PATTERNS OF DETECTABLE VIRAL LOAD IN A COHORT OF HIV-INFECTED ADOLESCENTS ON ANTIRETROVIRAL THERAPY

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I, Rebecca Yael Nthabiseng Sher, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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ABSTRACT

Background

Despite improved treatment and access to care, adolescent AIDS deaths are decreasing more slowly than in any other age group. There is lack of longitudinal data around adolescent adherence and the dynamics of viraemia over time. We aimed to describe patterns of detectable viral load in a cohort of adolescents attending an antiretroviral clinic in Cape Town, South Africa.

Methods

We conducted a retrospective cohort study of all patients on ART aged 10-19 years. Participants were included if they underwent at least two HIV viral load (VL) measurements and attended the Groote Schuur Hospital HIV Clinic for at least 24 months between 2002 and 2016. The primary outcome was two consecutive VL >100 copies/ml, in line with the lower limit of detection of assays in use over the follow-up period.

Results

Of 482 screened subjects, 327 met inclusion criteria. Most subjects were vertically infected (n= 314; 96%), and 170 (52%) were male.

Overall, 203 episodes of confirmed detectable VL involving 159 (49% [95% CI 43%–54%]) subjects were experienced during the follow-up period. A total of 111 (34%) subjects never experienced detectable VL, while 16 (5%) never suppressed throughout the follow-up period. Median age at first detectable VL was 14 (IQR 11-16) years. Of the 159 subjects who experienced detectable VL, 102 (64%) re-suppressed, of which 38 (37%) had a subsequent detectable VL.

Six subjects had genotyped resistance to protease inhibitors. Four of these never suppressed, while two suppressed on salvage regimens.

Total follow-up time was 1723 person years (PY), of which 880 (51%) were contributed by the 159 subjects who experienced detectable VL. Overall time with detectable VL was 370 PY. This comprised 22% of total follow-up time, but 42% of the follow-up time contributed by those who experienced detectable VL.

The rate of detectable VL was 11.8 (95% CI 10.3–13.5) episodes per 100 PY. The risk increased by 24% for each year of increasing age (RR 1.24 [95% CI 1.17-1.31]; p<0.0001).

Neither prevalence, duration nor rate of detectable VL was influenced by gender.

Conclusion

Detectable VL was seen in nearly half of adolescents, with the rate increasing with age. Viraemia was not a static process, and adolescents moved in and out of this state as adolescence progressed. Further study is warranted to correlate these findings with risks and clinical outcomes.

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Finally, I would like to acknowledge the patients who comprise the subjects of this study. Their resilience is an inspiration to me. I hope that this work can play its small part in the fight to end adolescent HIV.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
ARVs	Antiretrovirals
HIV	Human Immunodeficiency Virus
IQR	Inter-Quartile Range
LMIC	Low and Middle-Income Country
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
PI	Protease Inhibitor
PY	Person Years
VL	HIV Viral Load

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1 INTRODUCTION

1.1 Background

Since 2005, global deaths from AIDS have fallen by almost 40% across all age groups except adolescents. In this group, despite the massive upscaling of antiretroviral therapy (ART), overall AIDS-related deaths are decreasing at a much slower rate, and in older adolescents still show an increasing trend.(1-3) Globally, AIDS is among the top ten leading causes of death for adolescents.(3, 4)

Accounting for this relatively slow decline in adolescent mortality is challenging because of a lack of adolescent-specific data. HIV data are often presented as either children (younger than 15 years of age) or adults (aged 15 years and above). Disaggregated adolescent specific data are largely unavailable. Where estimates of HIV prevalence among adolescents are available, these tend not to distinguish between vertical and horizontal transmission.(5) The scale of the epidemic is such that of an estimated 1,2 billion adolescents alive in 2016, 2.1 million were living with HIV.(6)

Among vertically-infected children, models predict that without ART, a child who acquires HIV during pregnancy or birth will die at about one year of age. Those who are infected via breast-feeding (around half of all vertically infected children) are expected to survive for an average of fourteen years.(5) Without diagnosis and treatment, vertically infected children and adolescents are at high risk of mortality.(5) Unfortunately, ART coverage remains low in children. Of the 2.1 million HIV-infected children under 15 years worldwide, only 43% were receiving ART in 2017, meaning that most vertically-infected children are not on treatment as they enter adolescence.(6)

Among horizontally-infected adolescents, HIV testing is low, and as a result, treatment may be delayed until they have very advanced disease.(1) It thus seems likely that in Sub-Saharan Africa, where almost 80% of HIV-infected adolescents live, lack of diagnosis and access to care contribute to mortality.(1, 7)

However, even among adolescents who do access treatment, outcomes are poorer than those of children and adults on ART.(8) In general, adherence to medication in chronic disease tends to drop off in adolescence, causing a corresponding increase in morbidity and mortality.(9) The consequences of ongoing detectable HIV viraemia include immune destruction, disease progression with higher risk of mortality, and greater risk of onward transmission of HIV.(9-13) In comparison to adults, adolescents in southern Africa have been found to be less adherent to ART, and have displayed lower rates of virological suppression and immunological recovery as well a higher rate of virological rebound after initial suppression.(8, 14) The association between adherence to ART and viral suppression has been well-documented across age-groups.(8) It is estimated that for successful clinical, immunological and virological outcomes, ART adherence needs to be at least 95%.(9) There is evidence that world-wide, adolescents struggle to meet these adherence requirements.(15, 16)

1.2 Context

In Sub-Saharan Africa, where more than three quarters of the world's HIV-infected adolescents live, most facilities that treat adolescents do not have adolescent-specific services. (7, 17) Although the South African Department of Health has prioritised programming for adolescents and youth, many gaps remain.(18)

In contrast, the Groote Schuur Hospital Adolescent Clinic has had a dedicated adolescent service since 2005. The adolescent clinic grew out of one of the first paediatric HIV clinics in South Africa, which began prescribing ART to children in 2002, a full two years before

the national roll-out. Many of the adolescents attending the clinic were among the first children in the public sector to receive ART. There are thus detailed medical records pertaining to these children since 2002. Structured clerking notes ensure that psychosocial information such as the identity of the caregiver or scholastic failure is regularly documented. While most children attending this clinic are vertically infected with HIV, a small proportion of adolescents enter the service following horizontal infection. The clinical team has been relatively stable since the inception of the clinic. Each patient sees the same doctor and counsellor at each visit. In the few instances in which staff changes have taken place, each patient was handed over to a specific caregiver in order to maintain continuity of care. A modest transport subsidy was available to those in need for the most of the clinic's history. The excellent quality of patient records and the long period of follow-up mean that this site offers an unusual opportunity to conduct longitudinal research with relatively good quality retrospective data.

1.3 Aim

Our study aimed to determine the burden of detectable viral load in a cohort of HIV infected adolescents seen at the Groote Schuur Hospital Adolescent HIV Clinic.

1.4 Study Design

A retrospective cohort study using medical records was performed. The period under review dated from 1 January 2002 until 31 December 2016.

1.5 Rationale

Through this research, we hope to contribute to the understanding of virological outcomes in adolescents on ART. More specifically, we hope to address what we perceive to be a gap in the literature, namely the dynamics of viraemia – and, by extension -

adherence over time in a low and middle-income country setting (LMIC). We hope that the findings of the study can be used to identify adolescents at high risk of unsuppressed viral load and to offer extra assistance. Moreover, it is hoped that the data here generated will be more broadly relevant, and could be used to guide policy towards additional support for interventions that address adherence barriers.

1.6 Summary

The subsequent chapters will take the following format:

Chapter 2: Literature Review

This chapter reviews published literature on unsuppressed viral load in adolescents on ART. Epidemiological data concerning adolescent HIV are summarized, and conceptual difficulties with measuring adherence to ART discussed. Throughout, the lack of longitudinal data in the field will be highlighted.

Chapter 3: Methodology

This chapter describes the research design, methods and procedures, including how data were handled and analysed as well as the handling of ethical issues and study approval.

Chapter 4: Results

In which study findings are reported.

Chapter 5: Discussion

In which study findings are interpreted, and reviewed in the context of existing literature in the field.

Chapter 6: Conclusion

The contribution of this study to the literature is summarised, and its limitations noted. Recommendations are made for further study, and for the application of these findings to policy or clinical work.

2 LITERATURE REVIEW

2.1 Introduction

AIDS-related deaths in adolescents are decreasing much more slowly than in all other age-groups.(3, 6) Despite improved treatment and access to care, HIV/AIDS is listed as one of the top ten leading causes of death in adolescents globally.(2-4) This can be partially attributed to a lack of diagnosis and treatment, but even among adolescents who do access care, outcomes are worse than in other age-groups.(5, 8, 19) This is thought to result from poor adherence to antiretroviral therapy (ART).(9, 15)

This chapter will review the literature around the topic of adolescent adherence to ART, with a focus on viral load outcomes. Measures of adherence will be discussed, as well as the merits and shortfalls of using the HIV viral load (VL) as a proxy for adherence and an outcome variable. The epidemiology of detectable viral load in adolescents will be described, as well as demographic factors associated with it.

2.2 Methods

An electronic search for English-language articles was performed using Pubmed without restrictions. The initial search was performed in January 2017, and was repeated a number of times with the latest search conducted on 8 February 2018. A sensitive search strategy was adopted using various forms of combinations of text and MeSH terms of the following words: ‘adolescent’, ‘HIV’, ‘adherence’, ‘viral load’, ‘antiretroviral therapy’, ‘prevalence’, ‘incidence’ and ‘duration’. A total of 179 eligible studies were found, of which 65 were used.

