

**EVALUATION OF VIROLOGIC-IMMUNOLOGIC RESPONSES IN HIV-1
PERINATALLY INFECTED CHILDREN AT PUBLIC SECTOR CLINICS IN SOUTH
AFRICA**



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Declaration

I declare that this work is my own and is being submitted for the Degree of Master of Science in Medicine at the University of Witwatersrand, Johannesburg. This Dissertation has not been previously submitted for any degree or examination at this University or any other institution.



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07 January 2024

Dedication

I, “always giving thanks to God the Father for all things, in the name of our Lord Jesus Christ.” Ephesians 5:20

I am grateful to my family for their unwavering encouragement, support, and love throughout my studies. I would like to express my heartfelt appreciation to the following individuals:

To my wife, Anotidaishe Chinyanga, thank you for being my pillar of strength and for always believing in me.

To my mother, Lucia Chinyanga, your love and unwavering belief in my abilities have been instrumental in my journey. Thank you for your constant support.

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To my brothers and sisters, thank you for your support and for being there for me whenever I needed it.

Their presence in my life has made all the difference.

I thank my family for their encouragement, support, and love during my studies.

“Praise the LORD. Give thanks to the LORD, for he is good; his love endures forever.” Psalms 106:1

Abstract

Background

Despite achieving HIV viral suppression following start of combination antiretroviral therapy (cART), a significant proportion of HIV infected adults fail to restore CD4 Tcell counts to expected levels, a phenomenon known as a virologic-immunologic discordance. There is however paucity of data on the prevalence rate of virologic-immunologic discordance in HIV infected children. The study therefore aims to determine the prevalence and associated risk factors of virologic-immunologic discordance in perinatally HIV-1 infected children after initiation of a antiretroviral regimen.

Methods

The study is a retrospective longitudinal design that set out to describe the prevalence and risk factors of virologic-immunologic discordant responses in a large HIV paediatric public sector cohort of 1747 children initiated on cART from the January 1st 2004 to December 30th 2022.

Data Analysis

For data analysis, Chi-square and the Mann-Whitney U test were used. To explore factors associated with virologic- immunologic discordance, univariate and multivariate logistic regression analysis was used.

Results

Of the 1747 HIV-infected children included in the analysis, 895 (51.2%) were female and 852 (49.0%) male. One thousand two hundred and ninety-seven children on cART had HIV viral loads below the limit of detection and 72 (6%) displayed virologic- immunologic discordance. WHO stage III/IV, older age (10 – 17 yrs), CD4 T cell count <50 cells/μl and AZT or d4T based NRTI regimen, were factors associated with virologic-immunologic discordance.

Conclusion

HIV infected children who fail to achieve viral suppression and a resulting increase in CD4 T cell count, are at high risk of disease progression and death from AIDS and non-AIDS events and should be monitored closely, without effecting a regimen change.

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Nomenclature

AARMS	Academic Affairs and Research Management System
ART	Antiretroviral therapy
ARV	Antiretroviral
ABC	Abacavir
ANOVA	Analysis of variance
AZT	Zidovudine
cART	Combination Antiretroviral therapy
CDW	Central Data Warehouse
d4T	Stavudine
DTG	Dolutegravir
DoH	Department of Health
EVF	Efavirenz
FTC	Emtricitabine
HIV	Human immunodeficiency virus type-1/2
HIV VL	Human immunodeficiency virus viral load
IVD	Immunologic-virologic discordance
LLV	Low-level viraemia
LPV/r	Lopinavir/ ritonavir
NHLS	National Health Laboratory Service
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NRTI	Nucleoside reverse transcriptase inhibitor
NDHG	National Department of Health Guidelines
PI	Protease inhibitors
TLD	Tenofovir, Lamivudine and Dolutegravir
TDF	Tenofovir disoproxil fumarate
UNAIDS	The Joint United Nations Programme on HIV/AIDS
VL	Viral load
WHO	World Health Organization
WHREC	Wits Human Research Ethics Committee

3TC

Lamivudine

CHAPTER 1: INTRODUCTION

1. Literature Review

1.1 Epidemiology

Approximately 39 million individuals globally in 2022 [33.1 – 45.7 million] were HIV infected, with 37.7 and 1.5 million being adults and children respectively. With an estimated 67% of the 38 million HIV infected people in 2021 residing in sub-Saharan Africa, sub-Saharan Africa is recognised as the epicentre of the HIV pandemic (UNAIDS 2022).

There are variations in HIV prevalence rates between sub-Saharan African countries, with 20.6 million HIV-infected individuals living in Eastern and Southern Africa (UNAIDS 2023). In addition, an estimated 87% of children and 82% of adolescents are living with HIV (UNAIDS 2023). In 2021, the incidence of HIV and AIDS-related deaths in HIV-infected individuals in the two hardest-hit regions of Southern Africa (Eswatini, Lesotho and South Africa), were reported at 67 000 and 280 000 respectively (UNAIDS 2021).

Whilst new HIV-1 infections in adults have declined by 38% from 2010 to 2022, a significant decline by 58% was observed in children in 2022 (Simbayi et al., 2019). An estimated 13.9 million children in 2022 had experienced the death of one or both parents from AIDS-related causes.

1.1.1 South Africa

South Africa has 7.9 million of its citizens infected with HIV-1 (Simbayi et al., 2019). The highest HIV incidence is among adolescent girls and young women. Adolescents aged 10 to 19 years represent a significant proportion of the population at 17.4%, contributing to 2.1% of the total mortality (Simbayi et al., 2019).

1.2 HIV Prevention Strategies

In an effort to combat HIV, a number of combination HIV prevention interventions, including combination antiretroviral therapy (cART), male circumcision, condom use, and behaviour change have been adopted (UNAIDS 2020 – seizing the moment). The impact of these focused strategies is reflected in the 28.7 million HIV-infected people accessing cART treatment in 2021 (UNAIDS 2022), resulting in a significant improvement in access. Of concern, however, is that only 57% of the HIV infected children had access to antiretroviral in 2022 (UNAIDS 2022). Whilst great strides have been made through expansion in antiretroviral access, maintenance of long-term viral suppression is equally important and remains a challenge for HIV-infected children (Fitzgerald et al., 2013; Sigaloff et., 2011).

An additional ongoing concern has been the approximately 5.9 million people in 2021 who were unaware that they were HIV infected (UNAIDS 2021). In an effort to control the epidemic, the United Nations Programme on HIV/AIDS (UNAIDS) set global "95-95-95" targets that aimed to achieve 95% of HIV testing, treatment and durable viral suppression by 2030. To date, the updated "95-95-95" targets have been met by a Eswatini, Rwanda, Republic of Tanzania and Zimbabwe (UNAIDS 2023).

Ninety-five percent of HIV infected people in these countries therefore know that they are HIV infected. Improvements in paediatric HIV management has shifted outcomes of these children from HIV being a chronic condition with high mortality to a condition managed in outpatient's clinics (Hazra et al., 2010). The timely infant diagnosis together with improved access to antiretroviral therapy as well as advances in clinical management all have reduced mortality in the past decade (Chandiwana et al., 2018).

1.3 Virology of HIV

1.3.1 HIV Structure

The mature HIV virion is an enveloped spherical structure, approximately 100-120 nm in diameter. The envelope consists of a lipid bilayer and embedded within the lipid layer are approximately 72 glycoprotein heterodimers complexes, arranged as trimers (Collier et al., 1999). The trimeric viral glycoproteins (gp) include the external glycoprotein 120 and the transmembrane glycoprotein 41 bound together. Beneath the bilipid layer is the matrix protein containing an icosahedral capsid protein (Gelderblom et al., 1998) which houses the RNA genome with enzyme reverse transcriptase (**Figure 1**).

