, n= 17(47%). There were no clinically significant PI mutations, suggesting that patients should have responded to second line treatment.

Predictors of treatment failure were: patients who were never suppressed on first line therapy (figure 6) aHR 1.798, 95% CI 1.239 – 2.610) and patients older than 49 years (HR 1.577, 95% CI 1.159 – 2.942) (figure 7). Poor adherence was significantly higher in patients with treatment failure ($p < 0.0001$), however the statistical significance was lost when the model was adjusted for time to failure.

Discussion

This retrospective study at an urban hospital in the North West Province, South Africa, showed an extremely high rate of second line ARV treatment failure (53%) compared to reports from developing countries (6, 11, 14-16), which range from 8.6 – 37.3%. Patients who were never suppressed on first line therapy were more likely to fail second line therapy. Yet, more than 50% of patients who subsequently achieved virological suppression on second line therapy had been characterized as poor adherers, suggesting that clinic measure to address adherence and social issues may be effective in improving treatment outcomes.

Clinicians also contribute to sequelae of treatment failure by delaying appropriate switch in regimens. In this study population, only 18% of patients were switched within an appropriate time period – although this may be attributed to the multiple changes in ARV guidelines since the start of the ARV rollout programme.

Studies to determine the association between age and treatment failure have had contradicting results. Earlier studies showed that advanced age at the time of ARV initiation was associated with more advanced disease, more rapid progression to AIDS, smaller increases in CD4 counts and slower virological responses to treatment (17, 18). However, these studies were limited by the inability to determine the duration of HIV infection and account for its influence on disease staging, progression and success of treatment. In a prospective study which adjusted for duration of HIV infection, Weintrob at al (19) found that older age was associated with higher rates of adherence and virological suppression. Older age was a significant predictor of treatment failure in our study, however the duration of HIV infection was unknown and it was unclear what the premorbid functional status was and what the comorbidities of the older patients had, which contributed to virological failure.

These patients tended to have a higher rate of non-adherence and suboptimal social circumstances. Mills et al defined adherence as taking 95% of the prescribed medication (20). Due to the rapid evolution of HIV, adherence is a critical part of achieving and sustaining virological suppression, and ensuring the success of treatment. Poor adherence has consequences which impact the patient and the health system with the development of antiretroviral drug resistance, development of opportunistic infections and progression of disease, all of which are associated with increased rate of hospitalizations and added costs onto the health care system. Patient factors influencing adherence include substance abuse, illiteracy, food insecurity, subjective lack of clinical improvement, and depression and anxiety. Medication factors

include high pill burden, frequency of dosing, adverse side effects and poor patient-doctor-health care system interactions (20 - 22). Although poor adherence was not a significant predictor of treatment failure, there was a notable difference in the rates of adherence – patients with treatment failure had statistically significant poorer rates of adherence, which was consistent with the results of other studies.

Multiple studies have shown an association between the non-disclosure of HIV status, lack of social support and poor adherence to treatment (23, 24). Poverty and unemployment are strongly associated with treatment interruptions and poor adherence as the patient is unable to attend clinic visits, comply with treatment and take in adequate nutrition (25) – all of which are required for holistic management of HIV infection. In our study, there was no significant difference in the unemployment rate between patients who were virologically suppressed and those who were failing treatment. A greater proportion of patients with treatment failure may have had advanced stage disease which qualified them for social grants.

Friedland and Williams described the relationship between adherence and the development of drug resistance to be bell shaped – indicating that very high and very low levels of adherence posed no threat to the development of drug resistance and rather it is the 'in-between' levels of adherence which posed a higher risk to the development of resistance (26). Of the 16% of patients with viral resistance studies, 25% had no significant resistance mutations and

none had any significant PI mutations thus suggesting that there were high levels of non-adherence is this study population.

Although others have shown that CD4 count, HIV viral load and disease stage are markers of treatment failure (27-29); in this study population, baseline CD4 count was not a predictor of treatment failure. CD4 count criteria for initiation of ARVs have been increased in the guidelines over the years, however patients were initially started with low CD4 counts and advanced immunological suppression. CD4 count responses are variable and multifactorial and are difficult to generalize across different populations.