2.3 The Adolescent HIV Epidemic

The adolescent HIV epidemic is comprised of a mix of vertically-infected children who age into adolescence, and adolescents with new, horizontal infections.(2) In recent years there has been an increased focus on adolescents, although most data reporting at country level still does not provide disaggregated data on this group.(6) Moreover, adolescent data often does not distinguish between vertical and horizontal transmission.(5)

Of the 1.2 billion adolescents alive worldwide in 2017, 2.1 million were estimated to be living with HIV. This indicates an increase of 30% since the 2005 estimates.(6) In 2015, 80% of the world's HIV-infected adolescents lived in Sub-Saharan Africa, however 32% of new infections among 15-19 year olds occurred outside of this region. This suggests that adolescent HIV is indeed a global issue.(7) South Africa has the largest number of people living with HIV in the world, and in 2012 carried 20% of the global adolescent HIV burden.(2) In 2016 there were 370 000 adolescents living with HIV in South Africa, with 6200 adolescent AIDS-related deaths.(20)

Overall, AIDS-related deaths in adolescents have declined by a small amount each year since 2012, largely due to decreasing mortality in younger adolescents aged 10-14 years. In contrast, older adolescents aged 15 – 19 years are the only age group in which AIDS deaths have continued to rise.(2, 3) It is thought that this mortality is mainly made up of vertically-infected adolescents as horizontally-infected adolescents would have had relatively recent infections, though the evidence to support this is lacking.(2)

2.4 Measures of Adherence

Adherence to ART is notoriously difficult to measure, particularly as there is no gold standard.(19, 21-23) The most common means of monitoring adherence is by self-report.

This is vulnerable to over-reporting, as has been illustrated in trials comparing self-reporting to more objective measures such as electronic adherence monitoring devices or pharmacy refill.(22, 24-26)

As there are different patterns of non-adherence, self-report questionnaires may not screen for all patterns.(27) This is illustrated in the way that self-reported adherence estimates vary depending on the particular questions asked.(28) Additional challenges arise in adolescence, where there may be significant discordance between caregiver and youth estimates of adherence. The reliability of the estimate depends in part on the age of the adolescent. The caregiver report may be more useful in younger adolescents, while self-report may be more appropriate in an older age group.(21)

There is conflicting evidence on the usefulness of pill-counts, which can be confounded by pill-dumping (discarding pills in order to give the impression of better adherence). However, pharmacy refill data has been found to be a good predictor of virological failure across multiple studies.(22, 25)

There is general consensus that the most accurate means of monitoring antiretroviral adherence is through serial measurements of plasma HIV viral load (VL).(21, 22, 25) A high viral load is not synonymous with poor adherence, as it may result from virological resistance or more rarely, drug interactions or malabsorption. The interaction between rifampicin-based therapy for tuberculosis and protease inhibitors in particular has been associated with the development of resistance mutations.(9) Despite this, the association between adherence to ART and viral suppression has been well described in all age-groups.(9) In the context of effective ART, the HIV VL can be seen as both the outcome of adherence, and a sensitive means by which to monitor adherence. An undetectable HIV VL would indicate successful treatment, from which good adherence can be inferred.(10)

2.5 The HIV Viral Load

The HIV viral load is used as a "pivotal outcome variable" in a wide variety of studies of HIV infection, including drug trials, adherence studies and observational cohorts.(29) In addition to being a proxy marker of adherence, VL is frequently employed as a surrogate for disease progression.(10, 30) Evidence shows that HIV viraemia is associated with clinical deterioration independent of CD4 count, and that it predicts the development of resistance mutations which necessitate a change of treatment.(10) After 6 months on treatment, VL >400 copies/ml has been found to be a risk factor for death in vertically-infected children and adolescents.(11) The World Health Organization (WHO) recommends that VL be used as a gold standard for monitoring HIV treatment in all patients.(31)

Within a research context, single measures of VL are frequently used as an outcome variable (e.g. closest VL to month 12 after start of ART). Rose et al compared methods of analysing HIV VL data using single vs. repeat measures of VL, and concluded that repeat measurements provided more precision.(29)

Different assays also have different levels of detection, the limit of which has dropped over time from 500 copies/ml in 2000, to around 20 copies/ml since 2009.(32, 33) This may cause confusion, as the same level of VL may be classified as "detectable" or "undetectable" depending on the limit of detection, which varies according to time, context and assay used.(32)

The concept of treatment failure is based on confirming a detectable viral load.(10) Currently, the WHO and the South African Department of Health define treatment failure as consecutive viral loads of >1000 copies/ ml.(34, 35) However, there is evidence that even low level viraemia is associated with subsequent treatment failure and the development of resistance mutations, necessitating a switch to an alternative

regimen.(36-38) These findings have been well-established in higher income settings and have recently been validated in LMIC settings.(36-38)

In a recent prospective study from Lesotho, Labhardt et al found that 94% of patients with a confirmed VL >80 copies/ml but <1000 copies/ml had resistance mutations on genotype, suggesting that local definitions of virological failure are not sensitive enough.(38) Similarly, in a large observational cohort from South Africa, Hermans et al found that low level viraemia (defined as at least one episode of VL 51 – 999 copies/ml) was a strong predictor of subsequent virological failure and change to an alternative regimen.(37) While a virological "blip", or transient episode of low level viraemia has long been thought not to have clinical significance, this study found that even a single detectable VL >50 copies/ml was associated with poorer treatment outcomes.(10, 37)

It is important to note that virological "blips" may be subject to the performance of the particular quantification assay used. As Taiwo et al point out, not all VL assays are equal. Although there is generally good correlation at higher VL levels, "inter- and intra-assay variability may be significant around the lower quantification limits".(39) Whether or not a "blip" is recorded thus depends not only on the level of detection of the assay, but also on the type of assay used.(39)

Several large studies have shown the importance of undetectable VL in reducing HIV transmission. Undetectable VL therefore provides not only therapeutic benefit, but is also a strategy to reduce new HIV infections.(12, 13)

2.6 Virological resistance

Virological resistance has been described in African adolescents, with prevalence as high as 67% described in Zimbabwean patients with virological failure on non-nucleoside

reverse transcriptase inhibitors (NNRTIs).(40) In a cohort of children and adolescents in the Central African Republic, more than half of subjects with virological failure had resistance to first-generation NNRTIs and 24% had a major drug resistance mutation to a protease inhibitor (PI).(41) These findings were in settings without routine VL monitoring, where clinicians were guided only by clinical and immunological factors to diagnose treatment failure, potentially allowing resistant mutations to accumulate. In a Tanzanian cohort of children and adolescents, 90% of subjects with virological failure had at least one major drug resistant mutation, and NNRTI resistance was found in 81%. No PI mutations were found, despite 15% of the cohort being on a boosted PI. Routine VL monitoring was also not performed in this setting.(42)

In the South African context, where routine VL monitoring is available, a public health approach has been taken to virological resistance. As the drug resistance mutations that develop after failure of an NNRTI-based regimen are predictable, a standardised PI-based regimen has been chosen to achieve virological suppression in this case. National guidelines dictate a swift change to a PI-containing regimen for patients with two consecutive VL >1000 copies/ml on an NNRTI-based regimen. Within the public sector, HIV genotypes are performed only on patients with virological failure on a PI-containing regimen.(34) No studies describing prevalence of PI resistance in South African adolescents could be identified. A study of South African adults with virological failure on a PI-regimen found that 16.4% had at least one major PI mutation. The authors concluded that poor adherence is the leading cause of virological failure on PI-based ART.(43)

2.7 Epidemiology of Detectable Viral Load in Adolescents

2.7.1 Prevalence

In order to achieve virological suppression, adherence to ART needs to be at least 95%.(9) World-wide evidence suggests that adolescents struggle to achieve these stringent

adherence requirements, and have poorer virological outcomes than other age groups.(31) In a 2014 systematic review of cross-sectional studies in which adherence was defined as undetectable viral load or self-report of at least 85% of medication taken, Kim et al found that overall adolescent adherence was 62%, with much regional variation.(15) In North America, adherence was only 53%, while in Africa and Asia it was 84%, suggesting that high income countries do not necessarily have better adolescent adherence.(15) Similarly, in a French perinatally-infected cohort, Dollfus et al describe undetectable viral load in only 54,5% of adolescents on ART, compared to 45,9% in a similar American cohort reported by Kahana et al.(44, 45)

Ferrand et al performed a systematic review on the prevalence of virological suppression in adolescents after one year on ART. Results were extremely variable, and ranged from 27 – 89%. When the time constraint was removed and adolescents at any time since start of ART were included, similar results of 28 – 87% were found. However, the authors concluded that the main finding of the study was the "paucity of data on virological outcomes among adolescents with HIV".(16)

In 2009, Nachega et al analysed data from a private sector HIV-management programme in southern Africa and found that adolescents were less adherent than adults as well as having lower rates of virological suppression (at a level of detection of <400 copies/ml). In this cohort, 58% of adolescents had ever suppressed compared to 72% of adults. In addition, adolescents had lower rates of viral suppression at all time points after the start of ART, with a marked decline after the first VL at month 6. At month 6, virological suppression stood at 63% in adolescents compared to 69% in adults. At month 12, this had dropped to 46% compared to 62% in adults. By month 24 there was further decline to 44% in adolescents, while adults remained stable.(8)

A similar pattern of decreasing rates of virological suppression (<400 copies/ml) at increasing time on ART was found in a prospective cohort at a community clinic in Cape Town, South Africa. While ART-naïve adolescents had high rates of virological suppression

at week 16 on treatment (97.3%), this fell rapidly by week 32, declining to 27.3% by week 48 compared to 63% in young adults.(14)

In the Central African Republic, Mossoro-Kpinde et al performed a cross-sectional analysis on a mixed group of children and adolescents (median age of 12 years) receiving ART. At a level of detection of 20 copies/ml, only 40% of children had undetectable VL. Of the remaining children with detectable VL, 97% had VL >1000 copies/ml. (41) A similar high prevalence of non-suppression was found in children and adolescents in Togo, where 36% of subjects had VL <40 copies/ml, and 51.6% had VL >1000 copies/ml.(46)

More encouragingly, Collins et al examined prospective data from the United Kingdom and Ireland to determine clinical status at the last paediatric visit before transition to adult services. In a largely vertically-infected cohort with a median age of 17.4 years, 74% of subjects on ART had VL <400 copies/ml, while 60% had VL <50 copies/ml. These results did not include the 13% of the cohort which was not on ART due to treatment interruption at the time of transfer.(4)

A high prevalence of virological suppression was found in a recent cross-sectional study from Cape Town, in which 76% of vertically-infected adolescent subjects had HIV VL <50 copies/ml. However, it is worth noting that these were all younger adolescents, with a median age of 12 years.(30) The association between age in adolescence and VL will be discussed later in this chapter.