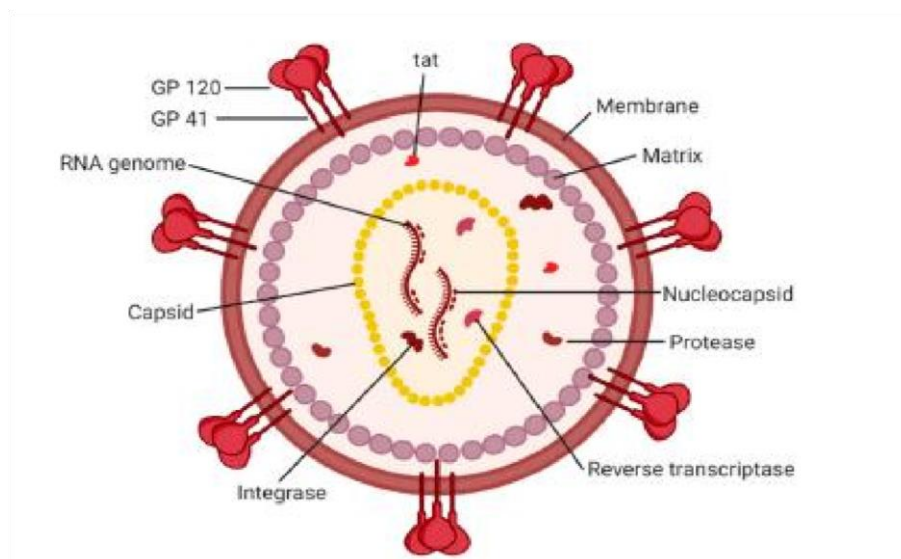


Figure 1: Cross sectional structure of the HIV-1 genome (Rossi et al., 2021)

1.3.2 Genome Characterization of HIV

The 9.2 kb HIV RNA genome is flanked by the 3'-long terminal repeats (LTR) and 5'LTR on either side and encodes both structural and non-structural proteins. The three structural proteins are gag, pol and env. In addition are non-structural proteins regulatory proteins: tat and rev as well as 4 accessory proteins: vif, vpu, vpr and nef. At the genome level, HIV-1 and HIV-2 types share similarities and only differ in terms of the proteins, with HIV-1 expressing vpu whilst HIV-2 expresses vpx (Figure 2).

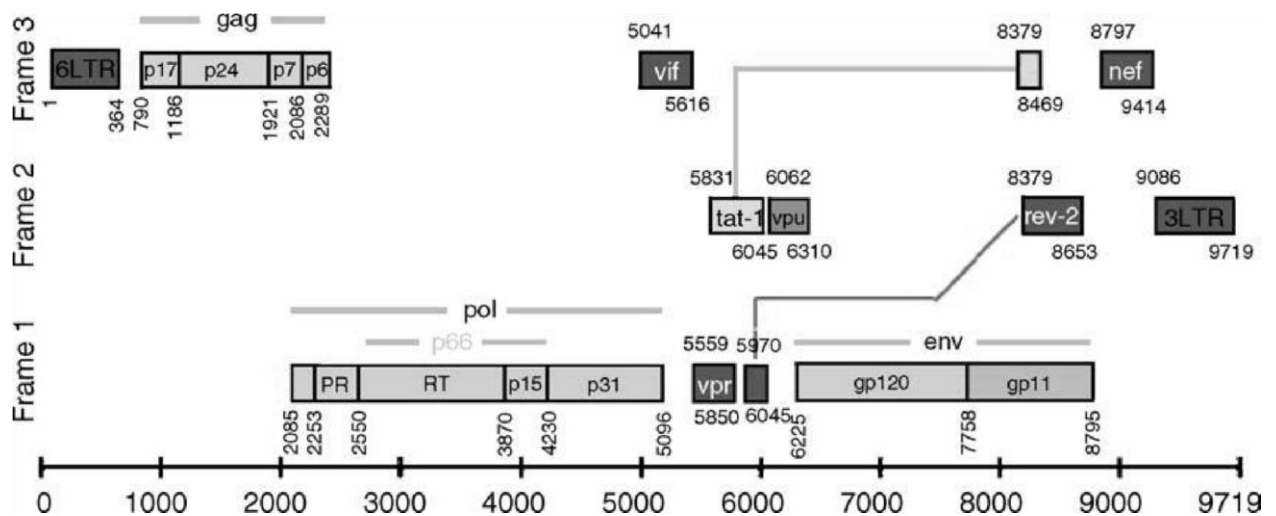


Figure 2: Genomic organization of HIV (adapted from Sierra et al., 2005)

1.4 Treatment of HIV

1.4.1 Highly Active Antiretroviral Treatment

It is generally acknowledged that combination antiretroviral therapy has significantly improved the clinical outcomes of HIV infected individuals. cART inhibits HIV replication resulting in CD4 T cell count recovery and ultimately a reduction in HIV related complications, morbidity, mortality as well as improving the quality of life of HIV infected people (Manaye et al., 2020; Kiros et al., 2022, Chan et al., 2006; Zoufaly et al., 2010; Braitstein et al., 2006).

1.4.2 Antiretroviral Mechanism of Action

There are six licensed classes of antiretroviral agents whose mechanism of action is to target the HIV cycle by inhibiting the gp41 mediated fusion, reverse transcription, integration and proteolytic cleavage steps in the HIV life cycle (Volberding, 2010). Entry inhibitors such as maraviroc and enfuvirtide block attachment and fusion respectively, whilst reverse transcriptase inhibitors NRTIs and NNRTIs, inhibit reverse transcription process. Other antiretroviral agents such as raltegravir and second generation integrase inhibitors such as dolutegravir, prevent the incorporation of HIV DNA into host chromosomes. Additionally, HIV protease inhibitors are utilized to impede the cleaving of polypeptide chains, thus preventing the virus from maturing (Volberding, 2010) (**Figure 3**).

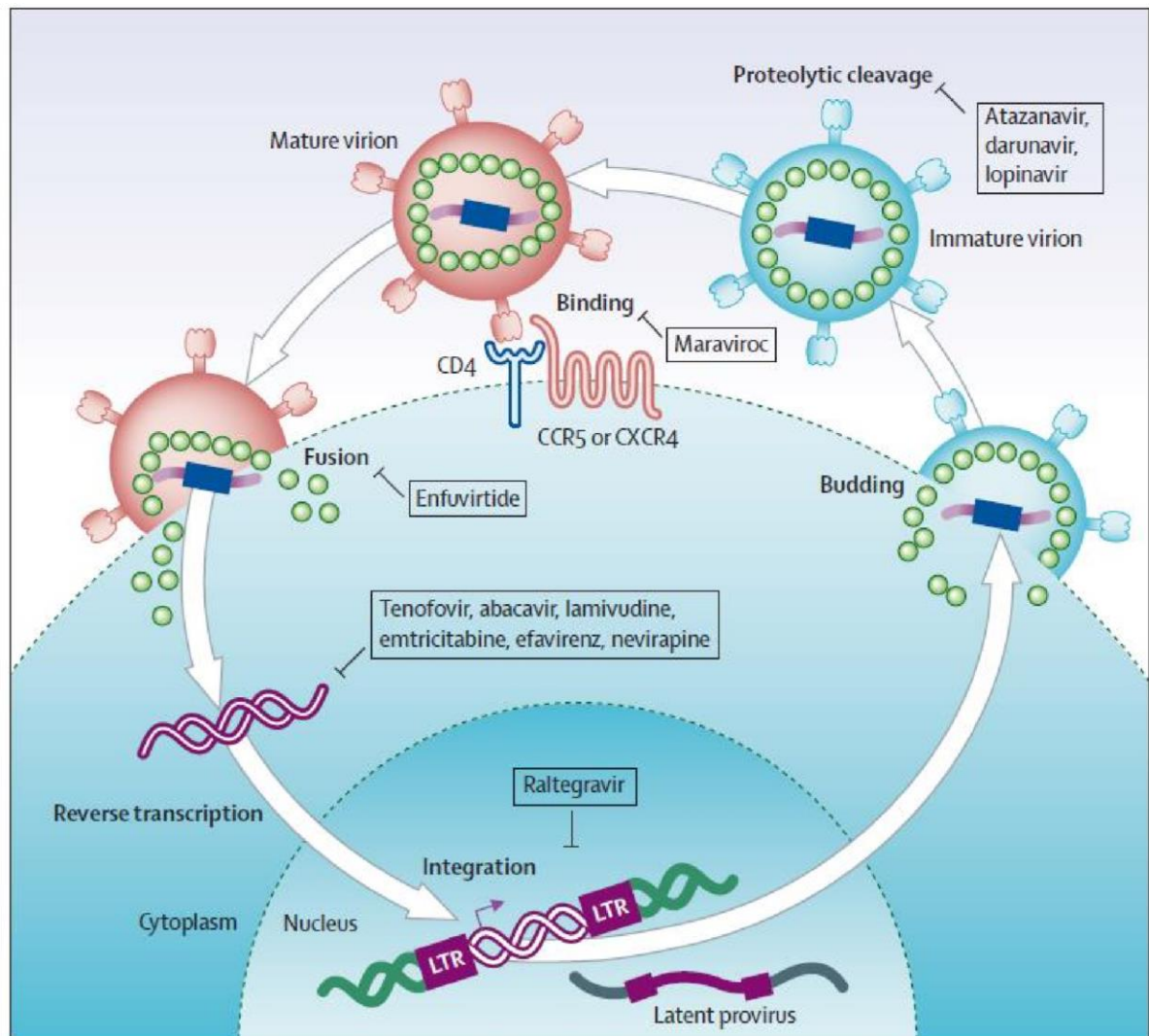


Figure 3: HIV life cycle and antiretroviral drug targets (Volberding, 2010).