Patients on treatment for more than two years and those with a longer duration of time between initiation and modification of treatment are at increased risk of treatment failure (30, 31). We found that the median duration on ARVs was in excess of four years and 40% of the patients were previously suppressed on first line treatment suggesting initial optimal compliance, which subsequently declined. However regression analysis did not show duration on treatment to be a significant predictor of treatment failure or time to treatment failure.

This study had several limitations. Firstly, missing data plagues the retrospective design. Secondly the clinic has a down-referral system in which patients who are stable and doing well on treatment are referred to local clinics and general practitioners; and it is also the referral centre for patients who are failing 2nd line treatment in the area. As a result, our findings may

overestimate the proportion of patients with treatment failure in the sub-district. Thirdly, few patients had viral resistance assays and therefore rates of resistance we report may not reflect the prevalence of drug resistance in this population.

Conclusion

This analysis of an urban referral ARV clinic showed that more than half the patients on second line ARVs fail treatment, highlighting the need for continuous patient and clinician education. Adherence remains a critical factor in the development of treatment failure and should be addressed by a multidisciplinary team consisting of health care and community workers, Rapid identification of patients failing treatment and institution of measures addressing adherence may improve the rates of virological suppression. Failing which, patients should be timeously referred for viral resistance testing. Further evaluation of patients on second line ARVs is necessary to determine efficacy of treatment and factors associated with treatment failure, including an analysis of drug resistance patterns and prevalence in urban clinics such as THWC, before one can comment on the need for expansion of third line treatment options in public sector treatment centres in South Africa.

Table 1: Patient Demographics and Clinical Parameters

Parameters	All patients	Virologically Suppressed	Virological failure
Gender – number (%)		n = 99	n = 124
Female	131 (58)	62 (63)	69 (56)
Male	92 (42)	37 (37)	55 (44)
Age – years			
Median (IQR)	42 (36 – 49)	42 (38 – 49)	42 (36 – 50)
19 – 35 (%)	49 (22)	19 (19)	30 (24)
36 – 49 (%)	122 (55)	59 (60)	63 (51)
50 – 64 (%)	46 (20)	21 (21)	25 (20)
> 65 (%)	6 (3)	0 (0)	6 (5)
Income source – number (%)			
Employed	34 (15)	18 (17)	16 (14)
Unemployed	59 (26)	28 (27)	31 (26)
Disability Grant	42 (18)	16 (15)	26 (22)
Child support grant/pension	29 (13)	15 (14)	14 (12)
Unknown	59 (26)	28 (27)	31 (27)
WHO Staging at Baseline			
no. of patients with available data	*207*	*94*	*113*
1 (%)	1 (0.48)	0 (0)	1 (0.9)
2 (%)	48 (23)	36 (38)	12 (11)
3 (%)	103 (50)	36 (38)	67 (60)
4 (%)	56 (27)	22 (24)	33 (29)
Baseline CD4 Count			
no. of patients with available data	*213*	*93*	*120*
Median (IQR)	96 (36 -160)	106 (44 – 159)	88 (26 – 161)
Duration on First Line therapy (months)			
no. of patients with available data	*222*	*98*	*124*
Median (IQR)	26 (18 – 42)	27 (16 – 42)	21 (19 – 42)
Time to Regimen Switch (months)			
Median (IQR)	12 (7 – 22)	10 (6 – 22)	13 (7 – 21)
Early switch (%) (< 3 months after a unsuppressed VL result)	26 (13)	15 (17)	11 (10)
Appropriate switch (%) (3-7 months after an unsuppressed VL suppressed)	35 (18)	14 (16)	21 (19)
Late switch (%) (>7 months)	138 (69)	60 (67)	78 (71)
CD4 count at time of regimen switch			
Median (IQR)	166 (88 – 247)	183 (117 – 266)	169 (91 – 233)
HIV viral load at time of regimen switch			
Median (IQR)	46838 (21341 – 151333)	42683 (18994 – 150000)	(60322 (26881 – 152666)
Total Duration on ARVs (months)			
Median (IQR)	62 (46 – 83)	57 (41 – 81)	65 (53 – 85)