In summary, the prevalence of detectable VL in adolescence varied widely by context and study. This variation could be due in part to differing thresholds of detection, but even those studies reporting at higher thresholds tended to find sub-optimal suppression. With a few notable exceptions, many studies reported undetectable VL in adolescents at

less than 50%. The consensus is that adolescents have a higher prevalence of viraemia than adults.

2.7.2 Retention in care

Retention in care varies across contexts but in general has been found to be worse among adolescents and youth than among older adults.(31, 47-50) This has clear implications for treatment outcomes, as adherence to treatment and ultimate virological suppression are only possible if there is retention in care.(48) A systematic review of youth (age 15 – 24 years) in South Africa found that overall retention in care was 83%.(49) Individual studies from elsewhere have found varying results. In a retrospective study from Uganda, adolescent retention in care was only 65%, which was similar to adult figures for the same district.(51) Much better retention was found in a study from Thailand, where Sudjaritruk et al describe loss to follow up (LTFU) in a cohort of vertically-infected adolescents as only 1,2%, or 0.3 events per 100 PY. However, as the endpoint of this study was virological rebound post-suppression, a substantial proportion of screened subjects (39%) were excluded as they did not have a sustained period of virological suppression. This may have introduced a bias towards more stable patients.(52) In a prospective South African adolescent cohort described by Nglazi et al, LTFU was 7.2 per 100 PY overall, although this was only 3.9 per 100 PY when restricted to vertically-infected adolescents.(14)

2.7.3 Incidence

The incidence of detectable VL in adolescence is not well understood due to a lack of longitudinal data. An adult study from Kwazulu Natal, South Africa, described incidence of "virological failure" (defined as a single VL >1000 copies/ml at least 6 months after commencing second-line, protease inhibitor-containing ART) of 12.9 per 100 person years (PY).(53)

In a South African study where “virological failure” was defined as two VL >1000 copies/ml after at least one VL <400 copies/ml, the findings were that adolescents experienced virological failure at a rate of 8.2 per 100 PY. This is significantly higher than the 5 per 100 PY experienced by young adults.(14)

Finally, in a vertically-infected Thai cohort, Sudjaritruk et al described the incidence of single VL >1000 copies/ml following sustained suppression, as 3.4 per 100 PY.(52)

Due to differing definitions, it is hard to compare incidence across studies. In the only study comparing adolescent to adult outcomes, the incidence of viraemia was higher in the adolescent group.(14)

2.7.4 Duration of detectable VL

There is paucity of data on duration of detectable VL over a specified time period. In the only identified study explicitly describing duration of detectable VL, person-time spent with VL >400 was described in a large cohort of vertically-infected subjects in the United States aged 7 to 30 years. Total follow-up time spent with VL >400 was 34% for the whole cohort but only 30% of the study time contributed by those aged 13 – 17 years old.(54)

2.7.5 Viral rebound and re-suppression

Collier et al performed one of the few studies to examine both re-suppression and duration of detectable VL. In this adult cohort from Kwazulu-Natal, South Africa, 57% of subjects with a single VL >1000 copies/ml on PI-containing ART re-suppressed, after a median of 8 months.(53)

A 2015 study by Childs et al examined outcomes after viral rebound (defined as a confirmed VL >400 copies/ml after suppression on first line ART) in a cohort of children and adolescents in the United Kingdom and Ireland. While half of the cohort was swiftly switched to an alternative regimen, a third re-suppressed on the same regimen. In the group that switched, 85% were suppressed at 12 months, and 73% at 24 months. In the group that re-suppressed without switching, 39% remained suppressed at the most recent follow-up (median 28 months), while 61% had a further VL >400 copies/ml.(55)

Conversely, Sudjaritruk et al studied outcomes after sustained virological suppression in a vertically-infected Thai cohort. A single raised VL >1000 copies/ml was experienced by 13% of the cohort.(52) Nachega et al found that in the early days of ART in southern Africa (between 1999 and 2006), adolescents who suppressed initially were at higher risk for viral rebound at all time points after commencing ART.(8)

The differing definitions and methodology make it difficult to compare studies, though all point to the notion that virological status is not static. Patients who are suppressed at one time point may rebound. Those with detectable VL may suppress at a later time point, even without a regimen change. Despite the potential for re-suppression displayed in the study by Childs et al, adolescents in Nachega et al's study were found to be at higher risk of virological rebound than adults.(8, 55)

2.8 Factors associated with detectable VL

In chronic illnesses as diverse as congenital cardiac disease, diabetes and HIV, it is well documented that medication adherence decreases during adolescence, while morbidity and mortality increase inversely.(9) Normal developmental characteristics such as the desire to be the same as one's peers, poor planning for the future and rebellion against parental figures can hinder adolescent adherence to medication in many chronic illnesses.(56) In the context of HIV, stigma and the effects of familial HIV on the

household pose additional adherence challenges.(48) Stigmatising medication side-effects such as lipoatrophy may also impact on medication adherence. In times of particularly high pill burden (such as during treatment for multidrug-resistant tuberculosis), polypharmacy may contribute to poor adherence.(9)

Multiple factors have been associated with poor adherence to ART and detectable HIV VL among adolescents, the most salient of which will be discussed at length below.

2.8.1 Age

In the adolescent population, increasing age has been associated with worsening outcomes across multiple studies. Both anti-retroviral (ARV) non-adherence, and its outcome of HIV viraemia, have been found to increase as adolescence progresses. A Tanzanian study found increased odds of poor adherence and virological failure on transition to adolescence, which only became more marked as adolescence advanced.(57) In a Thai study, Suaysod et al found that children older than 13 years were at higher risk of treatment failure (58), while Adejumo et al noted that older adolescents were at risk of poorer adherence compared to children younger than 15 years.(19) In a US study each year of age carried a 10% increase in odds of non-adherence measured by self-report.(56) More recently, a South African study found that compared to adolescents younger than 12 years, adolescents aged 12 – 14 years carried a 2.4 times higher risk of having a VL >50, and a 2.98 times higher risk of having a VL >1000.(30) Older adolescents have also been found to be at higher risk of attrition from care.(51)

A large US study found that adolescents aged 13 – 17 years as well as youth aged 18 – 30 years spent more time with a VL >400 copies/ml compared to a younger age-group of 7 – 12 years (30% and 44% compared to 22% of person-time respectively).(54)

A one year cohort analysing routine programme data from Uganda found that both children and adolescents experienced more virological non-suppression (defined as VL >1000 copies/ml for plasma or VL >5000 copies/ ml for dry blood spots) than adults. Children under 5 years had the highest prevalence (29%), followed by adolescents 15 – 19 years (27%). This was somewhat lower in children aged 5 – 9 and 10 – 14 years, at 23% for both. After adolescence, prevalence of non-suppression dropped steadily across five year age-bands until it reached 7% in those 35 years and older.(59)

Makadzange et al performed a similar analysis of children and adolescents in Zimbabwe, and found a 28% prevalence of a single VL >1000 copies/ ml in children aged 0 – 15 years. This was higher in older adolescents aged 15 – 19 years, at a prevalence of 37%. Within this study, the role of increasing age was complicated by whether ART was started in childhood or adolescence. In those who initiated ART in adolescence, each additional year of age at the time of evaluation was associated with a 2.4 times increased risk of VL >1000 copies/ml.(40)

In summary, the evidence from multiple contexts suggests that older adolescents are at higher risk of poor adherence, virological non-suppression and attrition from care.

2.8.2 Gender

Worldwide, there are more adolescent girls than boys living with HIV, with 65% of new infections occurring in girls.(7) The gender ratio tends to be equal among vertically-infected youth. (9) There have been conflicting findings on gender and adherence in adolescents, with some studies reporting better adherence associated with female gender, and others reporting the opposite.(9)

In a South African vertically-infected cohort, Brittain et al found that male gender was associated with a 2.2 times increased odds of having a VL >50 copies/ml.(30) In contrast, Muri et al found that female gender was associated with a 2.57 times increased risk of VL >1000 copies/ml in a cross-sectional study of children and adolescents in Tanzania.(42) In contrast Kahana et al described no association between gender and virological suppression in a large American study of over 2000 youths living with HIV, in both vertically and horizontally-infected cohorts.(44) Similarly, in a vertically-infected Thai cohort studied by Sudjaritruk et al, gender was not associated with virological rebound post suppression.(52)

2.8.3 Time on ART

Several studies of ART-naïve adolescents have found an increased rate of unsuppressed VL with increasing time since start of treatment. As mentioned previously, both Nglazi and Nachega noted that adolescent suppression tended to drop off steeply after the first VL measurement (at either 16 weeks or 6 months).(8, 14) However, it is less clear whether time on ART affects viral suppression in adolescents who initiated ART as children.

