NEURODEVELOPMENTAL DELAYS IN CHILDREN WITH PERINATALLY ACQUIRED HUMAN IMMUNODEFICIENCY VIRUS INFECTION, WITH RESPECT TO ANTIRETROVIRAL THERAPY INITIATION AND VIROLOGICAL SUPPRESSION

Renate Strehlau

A research report submitted to the Faculty of Health Sciences, the University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of

Master of Science in Medicine in Child Health Neurodevelopment

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DECLARATION

I, Renate Strehlau, declare that this research report is my own work. It is being submitted for the degree Master of Science in Medicine in Child Health Neurodevelopment in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signature _____

_____ day of _____ 2013

PRESENTATIONS ARISING FROM THE THESIS

Poster presentation (Effects of antiretroviral therapy and virological suppression on neurodevelopmental delays in children with perinatally-acquired HIV infection. P66) at the South African HIV Clinicians Society Conference, 25-28 November 2012, Cape Town, South Africa.

ABSTRACT

Context: Human Immunodeficiency Virus (HIV) infection in infancy may influence the developing brain and lead to adverse neurodevelopmental consequences.

Objective: We aim to describe the neurodevelopmental characteristics of a cohort of young children infected with HIV prior to antiretroviral therapy (ART) initiation and after achieving viral suppression.

Design, setting and patients: A retrospective analysis of data collected as part of a randomised equivalence trial between April 2005 and May 2009, at a hospital in Johannesburg, South Africa.

Methods: 195 HIV-infected children under 2 years of age were assessed. A simple, inexpensive screening questionnaire (Ages and Stages Questionnaire - ASQ) was used to identify neurodevelopmental delays. The ASQ was administered prior to ART initiation, and again after viral suppression on a protease inhibitor-based regimen had been achieved.

Results: Median age pre-ART was 8.8 months (range 2.2 - 24.9), 53.9% were male. Mean time to viral suppression was 9.4 months (range 5.9 - 14.5) and the ASQ was administered to 108 caregivers at this time. Compared to pre-ART, at viral suppression, there was significant reduction in the proportion of children failing the gross motor (31.5% vs. 13%, $p<0.01$), fine motor (21.3% vs. 10.2%, $p=0.02$), problem solving (26.9% vs. 9.3%, $p<0.001$) and personal social (17.6% vs. 7.4%, $p=0.02$) domains. The proportion of children failing the communication domain was similar at each time point (14.8% vs. 12%, $p=0.61$). At time of viral suppression 10.2% failed at least one of the five domains.

Conclusion: Achieving viral suppression on ART resulted in significant improvements in the neurodevelopmental function of young HIV-infected children, however, neurodevelopmental

problems still persisted in a large proportion. Appropriate screening for neurodevelopmental delay and timely referral could help improve outcomes.

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ABBREVIATIONS

3TC	-	Lamivudine
ABC	-	Abacavir
ASQ	-	Ages and Stages Questionnaire
AIDS	-	Acquired immunodeficiency syndrome
ART	-	Antiretroviral Therapy
ARV	-	Antiretroviral
CNS	-	Central Nervous System
CSF	-	Cerebrospinal fluid
CT	-	Computed Tomography
d4T	-	Stavudine
HAART	-	Highly Active Antiretroviral Therapy
HAMB	-	HIV and AIDS Malignancy Branch
HAZ	-	Height-for-Age Z score
HI	-	Human Immunodeficiency
HIV	-	Human Immunodeficiency Virus
LPV/RTV	-	Lopinavir/ritonavir
MRI	-	Magnetic Resonance Imaging
MTCT	-	Mother to Child Transmission
NNRTI	-	Non Nucleoside Reverse Transcriptase Inhibitor
NRTI	-	Nucleoside Reverse Transcriptase Inhibitor
NVP	-	Nevirapine
PCR	-	Polymerase Chain Reaction
PHE	-	Progressive HIV-encephalopathy

PI	-	Protease Inhibitor
PMTCT	-	Prevention of Mother to Child Transmission
RNA	-	Ribonucleic acid
SSA	-	Sub-Saharan Africa
RMMCH	-	Rahima Moosa Mother and Child Hospital
SD	-	Standard Deviation
UNAIDS	-	United Nations programme on HIV/AIDS
VL	-	Viral Load
WAZ	-	Weight-for-age Z Score
WHO	-	World Health Organization
WHZ	-	Weight-for-height Z Score
AZT	-	Zidovudine

CHAPTER 1

INTRODUCTION

1.1. Background

The World Health Organization (WHO) estimates that 3.4 million children under the age of 15 years, were living with the Human Immunodeficiency Virus (HIV) in 2011, and of these children 91% (3.1 million) were found to reside in Sub-Saharan Africa (SSA)(1, 2) The paediatric HIV epidemic in Southern Africa has greatly contributed to the burden of childhood disease in the region.