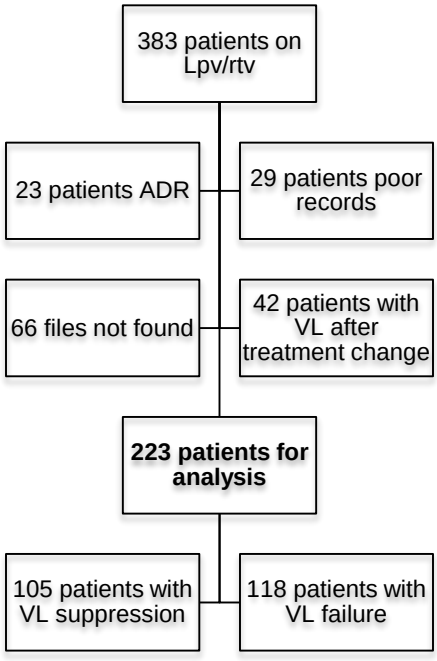


Figure 1: Flow diagram showing patients included in the study

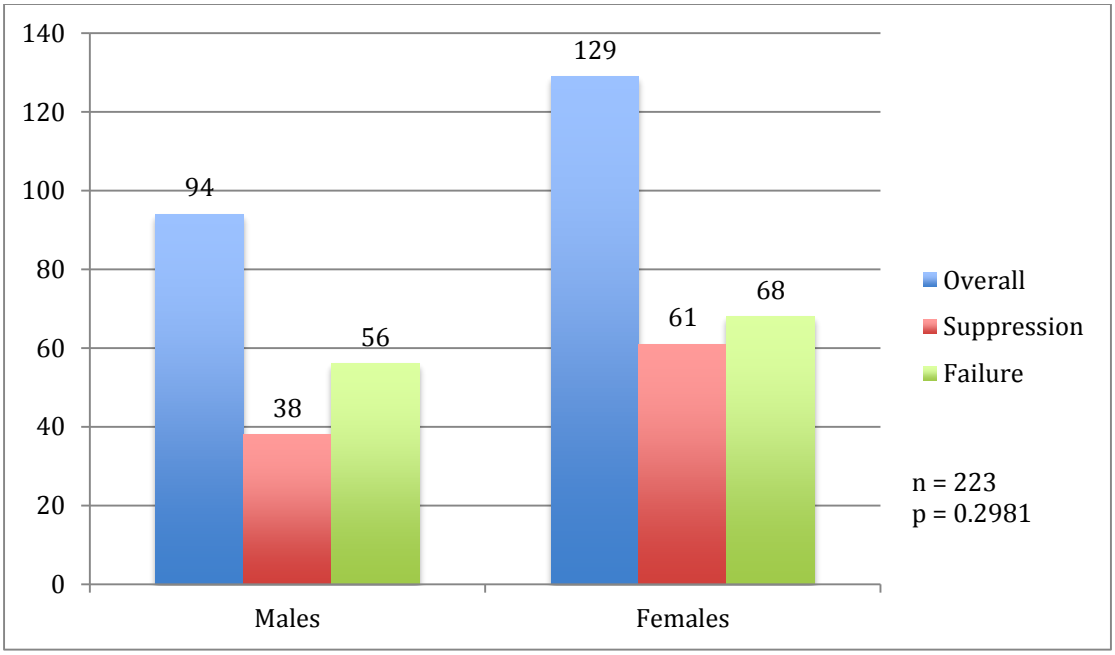


Figure 2: Virological suppression and failure in adults attending the Tshepong Hospital Wellness Clinic