An HIV viral load is a reliable indicator of adherence and adherence rates of 90% to 95% are deemed necessary to achieve HIV viral suppression (Edet et al., 2019).

1.5 Effect of combination antiretroviral therapy (ART) on CD4 cell count and HIV RNA.

HIV infected individuals on cART are normally monitored by measuring the CD4 T cell count and HIV viral load wherein a decrease in HIV RNA accompanied by an increase in CD4 T cell count, is an expected outcome and indicates a positive response to treatment (Gebremedhin et al., 2023).

1.5.1 Effect of cART on HIV Viral Load

With the “Test and Treat strategy, the CD4 T cell count is no longer used as a surrogate marker to commence ART but continues to be to assess the need prophylaxis in patients with CD4 T cell count < 200 cells/ul (Egger et al., 2002; Furrer et al., 2002). With adherence, the HIV viral load decreases to undetectable levels within three months in most individuals on cART. Combination antiretroviral therapy therefore typically leads to a decrease in circulating HIV RNA, often exceeding a 100-fold reduction within the first two weeks of antiretrovirals (**Figure 4**).

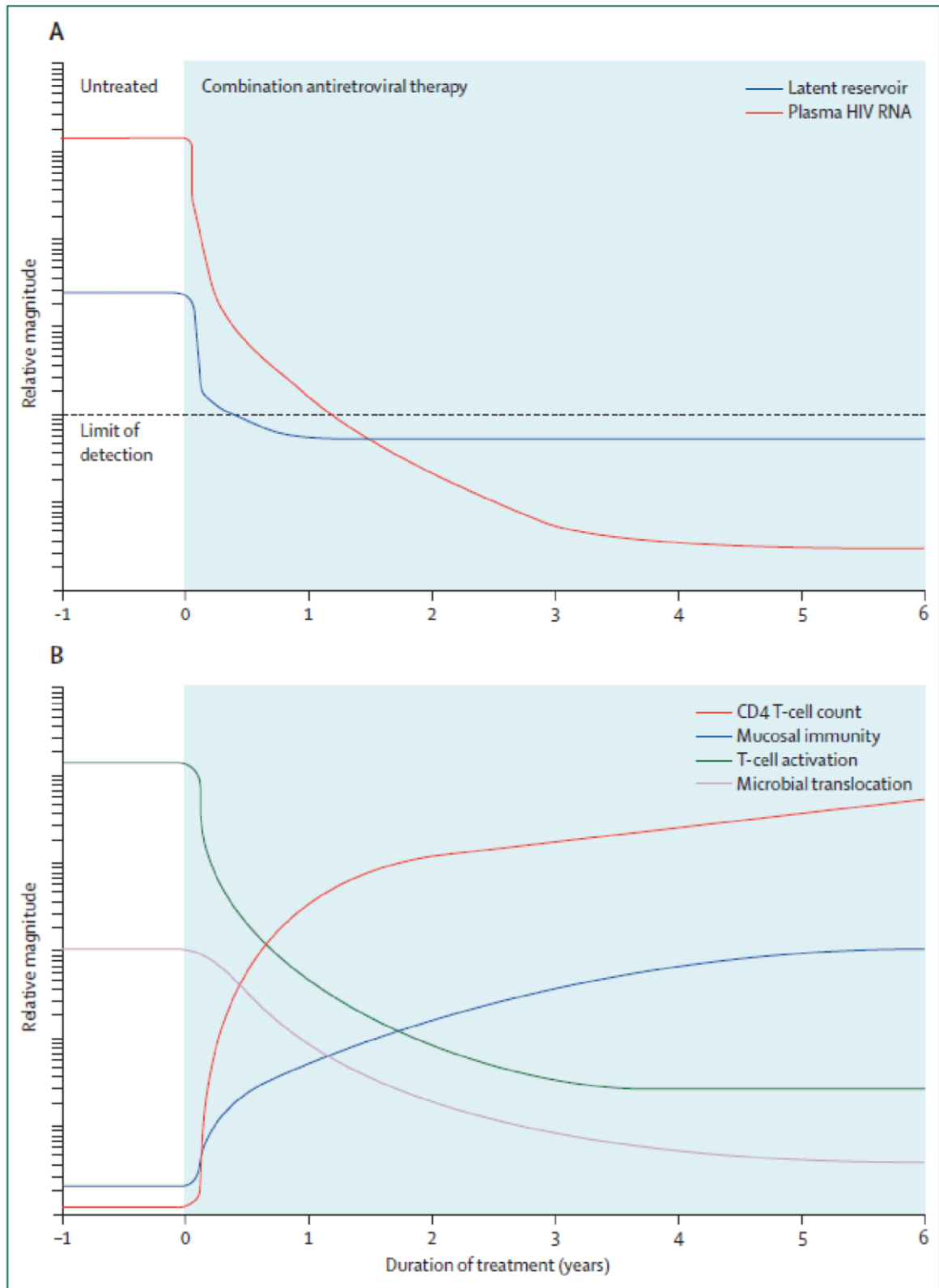


Figure 4: Effect of combination antiretroviral therapy (ART) on CD4 cell count and HIV RNA.

The initial decline in HIV RNA is followed by a gradual decrease, eventually reaching a new viral set point after a number of years on therapy (Mellors et al., 1996; Mellors et al., 1996). The presence of replication competent latent pool also diminishes, although the rate of decline is not as dramatic (Chun et al., 1997). Suppression of HIV replication through use of antiretroviral agents is accompanied by a sustained but variable increases in peripheral and mucosal CD4 T cell counts, with peripheral CD4 counts more frequently reaching normal values after several years of therapy (Bartlett et al., 2001; Kelly et al., 2009; Lok et al., 2010).

1.5.2 CD4 T Cell Reconstitution

Immunological reconstitution is biphasic. Initial rapid CD4 T cell count increases are seen within the first 3 – 6 months of cART, followed by slower but sustained increases (Pakker et al., 1998). However, variation has been noted in this response wherein CD4 T cell counts may remain below the critical threshold of 200 cells/ul in 10%–20% of individuals following placement of cART (Kauffman et al., 2005) when CD4 T cell recovery should display a sustained increase in CD4 T cell counts for ≥ 3 –4 years in individuals with advanced HIV-1 infection and who remain on cART (Smith et al., 2003; Kaufmann et al., 2005).

In addition, a decreasing CD4 T cell count predisposes to opportunistic infections (Kaplan 2002). CD4 T cell count reconstitution after HIV viral suppression correlates with improved T cell responses to antigens and mitogens and adequate protection against specific opportunistic infections (Egger et al., 1997; Palella et al., 1998; Egger et al., 2002; Ledergerber et al., 1999; Furrer et al., 1999). As such, the CD4 T cell count represents a surrogate marker for prophylaxis and discontinuation of prophylaxis once a CD4 T cell count of 200 cells/ul threshold has been reached.

1.6 Poor CD4 Reconstitution

With HIV RNA lower limit of detection maintained, peripheral blood CD4 T cell counts in most individuals with HIV will continue to increase. Whilst in most people on cART CD4 T cell count recovery of > 500 cells/ul is often evident, approximately 15 to 20% of those initiated with a low nadir CD4 T cell count experience a plateau at abnormally low CD4 T cell counts (Kelley et al., 2009; Lok et al., 2010). It is now widely accepted that initiation of cART early supports maximal CD4 T cell reconstitution (Le et al., 2013) and is the basis for the adoption of “Test and Treat”, a strategy now used in South Africa (NDoH 2015).

CD4 T cell counts that remain low over time despite viral suppression are associated with an increased risk of morbidity and mortality - with individual with CD4 T cell counts of < 200 cells/ul after three years of cART, having a 2.6-fold greater risk of mortality compared to those with higher CD4 T cell count (Engsig et al., 2014).

Regular assessment of both HIV viral load and CD4 T cell response is therefore essential to evaluate factors associated with poor clinical outcomes, including immunologic failures, among HIV-infected patients on cART (Murray et al, 1999; Kiros et al., 2022).