HIV is a neurotropic virus that enters the central nervous system (CNS) through alteration of the blood-brain-barrier, involving a complex cascade of events.(3) Direct viral invasion of the vulnerable developing brain gives rise to one of the most serious consequences of HIV disease, namely neurological damage;(4) with greatest risk in those with early foetal or neonatal infection.(5) Neurodevelopmental consequences of CNS disease have been well documented from early on in the epidemic, with a prevalence of 30 - 50% reported in antiretroviral treatment-naïve children.(6, 7)

Infection with the HIV can have devastating effects on the CNS. The clinical manifestations and severity of paediatric HIV-related CNS disease cover a broad spectrum of disorders. Infants and children may present with static or progressive encephalopathy, CNS compromise or no apparent CNS effects.(8) Children with static encephalopathy continue to acquire new skills, but function

consistently below the mean on standardised assessments,(9) while those with progressive encephalopathy continue to show decline in their level of functioning through the loss of acquired skills.(10) Possible developmental shortfalls include delays in motor, cognitive, and language development.(11) However, children with HIV-related CNS disease may present with a wide variety of neurologic complications that do not easily fit into simplified classification systems.(12)

HIV-infected infants have been shown to have more abnormal cognitive and motor neurodevelopmental outcomes than HIV-exposed, but uninfected infants.(13) Prevalence and degree of neurological deficit are increased relative to the degree of immunosuppression and stage of HIV disease.(14) Furthermore, HIV-affected families are frequently burdened by poverty and malnutrition which are known to exacerbate developmental delays and further increase risk of compromised brain development. (15, 16)

Antiretroviral therapy (ART) has been shown to alter the natural history of neurological outcomes of paediatric HIV infection. Early initiation of triple ART has been associated with delaying the onset of progressive encephalopathy as well as improving outcomes in children already presenting with CNS dysfunction.(17, 18) Cerebrospinal fluid antiretroviral (ARV) concentrations differ depending on the effectiveness of CNS penetration of individual drugs.(19) Drugs with higher CNS concentrations, such as abacavir (ABC) and zidovudine (AZT), have been shown to be more beneficial in preventing, or reversing, neurological damage caused by HIV.(20)

Childhood developmental screening tools are most likely under utilised in over-burdened, under-staffed clinics and treatment facilities in South Africa. However, young children infected with HIV have shown significant improvements in cognitive and motor development after being screened for

the presence of neurodevelopmental delay, and provided with a basic, locally developed, home management programme.(21)

The Ages and Stages Questionnaire (ASQ), second edition, is designed to be an inexpensive, easy to use screening questionnaire used to identify and monitor neurodevelopmental delays in young children. Completed at prescribed age intervals, the questionnaires are divided into five domains – Communication, Gross Motor, Fine Motor, Problem Solving, and Personal-Social. This screening test is published with reliability and validity data comparable to other childhood screening tests.(22) The ASQ can be completed in less than 10 minutes by any staff member of the clinical team with minimal orientation to this tool, and could, therefore, be a useful developmental screening tool in a busy paediatric HIV treatment facility or primary healthcare facility. A small preliminary South African study found the Afrikaans translated ASQ to provide a reliable assessment of development in urban, middle-class Afrikaans-speaking children. There is, however, no normative data available for the test when translated into other South African languages e.g. isiZulu, and conducted on rural or HIV-infected populations.(23)

In summary, HIV is a neurotropic virus which can cause severe damage to the developing brain. ART has been shown to alter the natural history of neurological outcomes in paediatric HIV infection, with early ART initiation delaying the onset of encephalopathy, as well as improving outcomes of infants already displaying signs of neurological compromise. Even though access to paediatric HIV treatment services in South Africa has greatly improved, the variable individual course of HIV infection, and the large numbers of children infected, make neurodevelopmental outcomes in these HIV-infected children a priority concern. Comprehensive HIV care should include developmental screening and regular assessments, so as to identify infants and young children with developmental delays and to institute timely interventions in an attempt to arrest

further neurological fallout. Children with persistent developmental delay and/or abnormal neurological signs require judicious referral to child development services so as to maximise their long-term developmental and educational progress.

1.2. Problem Statement

Few high quality studies, conducted in resource-poor settings, have described neurodevelopmental consequences of perinatal HIV infection, as recorded on a parent-reported developmental screening tool. It would be of interest to examine the results of such a screening tool in young HIV infected children prior to ART initiation, and to use the same tool to demonstrate changes after children have attained virological suppression on ART. Results could possibly alert treating practitioners, care-givers and those planning paediatric HIV treatment services, to the prevalence of developmental delay in this patient population, and the subsequent need for appropriate management in order to maximise neurologic outcomes.

1.3. Aim of Study

The aim of this study is to describe the prevalence, presentation and predictors of neurodevelopmental delays, in a cohort of perinatally HIV-infected children, before and after ART initiation.

1.4. Study Objectives

1.4.1. Primary Objective

To determine the prevalence of, and to describe, ASQ-defined neurodevelopmental delay in a cohort of children with perinatally acquired HIV infection,

a. before ART initiation

b. after achieving viral suppression on ART.

1.4.2. Secondary Objectives

- To investigate factors which may correlate with ASQ-defined neurodevelopmental delay present prior to ART initiation.
- To investigate factors which may correlate with ASQ-defined neurodevelopmental delay present after achieving HIV viral suppression on ART.
- To investigate whether ASQ-defined neurodevelopmental delay, present, pre- or post-ART initiation, affects treatment outcome with respect to HIV viral suppression and death.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

This chapter will discuss the epidemiology of HIV-1 infection as it pertains to children in SSA; the neuropathogenesis of the virus; risks of developing HIV-related CNS disease and the resultant consequences on childhood development. The effects of ART on paediatric neurological outcomes and use of childhood screening tests will also be discussed.

Articles referenced in this literature review were sourced from Pubmed, the Cochrane Collaboration, and Science Direct. Keywords used included paediatric, HIV, neurodevelopment, HIV encephalopathy, ART, and childhood neurodevelopmental screening tests.