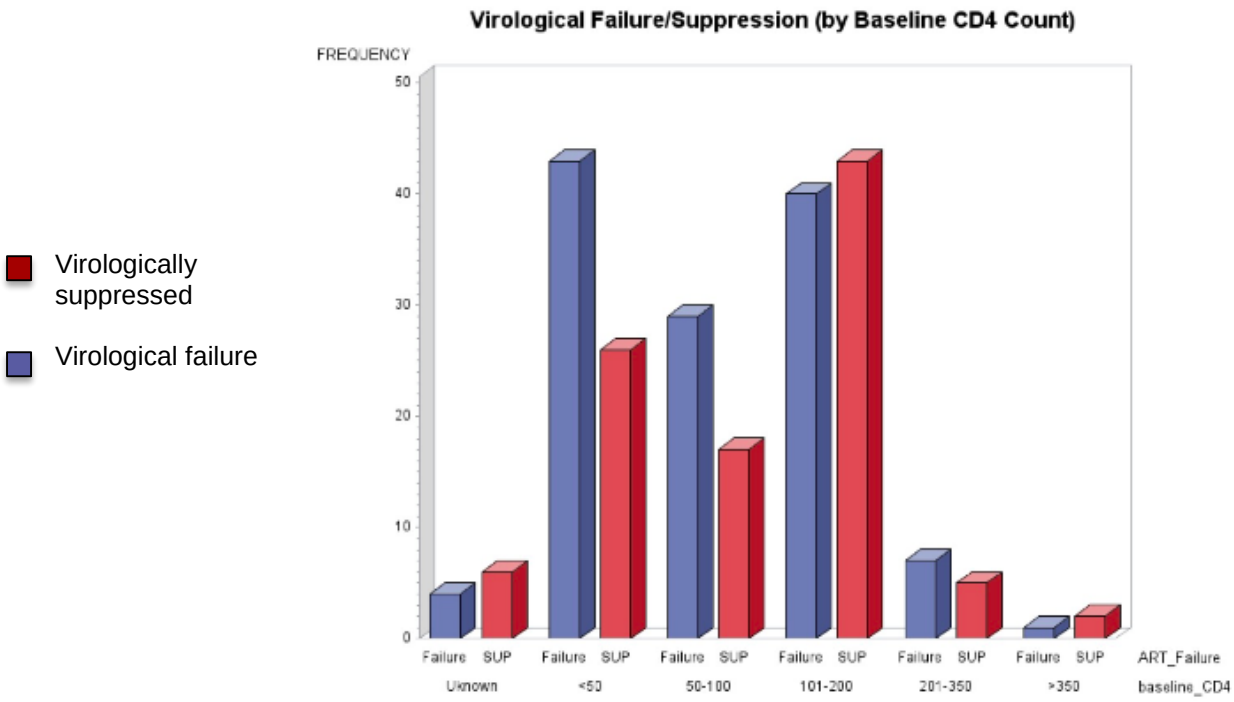


Figure 3: Bar graph comparing the baseline CD4 counts of patients with virological failure vs. those with virological suppression