1.7 Virological Immunological Discordance

Whilst It has been established that adherence to ART results in a robust increase in CD4 T cell count associated with an improved prognosis (May et al., 2007; Hunt et al., 2011), some individuals may not achieve the expected CD4 T cell count reconstitution, despite achieving viral suppression (Pakker et al, 1999). This paradoxical response, typified by an HIV viral load below the limit of detection and yet failure to achieve an adequate CD4 T cell count increase, is referred to by virologic-immunologic discordance (Gilson et al., 2010; Tuboi et al., 2010, Baizze et al., 2022).

The degree of immune activation at the time of cART initiation is associated with the development of immune response discordance (Hunt et al., 2011 Gandhi et al., 2006) and may ultimately predict mortality on cART (Kuller et al., 2008). Whilst the mechanisms promoting virologic-immunologic discordance have not been fully understood, destruction of CD4 T cells begins before initiation of cART due to direct effects of the HIV on thymic tissue and a resulting destruction of progenitor cells (Bandera et al., 2010). In patients whose nadir CD4 T cell count is low at time of initiation of cART, thymic output may be

disproportionally affected leading to underreconstitution of naïve CD4 T cells (Vrisekoop et al., 2008; Li et al., 2011). Approximately 20% to 40% of patients on HAART experience immunological discordance (Umar et al., 2020).

1.7 1 Factors Associated with Immunological Discordance

Identifying the factors associated with virologic-immunologic discordant is important. Virologic-immunologic discordancy in HIV-infected patients is linked to younger age, potential adherence issues, WHO clinical stage III and IV, opportunistic infections, lower baseline CD4 cell count (50-100/ μ L), higher baseline viral load (100,000 copies/ml), low-level viral rebound during the first year after achieving undetectable viral load (Hailu et al., 2021).

HAART composed of protease inhibitor (PI)-based regimens constructed of a protease inhibitor combined with NRTIs, have been reported to be associated with a higher risk of virologic-immunologic discordance compared to NNRTI-based regimens (Collazos et al., 2007). In addition, triple NRTI regimens, are associated with lower virologic efficacy and higher rates of treatment failure which increases the risk of virologic-virologic discordance (Landman et al., 2003). A regimen including saquinavir, use of an NRTI based regimen constructed with 3TC/ZDV, ddI/3TC, or ddI/stavudine as well as a ritonavir-boosted protease inhibitor-(PI) based regimen, and treatment compliance are associated with virologic-immunologic discordance (Kumar et al., 2012).

HIV infected individuals with virologic-immunologic discordance are at an increased risk of developing AIDS-related infections, and non-AIDS-related illnesses and resulting in higher mortality (Kacker et al., 2023). Changes of the ART regimen to prevent disease progression and enhance CD4 count increases in patients with virologic-immunologic discordance has been a strategy that has been employed (Zoufaly et al., 2010). Whilst the exact pathogenesis of virologic-immunologic discordance is still not fully understood, the discordance is postulated to be a result of failed immune reconstitution or the excessive destruction of CD4 T cell counts.

1.8 Research Project Background

The concordant response of HIV viral suppression with immunological reconstitution following initiation of cART is the expectation. Virologic-immunologic discordance is established when HIV viral load test results do not correspond to expected CD4 cell T cell count results and this discordance is associated with poor clinical outcomes. Up to 30% of patients on cART are reported to experience virologic-immunologic discordant response. (Gazzolla et al., 2009; Kaufmann et al., 2005; Moore et al., 2005; Falster et al., 2009).

1.8.1 Problem Statement and Significance of Study

The pathogenesis of the discordance in immune response remains unclear. It is thought to depend on the interaction of viral, host and treatment-related factors (Nicastri E et al., 2005; Moore et al., 2005; Kaufman et al., 2005). The concern over individuals with virologic-immunologic discordant response is the increased risk of developing AIDS defining events (Moore et al 2005; Lapadula et al., 2015). HIV related morbidity and mortality of patients with virologic-immunologic discordant response results from mainly acquisition or reactivation of opportunistic infections (Balkhair et al., 2012).

Risk factors associated with virologic-immunologic discordance indicate that older age and low baseline CD4 T cell count are important factors which may delay the immunological recovery (Molina-Pinelo et al., 2005). Identification of factors associated with poor immunological recovery is therefore of importance. There is also paucity data on the prevalence of virologic-immunologic discordant response in children as well as factors associated with discordant immune responses in the paediatric population in South Africa.

In this study, we therefore aim to investigate the prevalence and factors associated with the virologic-immunologic discordant responses in HIV-infected children who are on cART for at least 6 months post initiation of cART. The expected outcome of this study will provide evidence-based information to the Department of Health in South Africa, healthcare providers and policymakers in monitoring, improving treatment, policymaking and guidelines for the management of HIV-1/2 patients experiencing virological-immunological discordance.

1. 9. AIM AND OBJECTIVES

1.9.1. Aim

To assess the prevalence of virologic-immunologic discordant responses and associated risk factors in HIV-1 perinatally infected children on combination antiretroviral therapy (cART).

1.9.2 Objectives

To describe the proportion of children with an HIV viral load < 50 copies/ml and a CD4 T cell count increase of < 50 cells/uL, 6 month's post initiation of cART;

To describe a proportion of children who despite viremia at 6 months post initiation of cART, (HIV VL > 1000 copies/ml), display CD4 T cell count increases of > 50 cells/uL;

To determine the association of the different antiretroviral regimens with the different discordant virologic-immunologic outcomes;

To determine demographic and clinical factors associated with virologic-immunologic discordance among children on cART

CHAPTER 2: MATERIALS AND METHODS

2.1 Study Design

The study is a retrospective design intended to review demographic and HIV monitoring data (CD4 T cell counts and HIV viral loads) extracted from the National Health Laboratory Central Datawarehouse (CDW) from children initiated on combination antiretroviral therapy from 1st January 2004 to December 30th 2022.

2.2 Area of Study

2.2.1 Study Setting and Population

Gauteng province has approximately 16.1 million people (Statistics South Africa, 2019). Geographically, Gauteng province is bordered to the south by the Vaal river, Free State, to the west by the North West Province, to the north by the Limpopo Province and to the east by Mpumalanga Province.

The study involved data from patients managed for HIV in the public sector hospitals in the Gauteng province. The three main hospitals with less missing data sets from the NHLS CDW were Charlotte Maxeke Academic Hospital in Johannesburg, Steve Biko Hospital in Tswane and Dr George Mukhari Academic Hospital in Garankuwa – and as such were selected for inclusion into the study.

Steve Biko Academic Hospital is a tertiary health institution and a main teaching hospital of the University of Pretoria, alongside Kalafong Hospital in Atteridgeville and has an 832 bed capacity. Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), is an academic quaternary hospital located in the Johannesburg District.

Dr George Mukhari Hospital, also an academic hospital is situated 20 km near Pretoria north near the township of Ga-Rankuwa, and has a 1650 bed capacity. The National Health Laboratory Service (NHLS) laboratories are sited in these academic hospitals and with the oversight of Pathologists, test for CD4 T cell counts, HIV viral loads as well as safety monitoring tests for patients managed in the public sector hospitals.

Clinical care involves routine monitoring of HIV viral load, CD4 and the safety panel as well as managing various opportunistic infections patients present with. The three NHLS

laboratories use a number of automated platforms for both HIV viral load and CD4 T cell tests counts. As per NDoH guidelines, virological failure, defined as a viral load > 1000 copies/ml, should typically trigger working up the patient for adherence and repeating the viral load (NDoH 2015).

HIV-1/2 positive paediatric patients, who are viremic, in accordance with the National ARV guidelines, continue to be monitored using HIV viral loads. HIV-1 drug resistance testing is conducted on antiretroviral experienced paediatric patients with viral loads greater than 1000 copies/ml.

2.2.2 Study Definitions

As virological suppression and undetectable viral load in HIV clinical practice are used interchangeably, in our study a child that has an HIV viral load that was undetectable (< 50 copies/ml), was categorized as virally suppressed. According to the South African HIV guidelines, a patient is virologically suppressed when the HIV viral load is below 50 copies/ml. Virologic-immunologic discordance on the other hand, was described as an HIV-1 viral load that is undetectable but a CD4 count increase is < 50 cells/ μ l 6 months post initiation of cART. The other pattern of discordance we have not studied was described as a CD4 cell count of > 50 cells/ μ l despite a display of viremia at 6 months post initiation of cART, (HIV VL > 1000 copies/ml). For the study, low-level viremia was defined as viral loads between 51 and 999 copies/ml.