2.2. Epidemiology of HIV

Estimates from the WHO indicate that 34 million (range: 31.4 - 35.9 million) people worldwide were infected with HIV in 2011, of which 3.3 million (range: 3.1 - 3.8 million) were children under 15 years of age.(24, 25)

SSA continues to bear a disproportionate share of the HIV burden with 69% of all HIV-infected people living in this region in mid-2011.(24) Of the 3.1 million (range: 2.8 - 3.4 million) HIV-infected children residing in SSA, only 21% (range: 19 - 24%) were receiving ART.

South Africa remains one of the few countries in the world in which maternal and child mortality has increased since the 1990s,(26) although recent data reports an improving trend in the under-5 mortality rate.(27) Acquired immunodeficiency syndrome (AIDS) accounts for 35% of deaths in children under 5 years of age.(26) The Joint United Nations programme on HIV/AIDS (UNAIDS) and the WHO statistical modeling methods have estimated that only 36% (range: 32 - 40%) of the 300 000 South African children in need of ART, were receiving treatment in 2010.(24) More recent South African data however, suggests that access to ART in children is improving, with an estimated 152 000 children <15 years of age receiving ART in 2011.(28) Although ARV coverage among children in South Africa is greater than that in SSA, there remains a large population of untreated children, with resultant high morbidity and mortality.

Rahima Moosa Mother and Child Hospital (RMMCH) located in Johannesburg, Gauteng, is the site of the second largest paediatric HIV treatment facility in Gauteng. At present the clinic provides ART for approximately 1 300 children (Technau KG, senior medical officer HIV paediatric treatment clinic RMMCH, oral communication, 2012).

2.3. Neurotropism of HIV

HIV is a neurotropic virus – viral tropism refers to the specificity of a virus for a particular host tissue.(29) Brain studies of AIDS patients carried out since the beginning of the epidemic, have shown the HI virus to be neurotropic – meaning that the virus has an affinity for cellular

components of the nervous system.(25) It has been postulated that certain strains of HIV-1 might have an increased propensity to invade (neurotropism) and cause damage in the nervous system (neurovirulence).(30)

2.4. Neuropathogenesis of HIV

The mechanisms underlying the changes to the blood brain barrier and invasion of the CNS by the HI virus, involve a complex cascade of events and molecules including HIV envelope and regulatory proteins; products of activated monocytes; and activated brain endothelial cells.(3) The HI virus seeds the CNS during primary viraemia. The pathogenic mechanisms for HIV-related CNS disease have, however, yet to be clearly defined.

HIV-1 is able to persist, in a replication-competent form, in certain cell types and anatomical sites referred to as viral reservoirs, even in patients receiving ART.(30) In the CNS, macrophages (phagocytic white blood cells) and microglial cells (non-neuronal supporting cells in the CNS) have been shown to be the main, but not the only, cell reservoirs of the virus.(25) It is, however, still unclear whether the CNS can serve as a long-term reservoir for HIV-1 in patients with good suppression of viral replication.

2.5. Risk of Developing HIV-Related CNS Disease

The risks of a child developing HIV-related CNS disease can be attributed, among other factors, to the time point at which the infection was acquired;(5) the timing of ART initiation;(31) and individual patient and environmental characteristics.(32)

Mother-to-child-transmission (MTCT) of HIV occurs at three primary time points, namely: in utero, during labour and delivery (intrapartum), and postnatally through transmission of the virus via breast milk.(33) It has been found that the earlier the infant becomes infected with the virus, the higher the likelihood that the child will develop neurodevelopmental delay. In the Women and Infants Transmission Study (WITS) cohort, children infected in utero (indicated by a positive culture result for HIV within the first 48 hours of life) were more likely to display a rapid decline in neurobehavioral functioning, compared with those who acquired the infection perinatally.(5) It has also been postulated that due to rapid brain growth and development occurring in the latter stages of gestation and the early neonatal period, early HIV infection is associated with more rapid disease progression and earlier CNS manifestations.(10)

Early infant HIV diagnosis and prompt initiation of ART has been shown to reduce HIV progression by 75%, and also resulted in a lower incidence of HIV encephalopathy.(31)

2.6. Factors Affecting Normal Childhood Development

Early childhood development can be affected by a multitude of factors, resulting in devastating long-term consequences. Walker et al. compiled a systemic review of factors affecting childhood development and broadly defined two groups, namely: 1. biological factors – including, among others, nutritional status, comorbidity of infectious disease, and prematurity – and 2. psychosocial factors.(34)

HIV infection in the family potentially results in parental morbidity and mortality, poverty and food insecurity, thereby creating an environment which could compromise childhood development.(32) A recent study of children in Soweto, Johannesburg, showed one in five HIV-

infected children to be maternal orphans, and 30% of the infected children were not cared for by either parent.(35)

It is estimated that more than 200 million children under 5 years of age in resource-scarce countries do not reach their developmental potential.(36) Identification of developmental delays before the age of three years, and the subsequent implementation of early intervention programmes, has the potential to improve long-term outcomes.(37, 38)

2.7. Neurodevelopmental Consequences of HIV

Neurodevelopmental consequences of HIV have been well documented from early on in the epidemic, with a prevalence of 30 - 50% reported in ART-naïve children.(6, 7) Paediatric HIV-associated CNS disease can occur independent of systemic HIV disease, and has been reported as both the first AIDS-defining symptom,(10, 39) as well as occurring later in the course of the disease.(40) Natural history studies have indicated the highest incidence of onset of HIV-associated CNS disease as being in the first two years of life.(10) HIV-infected infants have been shown to have more abnormal cognitive and motor neurodevelopmental outcomes than HIV-exposed, but uninfected infants.(13)

The clinical manifestations and severity of paediatric HIV-related CNS disease cover a broad spectrum of disorders and children may present with a wide variety of neurologic complications that do not easily fit into simplified classification systems.(12) The HIV and AIDS malignancy branch (HAMB) of the National Cancer Institute, United States of America, has developed a classification system for assessing paediatric HIV-related CNS disease. As per this classification system, the child can be placed into one of three groups, namely: those with encephalopathy (static

or progressive); those presenting with CNS compromise; and those without any apparent CNS effects.