Pill-fatigue is a common complaint of vertically-infected youth (who have often been on treatment since early childhood), and has been associated with poor adherence.(60, 61) However, Xu et al found that number of years on ART was not associated with VL >1000 copies/ml among Thai vertically-infected adolescents.(62) Similarly, Kahana et al found that number of years since HIV diagnosis was not associated with viral suppression among a mixed group of vertically and horizontally-infected American adolescents. (44)

In a Zimbabwean adolescent cohort, Makadzange et al found that time on ART was associated with having a VL >1000 copies/ml. This however depended on whether the subject had started ART before or after age 10 years. In adolescents who initiated

treatment as children, time on treatment of 4 or more years was associated with an increased risk of raised VL. However, in those who initiated treatment as adolescents, increasing time on ART was associated with decreased risk of raised VL.(40) This stands in contrast to the findings of Nglazi et al and Nachega et al, who both found that adolescents initiating ART had reasonable virological suppression in the short term, but this was not sustained past the first test of VL.(8, 14)

2.8.4 Age at ART initiation

Conflicting evidence exists as to whether the age of ART initiation is associated with VL outcomes. Makadzange et al found that older age of starting ART was associated with raised VL. In this study, 39% of those who started ART between 10 and 19 years of age had a current VL >1000 copies/ml, compared to 26% in those who started below 10 years.(40) However, the opposite finding was reported by Muri et al, in a mixed cohort of children and adolescents (median age of 11 years) in rural Tanzania. In this cohort, older age at ART initiation decreased the odds of having VL >1000 copies/ml at cross-sectional analysis, with an adjusted odds ratio of 0,84 for each additional year of age.(42)

2.9 Conclusion

In summary, accounting for poor adolescent outcomes on ART is difficult due to the lack of data – and particularly disaggregated data - in the field. This is further confounded by the absence of a gold standard for measuring adherence, which contributes to the conflicting results found in different studies.

The HIV VL has been found to be the most sensitive measure of adherence to ART, as well as a proxy for disease progression and risk of onward transmission. However, there is a lack of standardisation across studies, and different VL thresholds have been used at different times and in different contexts. Moreover, depending on study design and local

definitions, outcomes such as virological failure may depend on single or multiple measurements. This variability makes it difficult to compare studies.

Worldwide, adolescents have been found to have poorer adherence and correspondingly worse VL outcomes than other age groups. As most studies are cross-sectional, prevalence is the most common outcome measured. This varies widely according to study design and context. However, all comparative studies reviewed found that the prevalence of virological non-suppression was higher in adolescents than in adults.

Incidence of detectable VL or virological failure is a less studied outcome, but what data exist show similarly worse outcomes for adolescents than adults. Virological rebound and subsequent re-suppression are common in adolescents and adults, although adolescents are at higher risk of rebound. There is very little data available concerning the duration of detectable viraemia.

Conflicting data exist around most factors associated with unsuppressed VL. The literature finds for and against age of ART initiation, time on treatment and gender as predictors of poor adherence or detectable VL. However, older age in adolescence has been strongly associated with poorer adherence and greater likelihood of non-suppression. This is in keeping with the evidence of high mortality in older adolescents.

A limitation of much of the literature is the high proportion of cross-sectional studies, which are not able to map dynamic processes of adherence and virological response. In light of this, multiple authors have called for additional longitudinal studies of ARV adherence, as well as its virological outcomes and associated factors.(9, 15, 63)

3 METHODS

3.1 Introduction

This study investigates the patterns of detectable viral load (VL) in a cohort of HIV positive adolescents on ART. Quantitative research methodology was used, in the form of a retrospective cohort study. An in-depth explanation of methodology used follows below.

3.2 Aims

The study aimed to describe the burden of persistent measurable viral load in a cohort of HIV positive adolescents on ART.

3.3 Objectives

The main objectives of the study were as follow:

1. To describe the prevalence of VL above 100 copies/ml (at two testing time points at least two months apart) in HIV positive adolescents on ART.
2. To describe the incidence of VL above 100 copies/ml in a cohort of HIV positive adolescents on ART.
3. To describe the duration of time spent with elevated viral load in the same population.
4. To analyse the association between demographic variables and detectable viral load.

3.4 Study Design

A retrospective cohort study using medical records was performed. The period under review was from 1 January 2002 until 31 December 2016.

3.4.1 Study Population

The study population comprised all patients age 10 – 19 years who attended Groote Schuur Hospital's Adolescent HIV Clinic from 2002 (when the clinic began) to the end of 2016. The age-limits of this study were chosen to reflect the WHO definition of adolescence, i.e. 10 – 19 years. Qualifying study subjects were required to have spent at least 24 months on ART after turning 10 in order for a trend within adolescence to be established. Data from patients who were subsequently transferred out of the service or who were older than 19 years at the time of review were included for that period of time that the subject met inclusion criteria.

Inclusion criteria

1. HIV-positive, on ART >24 months.
2. At least 10 years and no older than 17 years at study entry
3. Followed up for at least 24 months at Groote Schuur Hospital.

Exclusion criteria

1. Pre-ART, or on ART <24 months.
2. Not in the age range 10-19 years over the study period
3. Less than 24 months follow up at Groote Schuur Hospital.

3.4.2 Outcomes of Interest

The primary outcome was two consecutive detectable viral loads (viral load of at least 100 copies per millilitre), at least two months apart.

Over the period under review, the limit of virological detection varied between 20 and 100 copies per millilitre depending on the assay used. In order to standardise interpretation, detectable viral load was defined as greater than or equal to 100 copies per millilitre.

Regular viral load monitoring was always available in the clinic over the study period. Clinic protocols dictated that viral load was monitored annually in stable patients, while an unsuppressed viral load was repeated approximately two months after an adherence intervention. This protocol remained unchanged over the years under study. However, clinician discretion and variable patient attendance meant there was variation in the frequency of patient monitoring.

Where available, data on resistance genotyping were collated.

Time lost to follow-up was captured separately, and contributed to the duration of time spent with a high viral load. It was not, however, regarded as a new high VL event in a patient who was previously virologically suppressed. Loss to follow-up was defined as not returning to care for at least 12 weeks after the appointment date.

All deaths that occurred during the study follow-up period were recorded.

3.5 Study procedure

A search was performed using Groote Schuur Hospital's Information Systems as well as separately captured records from the clinic itself. All folders of patients who attended the clinic for any time during adolescence were pulled and reviewed against inclusion criteria.

Once eligible subjects were identified, relevant information was extracted from their medical records.

The data extracted included demographic data, mode of HIV acquisition, date of diagnosis, date of first ART, ART regimens, VL measurements, time lost to follow up and mortality. Other additional data that were collected but are beyond the scope of this project are shown in Annexure 2.

3.6 Patient safety and confidentiality

This was a non-interventional study with no direct patient contact using retrospective data. Strict confidentiality was maintained from the point of subject identification. Only the investigators were involved with data-capturing, and only they had access to the database throughout the study period. The database is stored in an access-controlled computer in a locked room.

3.7 Data management and statistical analysis

Data were directly entered electronically into a password protected database using KoBoToolbox, after which it was exported to STATA Version 14 (Stata Corporation, College Station, Texas, USA) to be checked and verified before analysis. Data were cleaned and queries addressed using standardised approaches.

Demographic characteristics and clinical findings were tabulated to provide baseline descriptions of the study population. After continuous variables were assessed for normality they were all summarised using medians with interquartile ranges as they showed significant skewness. Categorical variables were depicted as percentages and where necessary are shown together with their 95% confidence intervals.

The χ2 test or Fisher's exact tests were used to assess the strength of association between two categorical variables as appropriate while association between two continuous variables was tested with the Mann-Whitney test.

For estimation of incidence, time at risk was defined as total follow-up time. This was further stratified by gender, age at outcome, age at ART initiation, time on ART and calendar year.

The incidence of detectable viral load was estimated by calculating the rate of primary outcome events over the time period the subjects were in the study. All rates were calculated per 100 person years (PY) and included 95% confidence interval estimates. Relative risk ratios and their 95% confidence intervals were estimated using the Mantel–Haenszel method with and without adjustment for potential confounders.

Subjects who entered the study with detectable VL were categorised as having met the outcome at second consecutive detectable VL.

A significance level was set at a two-tailed $P<0.05$ for all analysis.

3.8 Ethical considerations

The protocol was approved by the Human Research Ethics Committee of the University of Cape Town (HREC REF: 899/2016), and a waiver of consent was granted. Institutional consent was granted by the superintendent of Groote Schuur Hospital.

4 RESULTS

4.1 Inclusions

Of the 482 patients who attended clinic during adolescence, seven were not on antiretroviral therapy (ART) for at least 24 months, while 148 did not attend clinic for at least 24 months as adolescents (this includes patients who transferred out or were lost to follow up in the first two years in care [n=91], as well as children who had not yet been adolescents for at least 24 months [n=57]). Therefore 327 patients met the entry criteria.

4.2 Baseline characteristic of study population

A total of 327 subjects were included for analysis of which 170 (52%) were male. The median age of study entry was 10.6 years (IQR 10 - 12.8), and exit was at 16.9 years (IQR 14.9 - 19.1). Median time spent in the study was 4.9 years (IQR 3.6 - 6.6). The number of patients followed up per calendar year increased over the follow-up period, peaking in 2014 at 296 subjects (Figure 4.1).

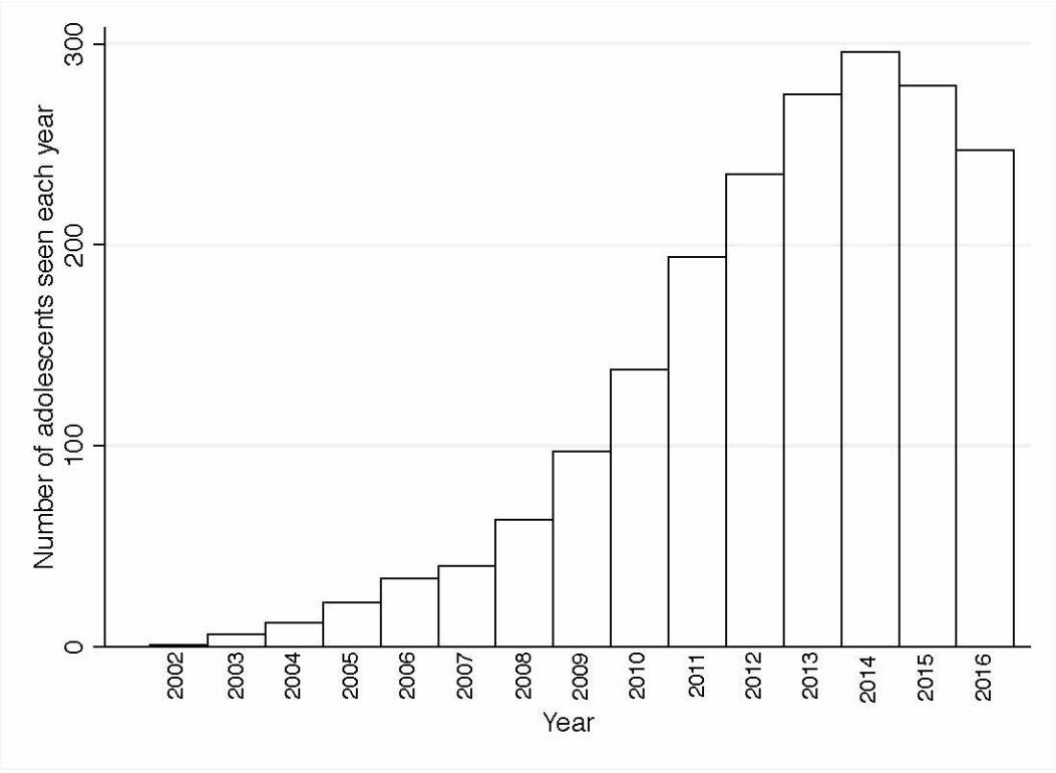

Figure 4.1 Number of adolescents seen per calendar year.