2.3 Eligibility Criteria

2.3.1 Inclusion criteria 2.3.1.1 Inclusion criteria

Children ≤ 17 years of age managed at an HIV public sector clinic in Gauteng;

Children with follow-up CD4 counts and HIV viral load data from initiation to 6 months post initiation.

2.3.1.2 Exclusion criteria

Follow-up period < 6 months.

Incomplete or missing medical records.

2.4 Sample Size Determination

To determine an appropriate sample size that would enable an accurate assessment of virologic immunologic discordance, the following formula was used:

$$N = Z^2 * P (1-P) / E^2 \quad (\text{Pourhoseingholi et al., 2013}) \text{ where}$$

N=minimum sample size

Z is a measure of the level of confidence. A value of 1.96 which reflects a 95% confidence interval was used;

P: is the estimated prevalence within the target population. The indicator of interest is the estimated prevalence of virologic-immunologic discordancy in HIV infected children on cART 6 months following initiation. P value of 30% was estimated from previous studies

published in literature with prevalence ranging from 24-30% in sub-Saharan Africa. (Hirigo AT et al., 2015; Anude CJ et al., 2013; Muza BP et al., 2012)

E is the margin of error. A recommended value of 0.05 was used.

Therefore sample size (N) = $1.96 \times 1.96 \times 0.3 (1-0.3) / 0.05 \times 0.05$

N=323.

2.5 Data Collection

Data were obtained by reviewing an Excel spreadsheet containing data on HIV infected paediatric outpatients who had been managed at HIV public sector clinics in Gauteng from 1st January 2004 to December 30th 2022. Demographic variables collected from CDW were age, gender as well as nadir CD4 T cell count and HIV viral load from CDW data. Baseline CD4 T cell counts and HIV viral loads and 6 monthly follow up CD4 T cell counts and HIV viral loads were also extracted. Prior HREC approved clinical data drawn from patient clinic files were used to support date of cART initiation as well as whether the patient was TB culture positive or negative and whether they were placed on antituberculous therapy concurrently with cART. Height and weight were not extracted from clinic files. Prior approved clinical data had information on whether the paediatric patient was positive for TB or not without indicating which TB assay had been conducted. However, testing for TB was furnished in CDW data and only culture and sensitivity data was used to indicate TB diagnosis.

2.6 Data Analysis

A descriptive analysis was conducted and categorical variables were summarized and expressed as medians and interquartile ranges or means with standard deviations. For the comparison of categorical variables, chi-square or Fisher's exact test was used. The independent t-tests or Mann-Whitney U test were utilized to compare averages between groups. For three or more groups ANOVA or Kruskal Wallis was used as appropriate. Variables with p values < 0.05 were considered statistically significant. Univariate and multivariate logistic regression analyses were used to examine the association of independent variables while logistic regression analysis was performed for both categorical and continuous variables. A p< 0.2 was included in the multivariable model

(Anude et al., 2013) using the entered model, and multivariate odds ratios (ORs) and respective 95% confidence intervals (CIs) were obtained.

2.7 Ethical Consideration

Permission from the National Health Laboratory Service (NHLS), Academic Affairs and Research Management System (AARMS) was obtained to access data on paediatric children infected with HIV on cART at least for six months from 1st of January 2004 to 31st of December 2022. An ethical clearance certificate was obtained from the Wits Human Research Ethics Committee (HREC; Reference number M230264 MED23-02028) upon the submission of the NHLS AARMS approval to use the data.

2.8 Data Confidentiality

Each episode number obtained from the National Health Laboratory Service (NHLS), Central Data Warehouse (CDW) was assigned a study number and the information linking the two was stored securely and separately. The data was reviewed and analysed by the researcher and supervisor only. Data was stored on a password protected laptop.

CHAPTER 3: RESULTS

3.1 HIV monitoring data for immunologic-virologic discordance

Of 2747 HIV-positive perinatally infected children initiated on combination antiretroviral therapy (cART) from 2004 to 2017, 620 children with HIV monitoring data missing were excluded from the analysis. Missing data ranged from gender, age, nadir CD4 T cell count and 6 monthly CD4 T cell count. An additional 380 children with HIV virological failure (viral load > 1000 copies/ml) at 6 month's follow-up after initiation with cART were also excluded, yielding 1 747 records for analysis (**Figure 5**).

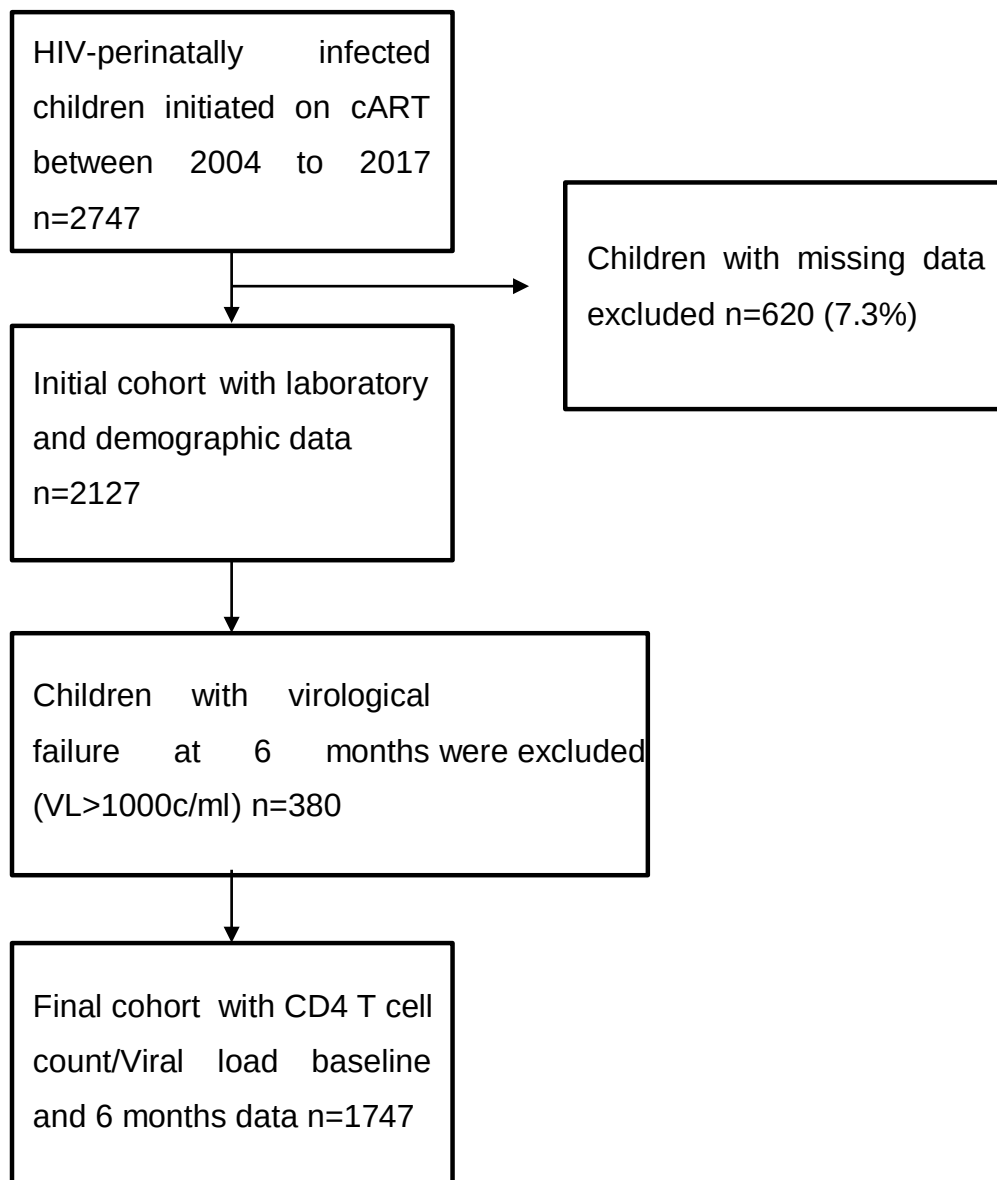


Figure 5: Flow chart of HIV monitoring data selected for immunologic-virologic discordance.

3.2 Baseline Demographic and Clinical Characteristics

Of the initial cohort of 1747 children, 895 (51.3%) were female and 852 (49.0%) male. Fifty nine percent of the children at the time of combination antiretroviral therapy (cART) initiation were aged <5 years (n=1037; 59.3%) Seventy-six percent of the children presented with a clinical WHO stage III/IV disease. The median (interquartile range (IQR) baseline median (IQR) CD4% T cell count of 474.0 cells/ μ l (217.5 – 919.5). A recent history of a diagnosis of tuberculosis or concurrent use of antituberculous therapy at initiation of cART was reported in approximately a third of the children in the cohort (n=554; 33.7%). Baseline and clinical characteristics are summarised in Tables 1.