HIV encephalopathy is defined as the presence of one or more of the following for at least two months, in the absence of another cause: 1. failure to attain, or the loss of, developmental milestones, or the loss of intellectual ability verified by standard neuropsychological tests;
2. impaired brain growth, acquired microcephaly (determined by head circumference), or brain atrophy determined by computed tomography (CT) or magnetic resonance imaging (MRI); and
3. acquired symmetric motor deficit manifested by two of the following: paresis, pathological reflexes, ataxia, or gait disturbances.(41)

Children who function consistently below the level of the mean on standardised assessments, but continue to acquire new skills, are defined as having a *static* type of HIV encephalopathy.(9) These children demonstrate fixed neurological deficits and skills are acquired at a stable, slower rate with no loss of acquired skills. At the other end of the spectrum are children who continue to show decline in their level of functioning through the loss of acquired skills, and are categorised as having a *progressive* type of HIV encephalopathy.(10) This type of encephalopathy can vary in its time course as being subacute; developing fairly rapidly over weeks to months; or as having a more indolent course.

The caregiver history regarding the loss of developmental milestones, or the failure to obtain expected developmental milestones, can be accompanied by a wide range of neurological abnormalities on clinical examination, including: oromotor dysfunction; facial paresis; abnormal eye movements; non-focal motor dysfunction (spastic quadriplegia or hypotonia in young infants, or spastic diplegia or hypotonia in older infants and children); and, less commonly, cerebellar signs.

Encephalopathy in the school-going child may present with a decline in academic achievement, change in behavior, and/or a psychomotor slowing.(8)

The second grouping in the HAMB classification system includes children with CNS compromise which may be evidenced by developmental shortfalls and delays in motor, cognitive, and expressive language development;(11) adaptive functioning (socialisation behaviour, quality of life); and memory.(8)

The third group in the HAMB classification system includes children who are apparently not suffering from any CNS abnormalities resulting from their HIV disease. Their cognitive development falls within normal limits; they have no decline in function; they have a normal neurologic examination; and have had no improvements in function related to the initiation of ART.(8)

HIV-associated psychiatric disturbances (bipolar mood disorder, anxiety disorders, adjustment disorder and depression) can also occur in the paediatric population, but may be under recorded.(42)

2.7.1. Effect of HIV on Cognition

Children with HIV infection have been shown to present with severe cognitive delays.(43) Abnormalities include behavioural impairments, learning difficulties, lower intellectual functioning and attention-deficit disorders.(13, 40, 44, 45) Poor cognitive outcomes have been associated with disease severity and growth failure(43) as well as CD4 count and viral load (VL).(44)

2.7.2. Effect of HIV on Motor Development

The motor system is often more severely affected as compared to other facets of development.(11)The effects are often detectable at a young age, prior to other developmental delays being evident.(13) Gross motor function has been reported to be more severely affected than fine motor function, and abnormalities may include hypotonia, abnormal reflexes and ataxia.(11) Muscle coordination and strength are affected in severely ill children. Low CD4 cell count and high VL are associated with poor motor development.(44, 46)

2.7.3. Effect of HIV on Language

Speech and language development has been shown to be adversely affected by HIV infection,(47)with expressive language ability more affected than receptive ability.(11, 14) The extent to which language is affected has been associated with disease severity, low CD4 cell count and high VL.(44)

2.8. Effect of ART on Neurodevelopment

ART has been shown to alter the natural history of neurological outcomes of paediatric HIV infection. Before highly active antiretroviral therapy (HAART) was used to treat HIV disease, the use of AZT monotherapy demonstrated improvements in neurodevelopmental abnormalities in children presenting with encephalopathy.(48) Moreover, early initiation of triple ART is associated with delaying the onset of progressive encephalopathy as well as improving outcomes in children already presenting with CNS dysfunction.(17, 18)

Not all ARV drugs demonstrate the same degree of penetration into the CNS. Letendre et al.(19) developed an ARV CNS penetration effectiveness score based on cerebrospinal fluid ARV concentrations. They reported AZT and nevirapine (NVP) as having the highest scores, followed by (in decreasing order of CNS penetration) ABC, lopinavir/ritonavir (LPV/RTV), and lamivudine (3TC) and stavudine (d4T). Drugs with higher CNS concentrations have been shown to be more beneficial in preventing, or reversing, neurological damage caused by HIV.(20)

The rate of progressive HIV-encephalopathy (PHE) in children, before the use of ART, was reported to be as high as 50%.(6, 9) However, the era of paediatric ART has decreased the incidence of PHE to less than 2%.(49) Yet, children with HIV still demonstrate significant delays in cognitive, language and motor development.(11, 35)

ART is, however, only one aspect of the multifaceted treatment approach for children infected with HIV because children taking a suppressive ART regimen *still* present with neurodevelopmental delays, and thus require further interventions in addition to drug therapy. The long-term nature of chronic HIV disease, and of childhood development, necessitates an intervention programme which can be instituted by the caregivers on a daily basis. Both the caregivers and the children benefit from home-based intervention programmes. A study by Spiegel and Mayers(50) found that a regular home-based physiotherapy programme provided a sense of purpose and competence for caregivers of children infected with HIV.(50) Young children have been reported to show significant improvement in cognitive and motor development after the provision of a basic home stimulation programme.(21)