The majority of the cohort acquired HIV infection vertically (n=314; 96%), with small proportions of horizontal infections or unknown mode of infection.

Median age of diagnosis was 5.2 (IQR 1.3 - 9.1) years and of starting ART was 7.2 (IQR 3.2 - 9.9) years. The median time spent on ART was (IQR 1.8 - 7.6) 4.4 years at study entry, and 10.2 (IQR 7.5 - 12.5) years at study exit.

Over the duration of follow-up, there were 783 drug changes. Of all regimens used, 49% contained non-nucleoside reverse-transcriptase inhibitors (NNRTIs), and 47% contained protease inhibitors (PIs). In addition, 3% of all regimens were non-suppressive "holding" regimens, and 0.5% were Darunavir/ Ritonavir – containing "third line" regimens, exclusively used in subjects with confirmed PI resistance on genotype.

Table 4.1 Baseline characteristics of HIV infected adolescents (N=327)

Variable		n (%)/ median (IQR)
Sex		
	Male	170 (52)
	Female	157 (48)
Mode of infection		
	Vertical	314 (96)
	Horizontal	9 (2.8)
	Unknown	4 (1.2)
Age (years)		
	Study entry	10.6 (10 - 12.8)
	Study exit	16.9 (14.9 – 19.1)
	Diagnosis	5.2 (1.3 – 9.1)
	ART initiation	7.2 (3.2 – 9.9)
Follow-up time		4.9 (3.6 – 6.6)
Duration of ART (years)		
	At study entry	4.4 (1.8 – 7.6)
	At study exit	10.2 (7.5 – 12.5)
ART Regimen at entry		327 (100)
	NNRTI	201 (61.5)
	Protease Inhibitor	124 (37.9)
	Holding	2 (0.6)

NNRTI = Non-nucleoside Reverse Transcriptase Inhibitor
ART = Antiretroviral therapy

4.3 Prevalence of detectable viral load (VL)

A total of 2468 viral loads were performed during the follow-up period of which 885 (36%) were detectable at a threshold of >100 copies/ml. Ninety-five subjects (29%) had detectable VL on entry, while 98 (30%) had detectable VL at exit. There was a median of 9 VL performed per subject (IQR 6 - 9), with a range from 2 to 21.

In total, 111 (33.9%) of patients had undetectable VL for the duration of the follow-up period, while 16 subjects (4.9%) never suppressed. A further 57 (17.4%) subjects had a single detectable VL followed by virological suppression at next bloods: they therefore did not achieve the primary outcome.

Over the follow-up period, 159 (49% [95% CI 43% – 54%]) subjects experienced confirmed detectable VL. A total of 86 (51%) out of 170 boys experienced detectable viral load compared to 73 (47%) out of the 157 girls; p=0.46.

At first detectable VL, median age was 13.8 (IQR 11.2 – 15.5) years, and median time on ART was 6.3 (IQR 3.4 - 9.0) years. Subjects who experienced detectable VL had a median age of starting ART of 7.8 (IQR 3.2 – 9.9) years, compared to 6.1 (IQR 2.6 – 9.6) years in those who did not (p=0.026). When age of starting ART was analysed as those <10 years compared to those 10 years or older, 47% of subjects younger than 10 years vs 53% of those older than 10 years experienced detectable VL (p=0.35).

Table 4.2 Prevalence of detectable viral load (N=327)

Variable	n (%)/ median (IQR)
Number of VL performed per subject	9 (6 – 9)
Number of subjects with:	
Any two consecutive VL > 100 copies/ml	159 (48.6)
Single VL > 100 copies/ml	57 (17.4)
No VL < 100 copies/ml	16 (4.9)
No VL > 100 copies/ml	111 (34)
Confirmed detectable VL outcome#	159 (100)
Male	86 (54)
Female	73 (46)
Episodes of study outcome# by subject	
0	168 (51.3)
1	121 (37)
2	32 (9.8)
3	6 (1.8)
Age of first study outcome# VL (years)	13.8 (11.2 – 15.5)
Years on treatment at first outcome VL	6.3 (3.4 – 9)
VL >100 copies/ml at study entry	95 (29)
VL >100 copies/ml at study exit	98 (30)
Confirmed detectable VL at exit	79 (24)
Genotyped PI Resistance	6 (1.8%)

VL = HIV Viral Load; PI = Protease Inhibitor; # Two consecutive viral loads > 100 copies/ml; IQR=interquartile range

4.4 Re-suppression and viral rebound

Of the 159 subjects who experienced detectable VL, 102 (64%) re-suppressed, of which 38 (37%) had a subsequent detectable VL. Of these, 22 (58%) re-suppressed, although 6 (27%) of these had a further detectable VL.

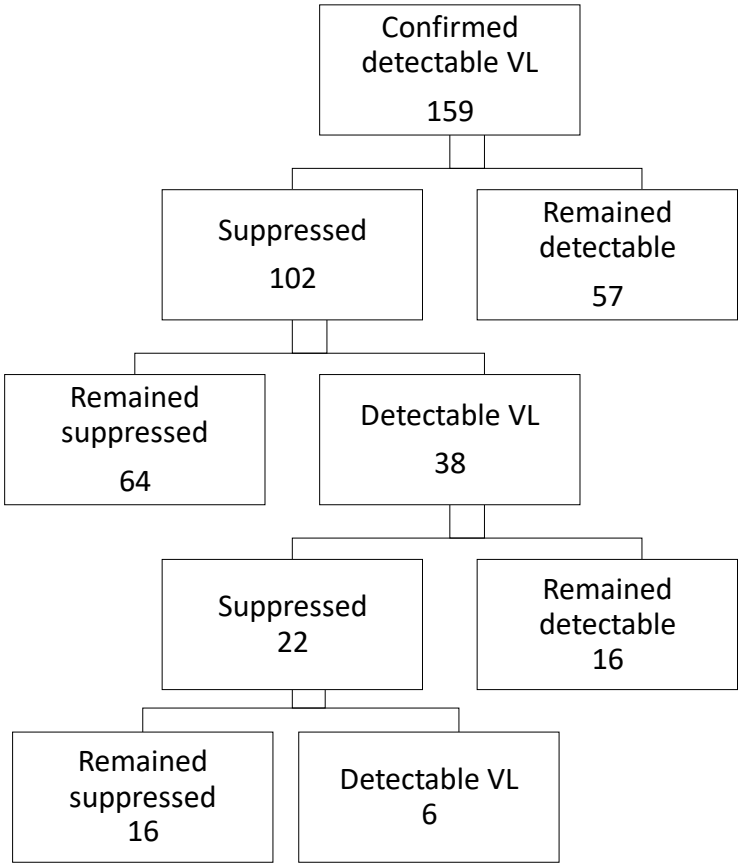

Figure 4.2 Re-suppression and viral rebound

Distinct episodes of detectable VL separated by at least one VL <100 copies/ml can be described. Of the 159 subjects who experienced detectable VL, 121 (76%) had a single episode, 32 (20%) had two episodes, and 6 (4%) had three episodes.

Six of the 159 (4%) subjects had genotyped resistance to protease inhibitors. Four of these never suppressed, while two entered the study with detectable VL, but later suppressed on Darunavir/ Ritonavir – containing “third-line” regimens.

The 16 subjects who never suppressed were comprised of nine females and seven males. Fifteen (94%) were infected vertically. For the 16 subjects who never suppressed, the median time on treatment before study entry was 4.2 (IQR 1.2 - 8.3) years. The median age at start of ART was 7.9 (IQR 3.5 - 12.2) years, and median age of study entry was 13.2 (IQR 11.1 - 13.6) years.

The 111 individuals who never had detectable VL were comprised of 55 (49.6%) females and 56 (50.5%) males. Five (4.5%) subjects were horizontally-infected, one (0.9%) had an unknown mode of transmission and 105 (95%) were vertically-infected. The median age of study entry for this group was 10.0 (IQR 10.0 – 12.6) years and age of starting ART was 5.2 (IQR 2.8 - 9.4) years. The median time on ART before study entry was 5.4 (IQR 2.6 - 7.8) years.