Table 1: Baseline characteristics of HIV-1 perinatally infected children at initiation of cART

Baseline Characteristics	All children (N=1747)
Gender females, n(%)	895 (51.2)
Males,n(%)	852(49.0)
Age at cART initiation (years), median IQR	
Age categories (years), n(%)	
0 – 3	736 (42.1)
3.1 -5	301 (17.2)
5.1 -10	514 (29.4)
10.1 – 17	128 (7.3)
Unknown	68 (3.9)
WHO stage	
I	114 (6.5)
II	298 (17.0)
III	935 (53.5)
IV	400 (22.9)
TB treatment history, n (%)	
Yes	554 (31.7)
No	1193 (68.3)
Baseline CD4 T cell count (cells/ μ l), median IQR	
CD4 T cell count in categories (cells/ μ l), n (%)	
<50	202 (11%)

101 – 200	210 (12%)
201 – 500	363 (21%)
501 – 1000	350 (20.3%)
>1000	289 (16.3%)
Baseline CD4 T cell percentage (cells/uL), median IQR	
CD4 T cell count in categories (cells/uL), n (%)	
0 – 10	548 (31.4%)
11 – 20	635 (36.3%)
21 – 30	346 (20%)
31- 50	218 (12.5%)
cART=combination antiretroviral therapy. IQR=interquartile range; WHO=World Health Organisation	

A state of significant immunocompromise requiring prophylactic cover was observed for in 46% of the children where CD4 T cells < 50 cells/ul, 50 – 100 cells/ul and 101 to 200 cells/ul were 11%, 19% and 12% respectively. Whilst opportunistic infections would be expected in children with CD4 T cell count < 200 cells/ul, this clinical information was not available.

Of the 1747 children, 554 (32%) were on antituberculous therapy at the time of initiation and 22.4% of these children had CD4+ T cell count < 200 cells/mm³ (n=267; 63.1%) In the severely immunocompromised group with CD4 T cell count < 50 cells/ul when pulmonary or extrapulmonary tuberculosis was detected, the median (IQR) CD4 T cell absolute count was 14 (8 – 37) and the corresponding median (IQR) CD4 T cell of 1.35% (0.68 – 2.40. cells/ul. As per National Paediatric Antiretroviral Guidelines, the year of initiation of cART influenced the NRTI backbone the children were placed on. Although

the total number of the cohort is 1747, 193 children had no regimen assigned and regimen was documented as unknown. Of the 802 (51.6%) children with documented regimen and initiated from 2004 to 2009, the majority (n=794; 99%) were on a stavudine (d4T) or zidovudine (AZT) based NRTI backbone, with 53% (n=424), 36% (n=286), 9% (n=71) and 1.6% (n=13) on efavirenz, boosted lopinavir, nevirapine and unboosted ritonavir as third antiretroviral agents respectively. Of the 802, eight children (1.0%) were on AZT+ddI NRTI backbone, traditionally reserved for as second line regimen. No documentation was furnished whether these children were transferred in and placed on second line or whether toxicity challenges necessitated placement on this regimen.

Seven hundred and fifty-two children were initiated from 2010 to 2017. The majority of these remaining 752 were initiated on an abacavir (ABC) based regimen (n=619; 82%), with 57% (n=351), 42% (n=262), 1% (n=6) on efavirenz, boosted lopinavir and nevirapine respectively.

3.3 Virological Response

Of the 1747 initiated on cART from 2004 to 2017, the majority (n=1225; 70.1%) had undetectable viral loads (less than 50 copies/mL) at month six post initiation. The majority of patients were not only were virally suppressed but had evidence of robust T cell reconstitution of > 350 cells/ μ L. Whereas the proportion of individuals with CD4 T cell counts <100 cells/ μ L was 31% at baseline, this proportion decreased to only 6% by week 6 months following use of cART.

Two hundred and sixty- five children (15.2%) had evidence of virological failure at 6 months, with a median viral load 4.3 (3.62 – 5.04) $\log_{10}$. Of the 265 children with virological failure, 22% (58/265) of the children had HIV viral loads higher than 5 $\log_{10}$, raising the possibility despite being documented as having been initiated on cART, antiretroviral therapy may not have been initiated.

Low level viremia (HIV viral loads between 51 and 999 copies/ml), were also observed, in 185 (10.6%) children, with a median viral load of 2.41 (2.195 – 2.675) $\log_{10}$. The

remaining 72 were HIV-1 infected children with HIV viral loads below the limit of detection but failed to display evidence of immunological reconstitution.

3.4 Immunological Responses

The mean CD4 T cell counts at after initiation on cART from baseline was calculated and the mean CD4+ cell count at baseline was 474 cells/ μ L. This increased to 659 cells/ μ L at 6 months. The calculated virologic-immunologic discordance for this cohort was 5.6% (n = 72).

3.5 Virologic-Immunologic Discordance

Seventy - two children in our cohort were considered to be having a paradoxical response and this total of 1225 children on cART who had HIV viral loads below the limit of detection, the prevalence of those with discordant virologic-immunologic responses in this cohort was 6%.

Table 2 displays characteristics of children with the expected outcomes of an HIV viral load below the limit at 6 months and adequate immunological reconstitution post initiation of cART (n=1225) and those with a paradoxical response, wherein the HIV viral load is below the limit of detection and yet immunological reconstitution is suboptimal (n=72).

Table 2: shows the descriptive characteristics associated with virologic-immunologic discordant and virologic-immunologic concordant responses in HIV-infected children.

Characteristics	Virologic-Immunologic Discordant Responses		Virologic-Immunologic Concordant Responses		P value
	N=72	%	N=1225	%	
Gender					0.044
Female	32	44.4	383	68.75	
Male	40	55.6	366	63.10	
Age (years)					0.264
<5	44	61.0	116	72.05	

6-10	23	32.0	260	67.18	
>10	5	7.0			
WHO staging					0.165
I	2	2.8	59	74.68	
II	15	21.0	128	65.15	
III	48	66.7	404	63.72	
IV	7	9.7	148	69.19	
Baseline CD4 cell count (cells/μl)					0.330
<50	4	5.6	73	70.19	
>50	68	94.4	676	65.44	
TB Co-Infection					0.799
TB	26	36.1	284	69.10	
No TB	46	63.9	3	75.0	
Regimen					0.048
NRTI backbone					
ABC, 3TC	19		249	60.88	
AZT, 3TC	15		134	69.07	
d4T, 3TC	38				
NNRTI as third agent					
EFV	58		363	68.85	
NVP	3		2	100	
PI as third agent					
Kaletra	11				

*p<.05 chi-square or Fisher's exact test, as appropriate.

Table 3: Factors Associated with Adequate Immunological Response`

Variable	Univariate Analysis			Multivariate analysis		
	OR	P value	CI	aOR	P value	CI
Male	1.0	1.0	1.0	-	-	-
Female	0.120	0.354	0.88- 1.41	0.312	0.38- 0.44	0.88
Age						
0-9yrs	1.0	1.0	1.0	1.0	1.0	1.0
10-17yrs	1.686	0.031	1.04- 2.71	0.513	0.039	0.027- 0.99
WHO staging						
I	1.0	1.0	1.0			
II	0.935	0.807	0.55- 1.60	1.10	0.942	0.09- 14.2
III	1.810	0.330	0.73- 2.851	1.05	0.953	0.23- 4.80
IV	1.252	0.572	0.415	0.010	0.986	1.31- 1.11
Baseline CD4 T cell count						
<50 cells/ µl	1.0	1.0	1.0	-	-	-
>50/cells/ µl	0.238	0.000	0.127- 0.448	-	-	-
No TB	1.0	1.0	1.0			

TB	0.620	0.663	0.07- 5.35	-	-	-
Regimen						
ABC/3TC	1.0	1.0	1.0	-	-	-
AZT/3TC	1.082	0.707	0.763- 1.27	-	-	-
D4T/3TC	1.054	1.443	0.72- 1.42	-	-	-

Factors associated with virologic-immunologic discordance were older age (10 – 17 years of age), WHO stages III and IV as well as low CD4 T cell counts.