2.9. Childhood Developmental Screening

Developmental screening tools are designed to assess the dynamic and complex process of childhood development by measuring skills in a variety of domains in order to identify children who should receive more intensive evaluations. Practitioners involved in the management of children often perform informal clinical assessments when determining childhood development and standardised assessment tools are not routinely used.(51) Only 30% of children with developmental problems are identified before school age when relying solely on clinical judgement,(52) thus an important window of opportunity is missed in identifying children's developmental problems and being able to intervene in order to alter their developmental trajectories favourably. The South African Paediatric Association does not currently advocate any specific standardised childhood screening tools, nor does it make guidelines available for developmental screening practices (Jacklin L, Department of Paediatrics, University of the Witwatersrand, oral communication, 2012).

The ASQ, second edition (1999), is a simple, cost-effective, easily administered, parent-completed screening questionnaire utilized to identify neurodevelopmental delays in young children.(53) The ASQ set, developed by the University of Oregon's Centre on Human Development,(54) has been developed to assist with the identification, and monitoring, of children with developmental delays between 4 months and 5 years of age. The ASQ was normed on more than 8000 children from diverse ethnic and socioeconomic backgrounds.(22) Questionnaires are completed at prescribed age intervals, and assess five domains – Communication, Gross Motor, Fine Motor, Personal Social and Problem Solving. Self-reporting of the occurrence of certain behaviours and skills is done by the parents when completing the questionnaire. The screening test is published with reliability and validity data comparable to other childhood screening tests.(22)

2.10. Conclusion

South Africa is home to a large population of HIV infected children, many of whom have yet to access ART. The neurotropic HI virus has been shown to result in a wide spectrum of neurological complications and developmental abnormalities in the paediatric population. Although ART has been shown to alter the natural history of neurological outcomes in children infected with HIV, developmental delays in children on a suppressive ARV regimen are still evident.

CHAPTER 3

METHODOLOGY

This chapter will elucidate the methodology employed in this research report.

3.1. Research Design

We conducted a retrospective, observational cohort study.

3.2. Location of Study

Data was collected from the Neverest 2 clinical trial which was conducted at the Empilweni Services and Research Unit (ESRU) located at RMMCH. Children enrolled in this trial were mainly from the urban neighbourhoods of Coronationville and Newclare surrounding the health facility, but also came from Soweto, Alexandra and Diepsloot. Children were mainly from poorer socio-economic backgrounds with most families receiving a child support grant.

3.3. Study Participants

The Neverest 2 trial was a randomised, open-label clinical trial investigating treatment options for nevirapine-exposed, protease inhibitor (PI)-treated children who initiated ART when less than 24 months of age (clinical trial.gov NCT01146873).(55) On the Neverest 2 trial, ART-naïve children were initiated onto PI-based ART as per Gauteng Department of Health guidelines in place at the

time.(56) Randomisation to either remain on the PI-based regimen or change to an NNRTI-based (NVP) treatment regimen, took place, as per study protocol, for those achieving and sustaining plasma HIV-1 ribonucleic acid (RNA), measuring VL < 400 copies/mL for at least 3 months within the first 12 months of treatment.

For this research report, the following inclusion and exclusion criteria were applied:

3.3.1. Inclusion Criteria

- Perinatal HIV infection confirmed by a positive HIV polymerase chain reaction (PCR) result;
- Two completed ASQs – one pre-ART initiation and the second once viral suppression had been maintained for three months;
- Participant < 24 months of age at time of initial assessment;
- Pre-ART initiation age corresponding with available ASQ.

3.3.2. Exclusion Criteria

- Prematurity (< 37 weeks gestational age at birth);
- Congenital abnormality;
- History of hypoxic brain injury at birth;
- History of head injury sustained prior to ART initiation;
- History of treatment for an opportunistic infection (excluding tuberculosis) or tumour after ART initiation.

3.4. Outcome Measures

3.4.1. Ages and Stages Questionnaires (2nd Edition)

The ASQ set comprises 19 different questionnaires administered to mothers or primary caregivers at specified age intervals between the ages of 4 - 60 months, with age adjustment according to gestational age up to 24 months. Each questionnaire is valid for one month on either side of the target age (ASQ time frame). Each questionnaire comprises 30 questions and is divided into five domains – Communication, Gross Motor, Fine Motor, Problem Solving and Personal Social – with six questions in each domain (Appendix I). Each domain is assessed by developmental milestones which are chosen so as to represent a developmental quotient of 75 - 100%. The response options for each questions is: yes/no/not yet, with a respective score of 10, 5 or 0 points. The ASQ scoring system uses statistically determined cut-off points developed for both normal children as well as those with elevated risk, (57) yielding an overall pass/fail score for each domain. Referral for further assessment is advised when the score on any domain falls below the cut-off point, which is set at 2 standard deviations below the mean of the reference group. In addition to the 30 items, each questionnaire has 7 - 8 open-ended questions, depending on the age of assessment, regarding parents' concerns about their child's general health. Completion of the questionnaire takes about 10 - 15 minutes of the caregiver's time, and 1 - 2 minutes for the staff member to score the responses. Overall sensitivity and specificity are 75% and 86%, respectively. In a recent multinational trial involving 18 countries in Asia, Africa, Europe, North- and South-America, sensitivity was 88% and specificity was 82.5%.(58) Test–retest reliability within two weeks was 94% for the original version. Inter-observer reliability between parents and professional examiners was 94%. Of the validation sample, 26% was taken from the two lowest income groups in the United States with 13.5% being African-American.(22)

A trained counsellor or study nurse conducted the questionnaires with the caregiver accompanying the child to the clinic. The same individual did not necessarily conduct the questionnaire with the participant at the two time points. In the case of the caregiver being unable to understand English, interviews were conducted using the preferred language of the caregiver. All data, and scores pertaining to the ASQ's, was then entered into the Neverest 2 database by the data capturer.