Table 4.3 Comparison by detectable viral load categories

A. Study outcome detectable VL (two consecutive VL > 100 copies/ml) versus no study outcome

Variable	Confirmed outcome VL# (n [%]/median [IQR]) n=159	No study outcome (n [%]/median [IQR]) n=168	P
Male sex	86 (54)	84 (50)	0.460
Horizontally acquired HIV	4 (3)	5 (3)	0.799
Age at ART initiation (years)	7.8 (4.3 – 10.2)	6.1 (2.6 – 9.6)	**0.026**
Age at study entry (years)	10.9 (10.0 – 13.6)	10.0 (10.0 – 12.5)	**0.024**
Follow-up time in study (years)	5.1 (3.8 – 7.1)	4.7 (3.4 – 6.2)	**0.021**

B. No single detectable VL versus at least one detectable VL (any VL > 100 copies/ml)

Variable	No single detectable VL (n [%]/median [IQR]) n=111	At least one detectable VL (n [%]/median [IQR]) n=216	P
Male sex	56 (50)	114 (53)	0.690
Horizontally acquired HIV	5 (5)	4 (2)	0.165
Age at ART initiation (years)	5.2 (2.8 – 9.4)	7.6 (3.4 – 10.1)	**0.048**
Age at study entry (years)	10.0 (10.0 – 12.6)	10.7 (10.0 – 13.0)	0.266
Follow-up time in study (years)	4.7 (3.5 – 6.1)	5.1 (3.7 – 7.0)	0.063

C. Always detectable VL (> 100 copies/ml) versus at least one undetectable VL

Variable	No undetectable VL (n [%]/median [IQR]) n=16	One or more undetectable VL (n [%]/median [IQR]) n=311	P
Male sex	7 (44)	163 (52)	0.499
Horizontally acquired HIV	1 (6)	8 (3)	0.381
Age at ART initiation (years)	7.9 (3.5 – 12.2)	7.1 (3.2 – 9.9)	0.395
Age at study entry (years)	13.2 (11.1 – 13.6)	10.3 (10.0 – 12.8)	**0.007**
Follow-up time in study (years)	4.0 (3.6 – 5.5)	5.0 (3.6 – 6.7)	0.159

VL = Viral Load; IQR = Interquartile Range; # Study endpoint of at least two consecutive detectable VL; **Bold type face** $p < 0.05$

4.5 Incidence of detectable viral load

A total of 327 adolescents were followed up for a median of 4.9 (IQR 3.6 – 6.6) years. The total follow-up time was 1723 PY of which 885 (51%) were contributed by males. The 159 subjects who experienced detectable VL contributed 880 (51%) PY of the follow-up time.

In total 203 distinct episodes meeting the study outcome of two consecutive VL > 100 copies/ml) occurred over the follow up period with an overall rate of 11.8 (95% CI 10.2 – 13.5) per 100 PY over the follow-up period.

The rate of detectable VL increased with increasing age (Table 4.4) with an overall relative risk of 1.24 (95% CI 1.17 – 1.31) for every increasing year of adolescence (p<0.0001). See Figure 4.3.

Table 4.4 Rate of detectable viral load by age

Age (years)	Rate per 100 PY (95% Confidence Interval)
10	1.8 (0.6-5.7)
11	5.7(3.2-10.3)
12	8.3(5.2-13.1)
13	12.4 (8.5-17.9)
14	8.3 (5.3-13.1)
15	12.7 (8.7-18.7)
16	15.4 (10.6-22.5)
17	17.3(11.7-25.6)
18	18.4 (11.7-28.8)
19	39.5(27.1-57.6)

PY = Person Year; VL = HIV Viral Load

The age of adolescents was categorised into age-bands to fit with early, middle and late adolescence. In this analysis, subjects aged 10 – 12 years had a rate of detectable VL of 5.6 (95% CI 3.9-7.9) per 100 PY. Those aged 13 – 15 had a rate of 11.1 (95% CI 8.8-14) per 100 PY and those aged 16 – 19 had a rate of 19.9 (95% CI 16.4-24.3) per 100 PY.

The rate of detectable VL was 12.1 (95% CI 10.0 – 14.6) per 100 PY in males and 11.5 (95% CI 9.4 – 14.0) per 100 PY in females. The trend in the age rate was similar in males and females (p=0.608). Figure 4.3.

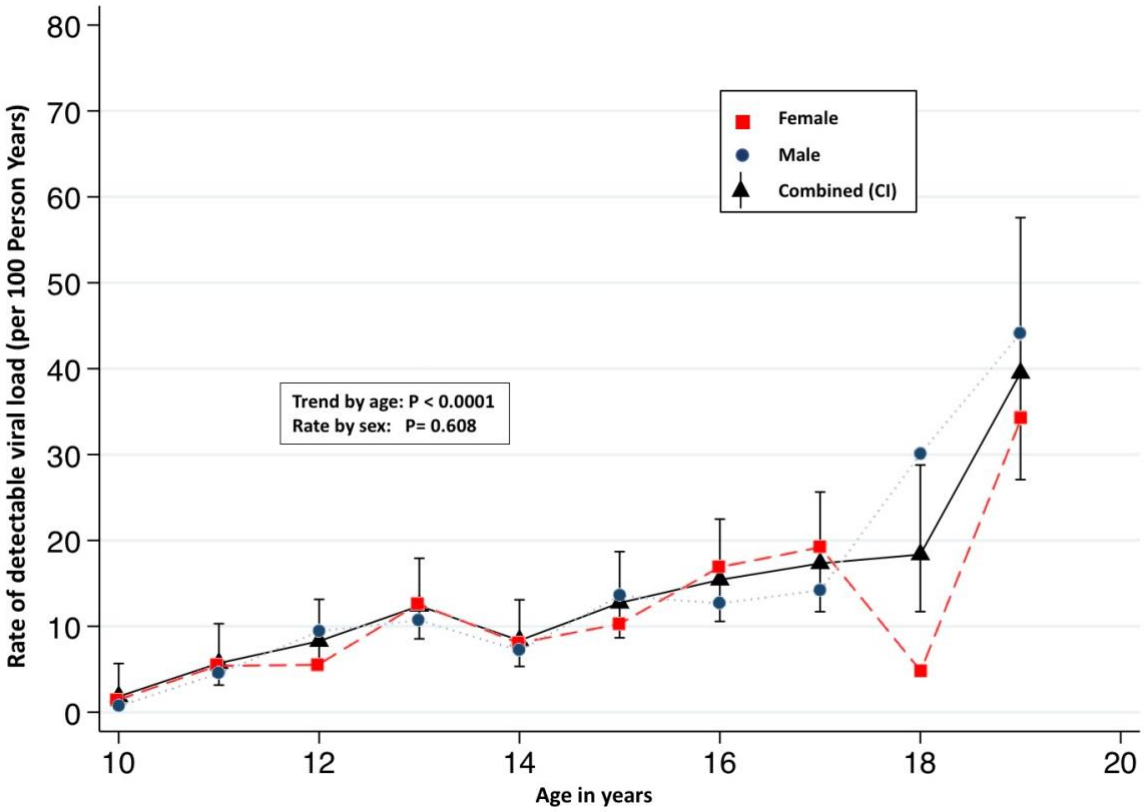

Figure 4.3 Rate of detectable VL by age and sex

The rate of detectable VL decreased with increasing time on ART. For subjects who had been on ART for less than 5 years, the rate was 23.7 (95% CI 17 – 33) per 100 PY, while for those who had been on ART between 5 and 10 years, the rate was 12.9 (95% CI 10.5 – 15.8) per 100 PY. The rate of detectable VL was lowest in subjects who had been on ART for greater than 10 years, at 8.9 (95% CI 7.1 – 11.1) per 100 PY.

The risk of detectable VL decreased by 13% per each additional year on ART [unadjusted RR 0.87 (95% CI 0.83 – 0.91)].

The rate of detectable VL increased by calendar year during the study period. Unadjusted risk ratio was 1.19 (95% CI 1.13 – 1.26). When adjusted for age, the risk ratio was 1.15 (95% CI 1.09 – 1.23).

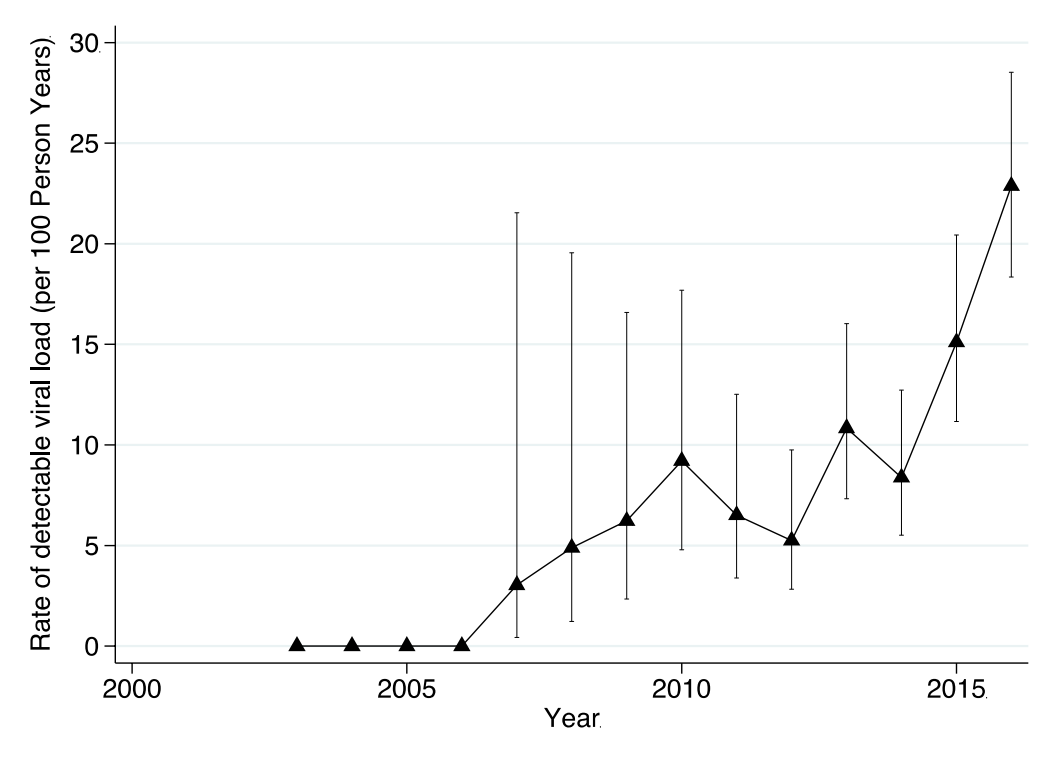

Figure 4.4 Rate of detectable VL by calendar year

4.6 Time spent with detectable VL

Of the total follow-up time of 1723 PY, the 159 subjects who experienced detectable VL contributed 880 (51%) PY. Of these, 370 years were actually spent with detectable VL: this comprised 22% of the total follow-up time, but 42% of the time contributed by those subjects who experienced detectable VL. Females contributed 173 (47%) of the 370 PY spent with detectable VL, while males contributed the remaining 197 (53%) years. Time with detectable VL comprised 21% of the total follow-up time contributed by females, and 22% of that contributed by males, (p=0.4).