CHAPTER 4: DISCUSSION

We investigated the prevalence and factors associated with the virologic-immunologic discordant responses in HIV-infected children at 6 month's post initiation of cART. A widely accepted clinical expectation is that initiation of cART generally will lead to a rapid drop in HIV-1 viral load and a subsequent increase in peripheral CD4 T cell count (Gulick et al., 1997., Hammer et al., 1997; Montaner et al., 1998). Placement of HIV infected individuals on cART in some HIV infected people results in HIV viral suppression, with small increases in CD4 T cell counts that deviate from the expected CD4 T cell kinetics. The phenomenon has been well described in adults, however, there is paucity of data of virologic-immunologic discordant responses in HIV-1 infected children. Our study set out therefore to focus on the paediatric population – in children with HIV-1 viral loads below the limit of detection at month 6 following initiation of cART but with the CD4 T cell count responses which remained blunted.

The HIV-1 viral load continues to be the key monitoring parameter used for determining virological suppression. Achieving virologic suppression is necessary to effect robust CD4 T cell count responses (Yeni et al., 2004; Wood et al., 2004). From our initial paediatric cohort of 1747, we reported virological failure rates of 15%. This is in keeping with findings from a large paediatric cohort (n=2234) managed in a South African public sector, where the incidence rate of virological failure was reported to be 18.5 per 100 persons-years follow up (Makatini et al., 2021). Lower virological failure rates of between 6 – 16% have also been reported in the South African public sector setting (Pillay et al., 2008; Orrel et al., 2013; Reddy et al., 2007; Barth et al 2020). In our cohort, low level viremia was observed in 10.6% (n=185) of the children. Failure of complete viral suppression remains a concern moreso with HIV drug resistance mutation emergence reported at these low viral loads (Swenson et al., 2014).

Complete virological suppression was observed in 1297 (74%) of the children. Of note is that whilst 1225 of these children displayed adequate CD4 T cell reconstitution as is the expectation, 72 remained virally suppressed but had inadequate CD4 T cell count increases – a paradoxical response we categorised as virologic-immunologic discordant response.

We therefore report findings of virologic-immunologic discordance of 6% in a large paediatric cohort managed for HIV-1 infection in South African public sector clinics and with monitoring data from the National Health Laboratory Service (NHLS). Our outcomes are consistent with findings from a number of studies conducted in Brazil, 9%, 9.5%, 11.5% and 15.4% conducted in Brazil, Northwest Ethiopia, Tigray Ethiopia and British Columbia respectively (Casotti et al., 2011; Kiros et al., 2022; Hailu et al., 2021; Moore et al., 2005).

Our results, however, are lower than findings virologic-immunologic discordance reported elsewhere in sub-Saharan Africa. Prevalence rates of 17%, 21%, 29%, 33% and 50.2% (Gunda et al., 2017) respectively have been reported (Grabar et al., 2000; Tuboi et al., 2007; Hirigo et al., 2015, Anude et al., 2013, Gunda et al., 2017). Three studies conducted in South Africa reported immunological discordance of 24%, 28% and 37% in HIV virally suppressed South African adults (Muzah et al., 2012; Edet et al., 2019; Julg et al., 2012) respectively. Muzah et al reported on a cohort of 810 adults residing in Johannesburg (Gauteng province) and found immunological discordant rates of 24%. Julg et al on the other hand reported on a cohort of 442 adults residing in Durban (KwaZulu Natal province), with 37% of participants failing to display immunological recovery of counts ≥ 200 cells/ μ l after 12 months post initiation of cART.

It is noteworthy that our study was conducted in a large paediatric population when all other studies cited were in the adult population. There are notable variations observed in prevalence rates and we propose that these variations could be due to differences in the definition of virologic-immunologic discordancy – where in almost all studies cited, the CD4 T cell threshold for discordance were based on CD4 T cell count incremental changes over time. We on the other hand adopted the National Institute for Health guidelines definition, and defined virologic-immunologic discordance as HIV-1 viral load < 50 copies/ml and a CD4 T cell count increase of < 50 cells/ μ l 6 month's post initiation of cART (DHHS 2015).

Despite the concern over prevalence variations, the clinical relevance to be derived from these studies remain similar in that patients with poor immune recovery are at risk of developing opportunistic infections and untimely death even with complete virological suppression (Garcia et al., 2007; Baker et al., 2008; Kaufmann et al., 2005).

The mechanisms involved in the failure of immune reconstitution has not been completely understood. A blunted CD4 T cell response despite HIV viral suppression has been hypothesized to be a result of impaired thymus (Ribeiro et al., 2008; Teixeira et al., 2001) and the possibility of an association with genetic factors is yet another consideration (Rauch et al., 2008). In addition, downregulation of interleukin-7 has been associated with discordant virologic-immunologic discordant responses – with an increase in CD4 T cell counts evidenced in patients who were treated experimentally with IL-7 in a phase I/IIa clinical trial study (Yang et al., 2020).

There are numerous different evidence based associations regarding the relevance of possible risk factors of virologic-immunologic discordance such as advanced age at the beginning of cART (Florence et al., 2003; Grabar et al, 2004; Gras et al; 2007; Huttner et al., 2007; Hanna et al 2008; Ledermann et al 2000; Moore et al., 2005; Nicastrì et al., 2005; Piketty et al., 2001; Tuboi et al., 2007), low, nadir CD4 T cell count (Florence et al., 2003), cART regimen (Moore et al., 2005; Wood et al. 2004) and, the presence of associated conditions (Grabar et al., 2004; Sachdeva et al., 2008). In terms of gender in our study, whilst there was an overall predominance of females (n=895; 51.3%) in the initial overall cohort of 1747 children, 55% (n=40/72) of males predominated the virologic immunologic sub-cohort. We found no significant differences of inadequate CD4 T cell count reconstitution between males and females. This is in keeping with studies reporting similar findings (Moore et al., 2003; Nicastrì et al 2005). There are however, reports of females displaying better immunological reconstitution compared to males (Collazos et al; 2007; Zaragoza-Maias et al 2010; Barber et al., 2011; Maskew et al., 2013) including females having higher mean CD4 T cell counts from baseline to 24 months when compared to males. The data on gender differences in trends of CD4 T cell count after commencement of cART remain inconsistent.

The debate on the effect of age on immune restoration in HIV-infected patients still persists. A number of studies have addressed the association of age with virologic immunologic discordance, supporting the evidence that CD4 T cell reconstitution is age dependent (Florence et al., 2003; Stuart et al., 2002) and that the increase in age was associated with a reduced chance to increase CD4 T cell counts (Falster et al., 2009). Other studies demonstrated that age had no significant impact on CD4 T cell recovery in HIV-infected patients (Ribeiro et al., 2008). Evidence from our study supports the evidence that increase in age at the time of diagnosis, is associated with immunological discordance - with older children aged ≥ 10 to 17 years in our cohort being 1.68 times at more risk of developing immunological discordance as compared to the younger age groups [$p = 0.031$; 95%CI (1.04–2.71)]. Whilst the slower CD4 T cell reconstitution observed in older patients may be related to an impaired thymic function (Douek et al., 1998), a preserved thymus function in younger age groups accounts for the improved CD4T cell restoration (Viard et al., 2001).

From clinical staging at first visit, fifty-five (76.4%) HIV-1 infected children in our cohort categorised as displaying immunological discordance and who were WHO stage III/IV, were 1.810 times more likely to have inadequate immunological reconstitution following initiation of cART compared to those in WHO stage 1 and 2 (OR = 1.810, $p = 0.067$). Similar findings supporting the evidence that patients in advanced HIV stage had poor immunological reconstitution compared to those in early stage of the disease were reported. Overall, these findings suggest that patients who present late and with severe immune suppression, need a much closer clinical follow up to improve their outcome (Kaufmann et al, 2002).

Baseline CD4 T cell counts are a robust predictor of likely CD4 T cell count recovery. Low CD4 T cell counts represent a severe immune destruction and patients who start ART at lower baseline CD4 T cell counts below 200 cells/ μ l, have been shown to have a poor immunological reconstitution (Le Moing et al., 2007; Kaufmann et al., 2005, Moore et al 2007). From our analysis, immunological discordant responses were observed to be

associated with low CD4 T cell count (< 100 cells/ μ l) at baseline ($p = 0.07$). Studies conducted in Nigeria (Anude et al., 2013), Oman (Ali et al., 2019), India

(Prabhakar et al., 2011) have reported similar findings. In addition a study by Hunt et al demonstrated that only about 21–49% of patients who were initiated on cART at CD4 T cell counts of <200 cells/ μ l achieved CD4 T cell counts of 350–500 cells/ μ l at 4 years while more than 93% of those who initiated HAART at higher CD4 T cell count >200 to 350 cells/ μ l attained CD4 T cell counts >350 to 500 cells/ μ l at four years (Hunt et al., 2003) This study adds more support to the value of starting cART at higher thresholds and with “Test and Treat” strategy, the National Department of Health Guidelines have addressed the challenge of delaying initiation of cART until CD4 T cells have dropped.