The age-specific ASQ's were conducted on many of the children enrolled on the Neverest 2 trial at specified time points. For this analysis ASQ data was taken from two time points. The first time point was at ART initiation. A window period of one month before or after this time point was allowed. The second data collection time point occurred when the participant achieved sustained virological suppression and was randomised (as part of the main trial).

3.4.2. Anthropometric Measurements

Weight (kg) and length (cm), or standing height, were recorded at baseline and at subsequent study visits. Anthropometric measurements were carried out by trained study nurses.

3.4.3. Physical Examination

Trained research doctors carried out thorough physical examinations at each visit. Based on clinical findings the presence or absence of developmental delay was recorded, independent of the ASQ.

3.4.4. Laboratory Analysis

CD4 T-cell counts were measured pre-treatment and every three months during follow-up. For this analysis we selected CD4 determinations done pre-treatment and closest to the time of

randomisation (viral load suppression). CD4 cell counts and percentages were obtained using the Beckman Coulter FlowCARE PLG CD4 Reagent System.

HIV-1 RNA quantity was measured pre-treatment and three monthly until randomisation. The standard assay was used for samples collected pre-treatment and the ultrasensitive assay for those collected after ART initiation (Roche Amplicor Assay, Version 1.5, Branchburg, NJ).

3.5. Ethical Considerations

Approval for this research was obtained from the University of the Witwatersrand Human Research Ethics Committee (Medical). Clearance certificate M111118. (Appendix II)

The Neverest 2 study received approval from the Institutional Review Boards of Columbia University (New York, United States of America) and the University of the Witwatersrand (Johannesburg, South Africa. Ethics Approval number 040912). Informed consent was signed by the parents or legal guardians of all participants enrolled onto the Neverest 2 trial. Access to the Neverest 2 database was granted by the principle investigators.

3.6. Statistical Methods

Outcomes were compared across time points using *t* tests for continuous variables (e.g. CD4 cell counts), and χ^2 or Fisher exact tests for categorical variable (e.g. WHO staging). The Mcnemar test was used to compare ASQ scores before treatment initiation and at viral suppression in the matched sample. Associations were examined using χ^2 tests.

Analyses were done using Epi Info version 7 and Microsoft Excel 2007. Weight-for-age (WAZ), height-for-age (HAZ) and weight-for-height (WHZ) Z-scores were calculated using Anthro software (Version 3.2.2; WHO). All statistical tests were 2-sided and $p<0.05$ was considered statistically significant.

CHAPTER 4

RESULTS

4.1. Study Population

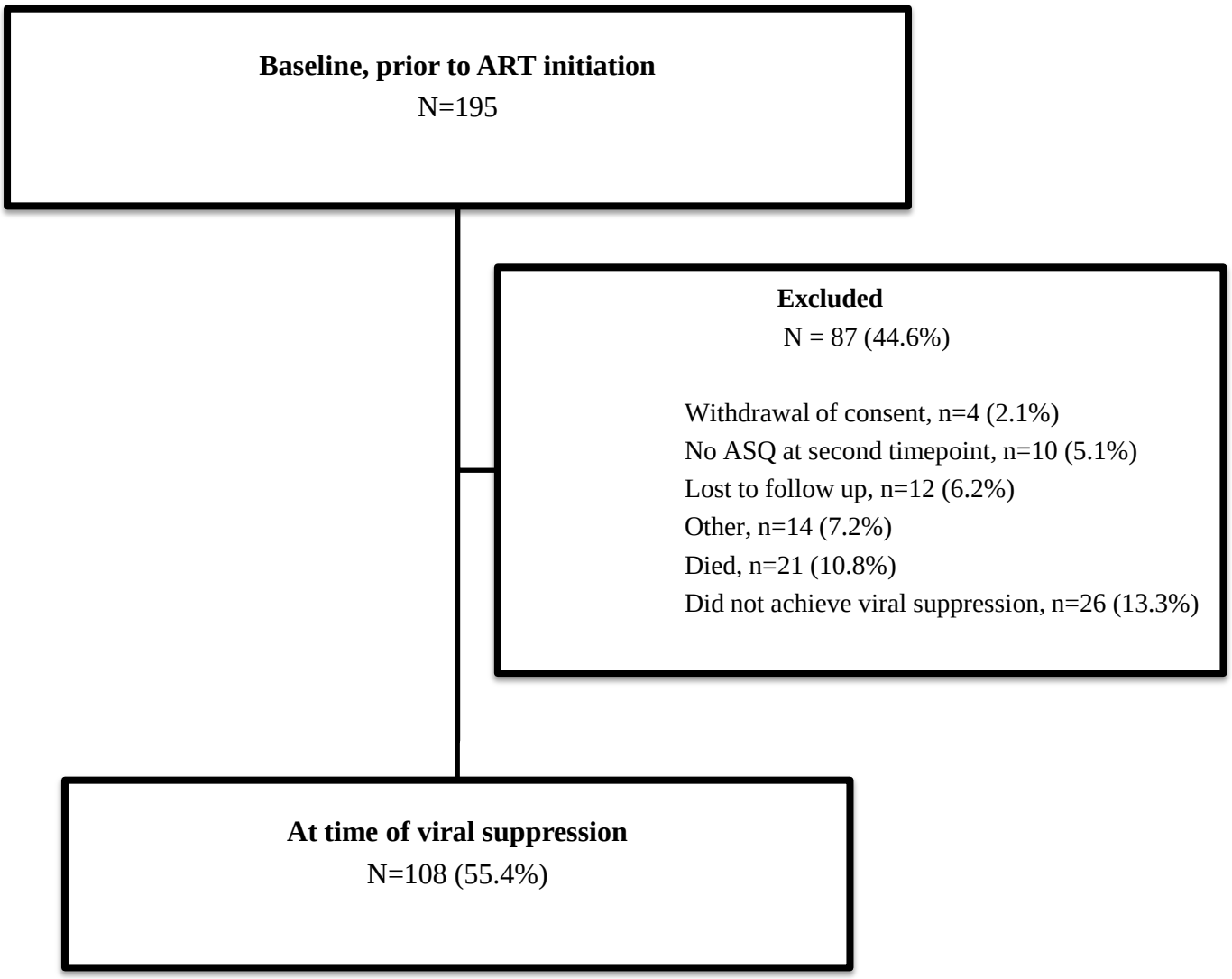
Of the 323 children enrolled onto the Neverest 2 trial, 95 had already started ART pre-enrolment, and 33 did not have baseline pre-ART ASQ data ,thus making them ineligible for inclusion in this sub-study. 195 participants, 105 (53.9%) male, completed an age-appropriate ASQ, and met inclusion criteria for this analysis prior to being initiated onto a PI-based ART regimen. Children had a median age of 31 weeks at first positive HIV PCR test result, and initiated ART at a median age of 8.8 months. Pre-treatment median VL and CD4 cell count percentage were 750 000 HIV RNA copies/mL and 17.8% respectively, and mean WAZ was -2.38. WHO HIV disease classification categorized 151 (77.4%) of the children as having stage 3 or 4 disease. Of those with stage 4 disease, 7.7% were diagnosed as having HIV encephalopathy (TABLE 4.1).