Participants experienced up to three separate episodes of detectable VL during the follow-up period. The median duration of the first episode was 1.5 (IQR 0.8 - 2.5) years, for the second was 1.3 (IQR 0.8 - 2.5) years and for the third was 1.1 (IQR 1.1 - 1.2). In all cases, the third episode was ended by study exit. The overall duration of time spent with detectable VL increased with increasing numbers of episodes, as can be seen in table 4.5.

The median duration of suppression after first detectable VL was 1.3 (IQR 0.7 - 2.8) years, while that of suppression following a second episode of detectable VL was 0.9 (IQR 0.4 - 1.8) years.

Table 4.5 Duration and proportion of time spent with detectable viral load

Duration (years)	n (%)/ median (IQR)
Total follow-up time (Person Years)	1723 (100)
Males	885 (51)
Females	838 (49)
Follow-up time contributed by subjects with:	
No detectable VL	843 (49)
Confirmed detectable VL	880 (51)
Time spent with detectable VL	370 (100)
Males	197 (53)
Females	173 (47)
Total time with detectable VL, by number of episodes	
1 episode (n=121)	1.5 (0.8 – 2.6)
2 episodes (n=32)	2.8 (2.1 – 4.4)
3 episodes (n=6)	3.5 (2.8 – 3.7)
Duration of suppression post detectable VL	
1st suppression (n=102)	1.3 (0.7 – 2.8)
2nd suppression (n=22)	0.9 (0.4 – 1.8)

VL = HIV Viral Load, NB. Detectable viral load=two consecutive VL> 100 copies/ml

4.7 Loss to Follow-up

Overall, there were 101 episodes of loss to follow up, involving 69 individuals (21.1% prevalence). Ultimately, 15 did not return to care within the study period (4.6%). The rate of total loss to follow up was 5.9 per 100 PY.

4.8 Mortality

There were 4 deaths during the study period: one traumatic, and three HIV related for a mortality rate of 0.2 per 100 PY (0.08 – 0.6). All deaths occurred among vertically-infected subjects. All three HIV related deaths occurred in males. One death occurred at the age of 14 years and two at the age of 18 years. The youngest death occurred in a subject who

did not meet criteria for confirmed detectable VL, as he had been virologically suppressed prior to a 19-month period of loss to follow-up shortly before his death. One of the subjects who died at 18 years had detectable VL for the full 4.7 years of follow-up. The other died with detectable VL after spending 6.2 of the 8.7 years of follow-up in a state of detectable VL.

5 DISCUSSION

5.1 Introduction

Our study shows that detectable viral load (VL) is common in a cohort of adolescents on ART being followed up at a specialised adolescent HIV clinic. Those who experienced detectable VL spent a significant proportion of their follow-up time in this state although the period was interrupted by periods of viral suppression. While there was no association with gender, there was a strong association with age, with older adolescents more likely to have detectable VL than the younger ones.

5.2 Prevalence

Our data show that almost half of adolescents experience detectable VL, although most also experience periods of virological suppression. In one published systematic review, Ferrand et al found adolescent virological suppression to range from 27 – 87% at any time since start of ART.(16) In a large meta-analysis, Kim et al estimated that world-wide adolescent adherence (based largely on VL, but also on self-report) was 62%.(15) . It is difficult to compare our own findings with published literature, as almost all identified studies are cross-sectional, with widely varying results. A further challenge arises from differing definitions of detectable VL depending on the limits of the assay or the study-specific definitions in use.

5.2.1 Subjects with no detectable VL

One third of the subjects in our study never experienced any detectable VL over the follow-up period. The only identified longitudinal study reporting similar findings reported that 29% of the cohort experienced no detectable VL during follow-up in a vertically-infected American cohort with a mean follow-up time of 4.6 years.(64) Similar

proportions of virological suppression were reported by several cross-sectional studies.(41, 44, 46) Our study was able to demonstrate that 34% of adolescents showed no rebound detectable VL over a sustained length of time. This group was similar to the rest of the cohort in its gender and age of study entry profile, but had a younger age of ART initiation and a larger proportion of horizontally-infected adolescents. This stands in contrast to the findings of Nglazi et al and Kahana et al, that horizontally-infected youth are less likely to be virologically suppressed.(14, 44) However, the small numbers of horizontally-infected adolescents enrolled in our study meant that this unusual finding could not be explored further.

5.2.2 Subjects with all detectable VL

There were only 16 (4.9%) individuals in this study who never experienced undetectable VL for the duration of the follow-up period. This is markedly lower than the proportion reported by Nachega et al, in which 42% of adolescents never suppressed during the follow-up period.(8) It is also lower than that found in a vertically-infected US cohort, in which 24% never suppressed.(64) In our study, subjects who never experienced undetectable VL were similar to the rest of the study population with respect to gender and mode of HIV acquisition. Time on ART prior to study entry was also similar, as was age at start of treatment. One area where this group differed from the rest of the cohort was in later age of study entry; p=0.007. This may have been due to these individuals being identified at other services as struggling on ART, and requiring referral to a specialised adolescent service. Four (25%) of these had genotyped resistance to a protease inhibitor, suggesting that for the other 75% of subjects, poor adherence was the most likely cause of non-suppression.

5.3 Incidence

Cumulative incidence of confirmed detectable VL was 11.8 per 100 person years (PY). Comparison with published literature is challenging due to few studies reporting this outcome and methodological differences between those that do. In addition, all studies discussed in the literature review used a VL threshold of 1000 copies/ml, in line with definitions of virological failure rather than non-suppression. The most striking finding regarding incidence in this study was its strong association with increasing age, which will be discussed below.

5.4 Duration

Our study found that 22% of the total study time was spent with a VL >100 copies/ml. A review of the literature revealed one other study that dealt with the duration of detectable VL during adolescence. Within this vertically-infected cohort of subjects aged 7 – 30 years, 34% of total study time was spent with VL >400 copies/ml, which is higher than what we found in our study. The age-stratification differed from our study, and was divided into children aged 7 – 12 years (22% of study time with VL >400 copies/ml), adolescents aged 13 – 17 (30% of study time with VL >400 copies/ml) and youth aged 18 – 30 years (44% of study time with VL >400 copies/ml). Our total study findings of 22% are equal to those of the lowest age group in this study even at a lower limit of detection of 100 copies/ml.

When follow up time is restricted to the 880 PY contributed by the 159 subjects who experienced detectable VL, the proportion of time spent with detectable VL rises to 42%. This indicates that those who have detectable VL tend to remain in this state for a considerable proportion of their follow-up time. For adolescents, who may be sexually active, time spent with detectable VL should be viewed not only as time at greater risk of disease progression, but also time of increased risk of HIV transmission.(10, 12, 13)

As previously indicated, duration of detectable VL is not commonly reported in the literature, possibly because of lack of longitudinal data. However, we believe it could be used as a potential marker of successful ART in adolescents. Although the duration of detectable VL can in part be affected by the frequency of virological testing, we believe that when used together with incidence and prevalence data, it has the potential to deepen our understanding of adherence patterns of adolescents on ART. Moreover, duration of detectable VL provides important additional information that is not reflected in prevalence and incidence estimates. As an example, if two adolescents are followed up for a similar period of time and each experiences a single period of sustained detectable VL, each will contribute a single episode to the prevalence and incidence of the study population. However, one may have been unsuppressed for 5% of the follow-up time, and the other for 90%. These patients would be considered equal under both prevalence and incidence studies of non-suppression, but a treating clinician would clearly have more reason to be concerned about the second patient.

The study outcome of duration of detectable VL thus adds important information that is taken into account by clinicians, but is not apparent in the literature.

5.5 Re-suppression and viral rebound

The longitudinal design of this study allowed us to look more closely at patterns of suppression and rebound detectable VL. Of the 159 subjects with detectable VL, approximately two thirds re-suppressed (67%). However, slightly more than one third of these (37%) went on to have further confirmed detectable VL. Of these, more than half (58%) suppressed once more, although almost a third of those left (27%) experienced detectable VL again. At study exit, half of the original 159 subjects with detectable VL had undetectable VL. Whether this pattern holds over longer periods of time or in contexts other than ours deserves further study.

The dynamic nature of adherence and detectable VL has been noted elsewhere. Childs et al found high rates of re-suppression after confirmed VL >400 copies/ml in a paediatric and adolescent cohort in the United Kingdom and Ireland. In this cohort, 69% of subjects had re-suppressed 24 months after rebound, comprising those who remained on the same regimen as well as those who switched to an alternative regimen. In the group that re-suppressed without regimen switch, 61% had virological rebound at most recent follow-up.(55)

In a Thai cohort, Sudjaritruk et al found that 13% of a cohort of vertically-infected adolescents experienced virological rebound of >1000 copies/ml after a period of sustained suppression.(52) The comparatively lower rate of viral rebound could be due to a higher threshold for detectable VL, or to a bias towards more stable patients arising from the entry criterion of sustained virological suppression.

In combination with the literature, our study adds to the growing body of evidence relating to the dynamic nature of adherence and virological outcomes. During adolescence, those with sustained virological suppression may become unsuppressed, while those with viraemia may re-suppress. Clinicians need to be alert to the fact that adherence behaviour may change over time, for better or worse. Further research is needed to examine factors that influence these changes for or against virological suppression.

5.6 Associations

This study was primarily descriptive, and its main objectives were to examine the rate, duration and prevalence of detectable VL. We did, however, examine demographic data for associations with these outcomes. The following variables will be discussed: age, gender, time on ART, age at ART initiation and calendar year.