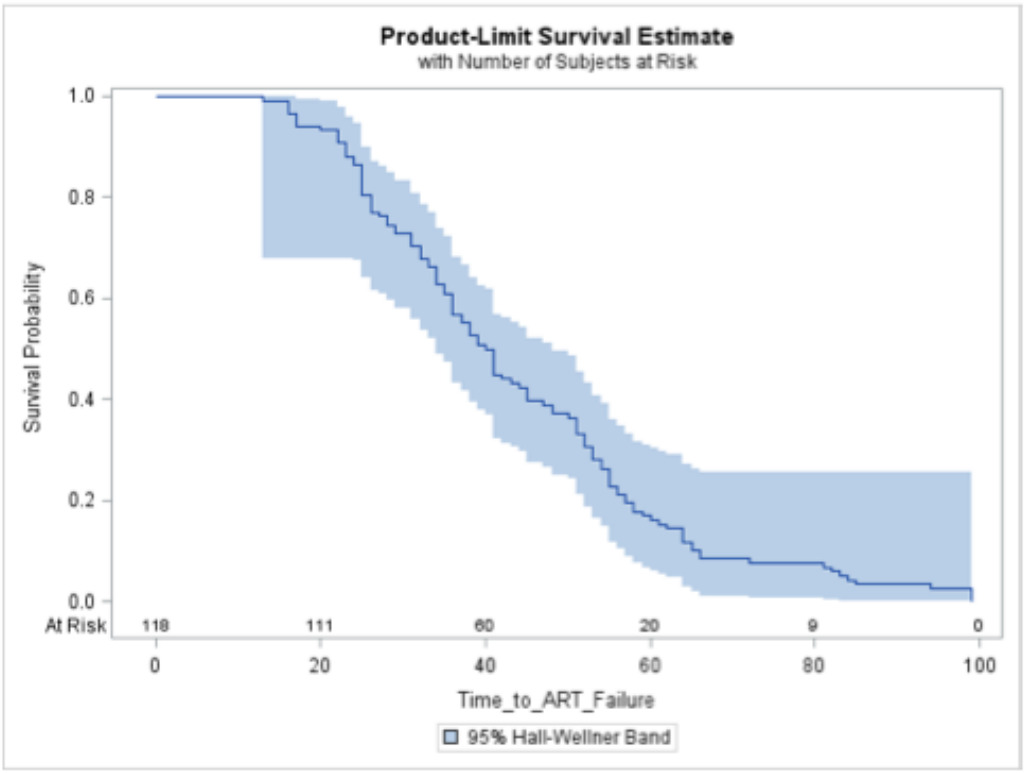


Figure 4: Kaplan Meier analysis showing the overall median time to treatment failure of 43.5%: The product limit band indicates the 95% confidence interval.

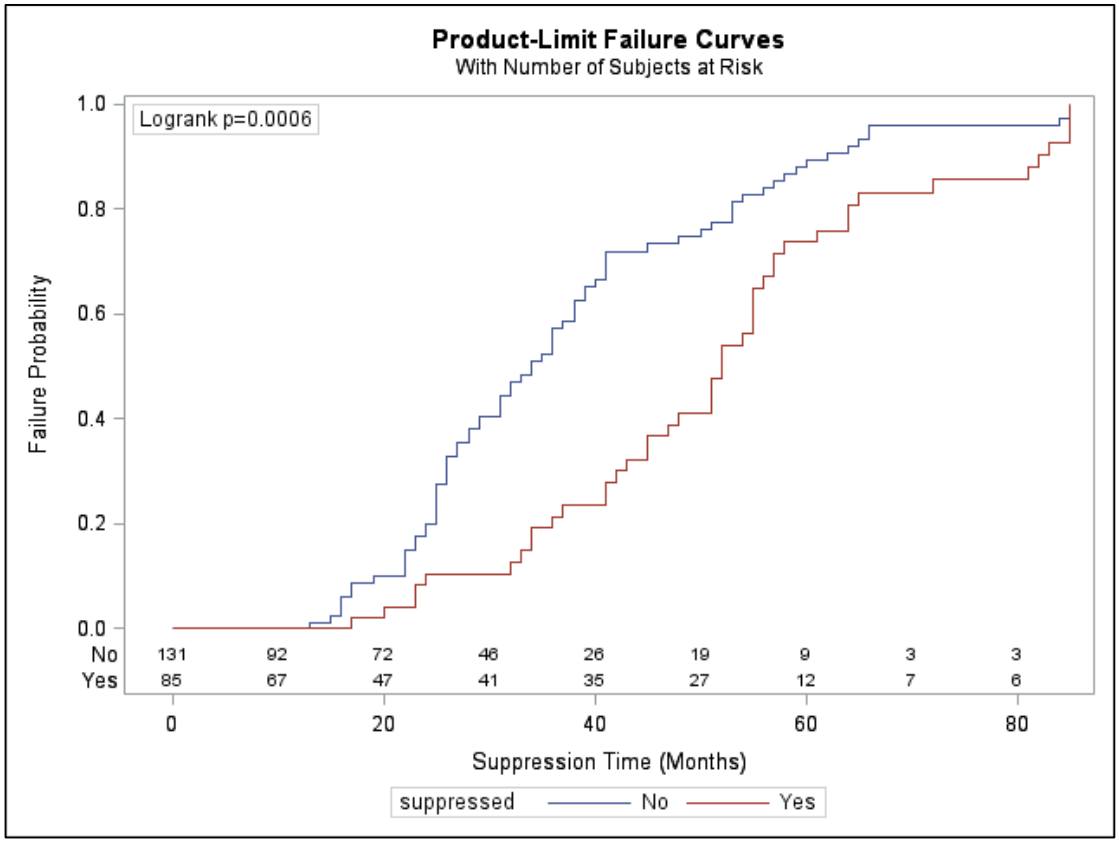


Figure 5: Kaplan Meier curve showing the statistically significant difference (according to the Logrank p test) in time to suppression between patients who were suppressed on first line therapy and those who were never suppressed..

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