TABLE 4.1 Characteristics of 195 HIV-infected children prior to ART initiation (pre-treatment)

	N (%)
Sex	
Male	105 (53.85)
Female	90 (46.15)
Age (months)	
0 to <12	133 (68.21)
12 to 24	62 (31.79)
Median [Range]	8.83 [2.24 - 24.92]
CD4 percentage, N	177
Median [Range]	17.8 [1.26 - 43.0]
CD4 count cells/mm^3, N	177
Median [Range]	872 [10 - 4241]
HIV RNA copies/ml, N	187
Median [Range]	750 000 [400 - 3 million]
WAZ, N	195
Mean [std]	-2.38 [1.78]
HAZ, N	195
Mean [std]	-3.45 [1.72]
WHZ, N	195
Mean [std]	-0.27 [2.2]
Age at first positive HIV PCR result (weeks), N	147
0-6	40 (27.21)
> 6	107 (72.79)
Median [IQR]	31 [12 - 33]
Age at ART initiation, (months), N	195
Median [IQR]	8.83 [5.65 - 14.04]
WHO stage pre-treatment, N	195
Stage 1	34 (17.44)
Stage 2	10 (5.13)
Stage 3	99 (50.77)
Stage 4	52 (26.67)
WHO stage 4 with HIV encephalopathy	4 (7.69)

Among the 195 children assessed pre-ART, 30 (15.4%) did not remain in follow-up; 26 (13.3%) failed to achieve viral suppression in the required time period; 21 (10.8%) died, and 10 (5.1%) did not complete a second ASQ after viral suppression had been achieved (FIGURE 4.1).

FIGURE 4.1 Flow Diagram of Study Participants



Of the initial 195 children included in the analysis, 108 achieved viral suppression and completed a second ASQ a mean 9.4 months after starting ART. Median age of the cohort at viral suppression was 18.6 months. There were significant increases ($p<0.001$) in the median CD4 percentage and mean WAZ scores at 29.9% and 0.68 respectively. Median HIV RNA had decreased to 52.5 copies/mL (TABLE 4.2).

TABLE 4.2 Comparison of 108 HIV-infected children pre-ART (Pre-treatment) and when attaining viral suppression (Suppressed).

	Pre-treatment	Suppressed	P†
Age in months			
Median [Range]	9.3 [2.24 - 24.16]	18.62 [9.07 - 34.45]	**<0.001**
CD4 percentage, N	99	107	
Median [Range]	19.75 [2.62 - 41.8]	29.9 [11.6 - 55.7]	**<0.001**
CD4 count cells/mm^3, N	99	107	
Median[Range]	1003 [10 - 3762]	1925 [512 - 5423]	**<0.001**
HIV RNA copies/ml, N	104	108	
Median [Range]	750 000 [400 - 3million]	52.5 [49 - 399]	**<0.001**
WAZ, N	108	108	
Mean [std]	-2.12 [1.74]	-0.68 [1.16]	**<0.001**
HAZ, N	108	108	
Mean [std]	-3.1 [1.51]	-3.17 [1.59]	0.66
WHZ, N	108	108	
Mean [std]	-0.26 [2.33]	1.35 [1.48]	**<0.001**

† Paired t-tests were used to compare appropriate parameters pre- and post-treatment initiation

4.2. Ages and Stages Results

Pre-ART, the examining physician classified 39.9% of the children as having developmental delay, according to findings on history and examination. On the ASQ, children scored least well in the gross motor domain with 33.9% failing this domain. The next most commonly affected domain was problem solving (29.7%), followed by fine motor (26.7%), and personal-social (23.7%). Communication was the least affected domain with only 16.4% obtaining a failed score (TABLE 4.3).