5.6.1 Age

Our results show a clear trend of increasing rates of detectable VL as the cohort ages. The risk of detectable VL increased by 24% for each year from two per 100 PY at age 10 to a twenty-fold increase to 40 per 100 PY at 19 years of age. This is in keeping with the literature, in which multiple studies have found that older adolescents are at higher risk of detectable VL.(19, 30, 40, 57-59) Compared to those aged 10 – 12 years, adolescents aged 13-15 and 16 – 19 years had a double and four-fold higher risk of detectable VL respectively.

The extremely high rates of detectable VL in the older adolescents within this cohort is of concern, as this occurs at the time prior to transfer to adult services where they are expected to be more independent. It would be useful to know whether this high risk of detectable VL is sustained into young adulthood, or whether this represents a peak that declines thereafter.

5.6.2 Sex

Studies exploring associations between sex, adherence and VL have mixed findings, with positive, negative and no associations being reported.(9, 30, 42, 44, 52) In our study sex was not associated with prevalence, incidence or duration of detectable VL.

5.6.3 Time on ART

This study found that longer time on ART was associated with decreased risk of detectable VL. This stands in contrast to published literature, which shows either no association or a decline in virological suppression with increasing time on ART. (8, 14, 40, 62) It is not entirely clear why our findings differ from other published studies. The nature

of services offered by the specialised HIV adolescent clinic in which this cohort was based could partially account for the observation. The clinic offers a relatively well-resourced, adolescent-friendly service, with strong continuity of care in which each patient is allocated their own counsellor and physician. Subjects who had been on treatment for more than ten years are likely to have commenced treatment in the same clinic, and to have benefited from the advantages offered by this service.

5.6.4 Age at ART

We found that subjects who experienced detectable VL started ART later than those who did not; p=0.026. This is in keeping with the results of Makadzange et al, who found that older age of ART initiation was associated with VL >1000 copies/ml.(40) When we performed a similar analysis by those who initiated ART younger than 10 years compared to 10 years and above, we also found increased prevalence of detectable VL in the older group, though our findings were not significant.

5.6.5 Calendar year

The rate of detectable VL shows an upward trend from 2007 onward, and this rise is sustained until the end of the study. The reasons for this are not well understood, but we believe that they relate to the increasing numbers of “high risk” patients referred into the clinic from other services. As ART became more available in South Africa, and a decentralised approach was rolled out across the Cape Town metro, the Groote Schuur Hospital Adolescent Clinic evolved into a referral centre for adolescents with complex adherence problems. These adolescents are expected to be at higher risk for detectable VL than the general clinic population. This was an unexpected finding which deserves further investigation.

5.7 Mortality

The mortality rate in this study was 0.2 per 100 PY. This is identical to that reported by Sudjaritruk et al in a cohort of vertically-infected adolescents.(52) Our population differed from that in Sudjaritruk's study which only included subjects with a history of sustained virological suppression, thus biasing selection towards more stable patients. The mortality rate in our study is considerably lower than the 1.2 per 100 PY reported by Nglazi et al in another South African adolescent cohort.(14) However, our study excludes subjects who had not spent at least 24 months on ART, so any early deaths on treatment would have been excluded. By including both adolescents who were already on ART (29%) as well as those initiating treatment, Nglazi's study captures both early and late mortality.(14)

In a recent cohort study, Judd et al found that the overall mortality rate for European and Thai children and adolescents enrolled between 1997 and 2013 was 2.5 per 100 PY in the first six months on ART, and 0.27 per 100 PY thereafter.(11) The mortality rate after six months on ART is similar to the rate in our cohort.

The mortality rate in our study can thus be viewed as a late outcome on ART, and as such is likely to be lower than the true mortality rate for the clinic overall. However, it is in keeping with late outcomes reported by middle and high-income countries in Europe and as well as Thailand. This may reflect the relatively well-resourced nature of the clinic under study.

6 CONCLUSIONS

This study has found that detectable viral load (VL) is experienced by half of adolescents, with a cumulative incidence of nearly 12 for every 100 adolescents in a year. Although other explanations exist for detectable viral load, in the context of effective antiretroviral therapy, this finding largely reflects poor adherence. The most concerning finding is that those with detectable VL spent almost half of their time in this state. This risk is further amplified by the fact that rates of detectable VL are higher in older adolescents who are more likely to be sexually active, increasing the risk of transmitting the virus.

This study contributes the seldom reported phenomenon of "time spent with detectable VL" to the existing body of work on adolescent adherence. This is a concept that has not been well-explored, but may be useful in defining periods of particularly high risk of disease progression and transmission of HIV infection.

6.1 Limitations

The findings of this study may not be generalisable to other contexts and for a number of reasons, the results may not be representative of HIV-infected adolescents as a whole. The study was based in a single centre which takes referrals of "difficult" patients from other services. It is possible that the study subjects experienced more challenges than their peers followed up in primary health care settings.

Though the quality of data was generally good, the retrospective design of the study meant that not all the data we would have liked was readily available, in particular that pertaining to adherence as well as HIV genotyping. While six patients were found to have resistance to protease-inhibitor containing regimens, it is possible that others also

experienced undiagnosed resistance, and ineffective ART may have been responsible for persistent detectable VL in their case.

Inasmuch as the findings of this study are strengthened by the inclusion criterion of a minimum of two years' follow-up, this also brings certain limitations. Firstly, 57 younger adolescents were excluded as they were younger than 12 years at the study endpoint. Secondly, a total of 91 subjects were excluded as they did not remain in care for long enough: this figure includes patients who transferred out to other services, as well as those who were lost to follow-up (LTFU). As a result, it is possible that the study may have underestimated both mortality and LTFU rates. The reported mortality and LTFU rates thus reflect long term rather than early ART outcomes in adolescence.

6.2 Recommendations

6.2.1 Clinical recommendations

As older adolescents are at high risk of HIV viraemia, clinicians should anticipate this, and offer appropriate adherence support to this group. Community-based adherence support and group adherence counselling have shown promise in improving adolescent adherence to ART.(65) It is our experience that having a dedicated clinician and counsellor allocated to each individual helps to prevent adherence difficulties from going undiagnosed, while referral to a psychologist improves complex adherence problems.

6.2.2 Recommendations for further study

This report restricted itself to detailed descriptions of patterns of viral load and only minimally analysed the effect of demography and time on this pattern. There is a need to

analyse other risk factors that may impact on adolescent adherence to ART (such as mental health, orphanhood or stigmatizing medication side effects like lipoatrophy).

In addition, although detectable VL is widely used as a marker of disease progression, this study did not report on immunological or clinical outcomes (aside from death). Data for making sense of these important aspects have been collected and plans are underway to analyse and report on these in the near future.

Detectable VL rose sharply with increasing age, and was highest among 19 year olds, but this study did not include ages above 19. A follow up study to determine if the observed patterns of viraemia persist beyond 19 are warranted. There is also a need for independent longitudinal studies in other adolescent cohorts especially in low and middle-income settings to see if the patterns differ from those observed in this cohort.

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APPENDICES

Appendix 1.

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Human Research Ethics Committee

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11 January 2017

HREC REF: 899/2016

Dr R Muloiwa
Division of Paediatric and Child Health
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Dear Dr Muloiwa

PROJECT TITLE: FACTORS ASSOCIATED WITH DETECTABLE VIRAL LOAD IN A COHORT OF HIV POSITIVE ADOLESCENTS ON HAART (MSc Candidate Rebecca Sher)

Thank you for your response letter dated 10 January 2017, addressing the issues raised by the Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 January 2018.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student, Dr J Rusch will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

HREC 899/2016

Appendix 2.

new version friday

Name

_____

Surname

_____

Folder number

_____

Date of birth

yyyy-mm-dd

Gender

- ◯ Male
- ◯ Female

Date study entry

yyyy-mm-dd

Date study exit

yyyy-mm-dd

Date of diagnosis

yyyy-mm

Transmission

- ◯ Vertical
- ◯ Horizontal
- ◯ Unknown

Date of disclosure

yyyy-mm

Date started HAART

yyyy-mm-dd

ARV Regimens during adolescence

1

*** Drugs**

- [ ] Abacavir
- [ ] Lamivudine
- [ ] Tenofovir
- [ ] Emtricitabine
- [ ] Zidovudine
- [ ] Stavudine
- [ ] Didanosine
- [ ] Efavirenz
- [ ] Nevirapine
- [ ] Lopinavir/ Ritonavir
- [ ] Atazanavir/ Ritonavir
- [ ] Darunavir/ Ritonavir
- [ ] Raltegravir
- [ ] Ritonavir

*** Start date ARV regimen**

yyyy-mm-dd

*** Dosing**

- ◯ Daily
- ◯ Twice a day

CD4 count during adolescence

1

* **CD4 count**

* **Date**

yyyy-mm-dd

Viral load during adolescence

1

* **Viral load**

* **Date**

yyyy-mm-dd

Time lost to follow up (3 or more months late for date)

1

Date expected back

yyyy-mm-dd

Date attended

yyyy-mm-dd

Orphan status

- ◯ Not an orphan
- ◯ Maternal orphan
- ◯ Paternal orphan
- ◯ Double orphan
- ◯ Unknown

Date of orphanhood (if occurred between 10 and 19 years)

yyyy-mm

Caregiver

1

*** Caregiver type**

- ◯ Both parents
- ◯ Mother
- ◯ Father
- ◯ Grandmother
- ◯ Aunt1
- ◯ Aunt2
- ◯ Aunt3
- ◯ Male relative
- ◯ Non relative
- ◯ Children's home
- ◯ Temporary placement
- ◯ Other

*** Date**

yyyy-mm

School failure ever

- ◯ Yes
- ◯ No

Referral to/ attendance at Neurology ever

- ◯ Yes
- ◯ No

New referral for extra support

New referral for extra support

- ◯ Psychiatry
- ◯ Psychology
- ◯ Social Work

Date

yyyy-mm-dd

Notes

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by Rebecca Sher

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Date: 18th February 2018