We also reviewed the association of NRTI backbones with a discordant immunological response. In our study, in keeping with National paediatric guidelines, HIV infected children initiated between 2004 and 2010, were placed on stavudine (d4T) or zidovudine (AZT) with 3TC NRTI backbone. From 2010, d4T was replaced with abacavir. Nucleoside reverse transcriptase inhibitors (NRTI) backbones were not found to be associated with virologic-immunologic discordance, although a significant association with zidovudine-lamivudine-based regimens was evidenced. The use of zidovudine or didanosine as part of the NRTI backbone, has been associated with inadequate CD4 T cell responses despite suppression of viral replication (DHHS 2005). Although there are no published controlled studies evaluating the impact of different antiretroviral regimens on the incidence of discordant responses, there are data to indicate that the degree of impairment in viral replicative capacity in virus isolated from patients with discordant responses may depend on the type of antiretroviral regimen being used (Linden et al., 2003). For clinical management of patients, to date, there is no published data to support switching of cART regimen in patients with immunological discordance.

Our final finding was that co-infection with tuberculosis (TB) was not a significant factor for discordant virological response to cART. The findings are again consistent with studies conducted in other settings which failed to confirm the association with tuberculosis (Karo et al., 2017; Saila et al., 2021).

4.1 Conclusion

In our cohort of HIV-1 infected children, 71% had achieved viral suppression, had evidence of robust T cell reconstitution of > 350 cells/ μL . However, 6% displayed virologic-immunologic discordance as they failed to achieve adequate immunological reconstitution – placing them at high risk of disease progression and death from AIDS and non-AIDS events. In the public sector setting, failure to achieve adequate immunological reconstitution despite HIV viral suppression must be addressed to allow for an improved and rational approach to the clinical management of HIV-1 infected children with discordant responses to cART.

4.2 Strengths and Limitations:

The strength of our study is that it is based on a paediatric population and whilst the study may be limited by the relatively small number of patients, it provides insight into risks factors for immunological discordance among HIV infected children.

We also recognize some limitations in our study. The study is retrospective in nature and invariably has a number of variables missing. Data was retrieved from the NHLS CDW, encompassing only data from only three public sector clinics in Gauteng. A significant number of the data sets advanced from the NHLS had no baseline CD4 T cell counts and consequently, the resulting data set is relatively small and may impact the statistical power to discriminate the effects of tested predictors. In addition, the findings may therefore not be generalizable to the general population.

Adherence to cART was not assessed in this study and as such children with poor adherence could have been included in the sub-cohort with a discordant immunological response. In addition, the overall study cohort did include children who children who displayed low level viremia and who could have had adherence challenges and these children were not included in the discordant sub-cohort.

CD4 T cell count measurements in public sector clinics are generally undertaken approximately six months apart. However, many of the children had CD4 T cell counts

taken three monthly and It is therefore possible that this may have affected the classification given the fluctuation of CD4 T cell measurements.

Finally, the national antiretroviral guided changes in the NRTI backbone as a result of the availability of newer less toxic antiretroviral agents over the study period may be regarded as a limitation.

CHAPTER 5: REFERENCES

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CHAPTER 6: APPENDICES.

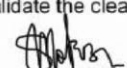
6.1 Appendices.

Appendix 1: Data collection sheet.

Appendix 1: Data Collection Sheet

Demographic Data				
	Gender			
	Height			
	DOB			
Clinical Information	WHO Stage	Clinical condition	☐	
		Treatment	☐	
Nadir CD4 T cell count				
Baseline Viral Load				
Regimen initiated				
D4T+ 3TC& EFV or LPV/r				
ABC+ 3TC &EFV or LPV/r				
Other regimen				
Baseline Safety Panel				
Six monthly follow up	CD4 T cell count	HIV VL	Hb	ALT
Six monthly follow up	CD4 T cell count	HIV VL	Hb	ALT
Six monthly follow up	CD4 T cell count	HIV VL	Hb	ALT

Appendix 2: Ethics clearance certificate.

	
UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	
R14/49 Mr Nelson Chinyanga	
HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)	
<u>CLEARANCE CERTIFICATE NO. M230264 MED23-02-028</u>	
<u>NAME:</u>	Mr Nelson Chinyanga
<u>(Principal Investigator)</u>	
<u>DEPARTMENT:</u>	Virology National Health Laboratory Service
<u>DEGREE:</u>	MSc Virology
<u>PROJECT TITLE:</u>	<i>Evaluation of virologic-immunologic responses in HIV-1 perinatally infected children at public sector clinics in South Africa</i>
<u>DATE CONSIDERED:</u>	24/02/2023
<u>DECISION:</u>	Approved unconditionally
<u>CONDITIONS:</u>	
<u>NOTE:</u>	If contact information regarding student study participants is required, please contact the Registrar's office - Nicoleen.Potgieter@wits.ac.za
<u>SUPERVISOR:</u>	Dr Z. Makatini and Miss H. Mthiyane
<u>APPROVED BY:</u>	 _____ Professor Paul Ruff, Chairperson, HREC (Medical)
<u>DATE OF APPROVAL:</u>	13/11/2023
<u>EXPIRY DATE:</u>	13/11/2028
This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.	
<u>DECLARATION OF INVESTIGATORS</u>	
To be completed in duplicate and ONE COPY returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. I agree to submit a yearly progress report. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in February and will therefore be due in the month of February each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).	
 _____ Principal Investigator Signature	<u>13/11/2023</u> Date

Appendix 3: AARMS NHLS approval letter.



**NATIONAL HEALTH
LABORATORY SERVICE**

Academic Affairs and Research
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Email: babatyi.kgokong@nhls.ac.za
academic.research@nhls.ac.za
Web: www.nhls.ac.za

19 September 2023

Applicant: Nelson Chinyanga
Institution: University of the Witwatersrand
E-mail Address: chinyanganelson@gmail.com
Tel: 011 489 8852 **Cell:** 078 384 0647

Project Title: EVALUATION OF VIROLOGIC-IMMUNOLOGIC RESPONSES IN HIV-1 PERINATALLY INFECTED CHILDREN AT PUBLIC SECTOR CLINICS IN SOUTH AFRICA.

Reference Number: PR2343130

Research Application Type(s):

1. Request for Data

RE: APPROVAL LETTER: REQUEST TO ACCESS NHLS RESOURCES FOR RESEARCH PURPOSES

This letter serves to advise that the application requesting permission to conduct the above-mentioned research using the listed NHLS resources has been reviewed and "Approved". Please note that the approval is granted on the condition that you comply with the NHLS Research Material and Data Access Policy and requirements stated below.

1. All material and data requested shall be used as per the research protocol submitted to the NHLS and as approved by the relevant Health Research Ethics Committee (HREC) in South Africa.
2. Access to the NHLS material and/or data shall be limited to the minimum required for successful completion of the approved study and shall be made available *without patient names and other patient identifiers (including, but not limited to, national identity numbers, hospital/clinic file numbers, addresses and telephone numbers)*.
3. Confidentiality shall be maintained at the participant and institutional level and there shall be no disclosure of personal information or confidential information.
4. Data and/or material shall not be shared with other parties unless approved by the NHLS
5. The material and/or data obtained from the NHLS shall be anonymised and not, for any reason, be used to track or recruit patients as no pre-approval/consent is obtained from patients.
6. Processes shall be discussed with the relevant NHLS departments (i.e. Corporate Data Warehouse (CDW), NHLS Laboratory Management, Operations Office, etc.) and agreed upon.
7. Any amendments to the study requirements, including the use of the material and/or data for purposes not initially disclosed to the NHLS) shall be cleared by an approved HREC and submitted to the NHLS for approval via the AARMS system – <https://aarms.nhls.ac.za>.
8. The NHLS shall be acknowledged as a source of material and/or data in any output, such as abstracts and journal articles, emanating from the project.
9. A final report of the research study and any published output resulting from this study shall be submitted to the NHLS via AARMS

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. The NHLS entities tasked with providing the material and/data may have additional requirements for access. Data related queries may be directed to NHLS CDW, email: zarina.sabat@nhls.ac.za; contact number: 011 386 6074 and sample related queries (if applicable) shall be directed to the relevant business manager.

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