TABLE 4.3 Prevalence and type of ASQ determined neurodevelopmental delay in 195 ARV naïve children

	N (%)	
Physician assessment of developmental delay	193	
Present, N (%)	77 (39.9)	
Age at first ASQ (months)		
Median [Range]	8.84 [2.83 - 25.18]	
Number of children failing each domain		95% CI
Communication	32 (16.41)	0.12-0.22
Gross Motor	66 (33.85)	0.28-0.41
Fine Motor	52 (26.67)	0.21-0.33
Problem Solving	58 (29.74)	0.24-0.37
Personal-social	46 (23.71)	0.19-0.3

Of the 108 children attaining viral suppression, significantly fewer were classified by the examining physician, after history taking and examination, as having developmental delay (9.3%, $p<0.001$). The second ASQ, conducted at viral suppression, revealed significant ($p<0.001$) decreases in the number of children failing individual domains (gross motor 13%; fine motor 10.2%; problem solving 9.3%; personal social 7.4%). The only domain not showing significant improvement was that of communication; with 12% of the children obtaining a failed score (TABLE 4.4).

TABLE 4.4 Prevalence and type of ASQ determined neurodevelopmental delay in 108 children assessed pre-ART initiation (Pre-treatment) and after attaining viral suppression (Suppressed)

	Pre-treatment, N (%)	Suppressed, N (%)	P
Physician assessment of developmental delay			
Present	41 (37.96)	10 (9.26)	**<0.001**
Number of children failing each domain			
Communication	16 (14.81)	13 (12.04)	0.61
Gross Motor	34 (31.48)	14 (12.96)	**<0.001**
Fine Motor	23 (21.3)	11 (10.19)	**0.02**
Problem Solving	29 (26.85)	10 (9.26)	**0.001**
Personal Social	19 (17.59)	8 (7.41)	**0.02**

Groups compared using the McNemar test

Longitudinal data of children failing domains pre-ART showed that 43.7% (communication), 29.4% (gross motor), 26.1% (fine motor), 20.7% (problem solving) and 21.1% (personal-social), also failed that same domain at the second time point. Fewer children had worse outcomes over time i.e. passing a domain on the initial ASQ, and then failing the domain at the time of viral suppression (TABLE 4.5).

TABLE 4.5 Longitudinal data for 108 children passing and failing each domain, across two time points (Pre-treatment, At viral suppression).

	Pre-treatment, N	At viral suppression, N (%)
Developmental delay		
Present	41	7 (17.1)
Absent	67	64 (95.5)
Communication		
Failed	16	7 (43.8)
Passed	92	86 (93.5)
Gross Motor		
Failed	34	10 (29.4)
Passed	74	70 (94.6)
Fine Motor		
Failed	23	6 (26.1)
Passed	85	80 (94.1)
Problem Solving		
Failed	29	6 (20.7)
Passed	79	75 (94.9)
Personal-Social		
Failed	19	4 (21.1)
Passed	89	85 (95.5)

4.3. Pre-ART Factors Associated with Failure of Specific ASQ Domains

A comparison of children failing and passing specific domains was carried out in order to elucidate possible predictors of failing specific domains (TABLE 4.6).

4.3.1. Communication

Children failing the communication domain: were of an older age at ART initiation i.e.12 - 24 months; had WAZ and HAZ scores ≥ 2SD and ≥ 3SD below the mean, respectively; were more likely to have WHO stage 3 or 4 disease and be assessed as having HIV encephalopathy; and were more likely to have had a history of previous hospital admissions.

4.3.2. Gross Motor

Pre-ART initiation, children failing the gross motor domain were more likely to have a CD4 percentage < 25; WAZ ≥ 2SD below the mean; WHO stage 3 or 4 disease; HIV encephalopathy; and a history of previous hospital admissions. Children failing this domain were also more likely to have been diagnosed with HIV infection when they were older than 6 weeks of age, and to have initiated ART after 12 months of age.

4.3.3. Fine Motor

Children failing the fine motor domain differed significantly from of those passing this domain in terms of: being of older age at ART-initiation i.e. 12 - 24 months; having a CD4 percentage < 25; having a WAZ ≥ 2SD below the mean; presenting with WHO stage 3 or 4 disease; assessed as having HIV encephalopathy; and having a history of previous hospital admissions.

4.3.4. Problem Solving

Children initiating ART between 12 - 24 months of age; having a pre-ART CD4 percentage

of < 25; with WAZ and HAZ ≥ 2SD and ≥ 3SD below the mean, respectively; diagnosed with WHO stage 3 or 4 disease or with HIV encephalopathy; and having a history of previous hospital admissions, were more likely to fail the problem solving domain.

4.3.5. Personal-Social

Fewer factors were found to significantly affect the personal social domain. Children with WAZ and HAZ ≥ 2SD and ≥ 3SD below the mean, respectively; and WHO stage 3 or 4 disease were more likely to fail this domain.

TABLE 4.6 Risk factors associated with failing specific domains of the ASQ in 195 children pre-ART initiation

		Communication		Gross Motor		Fine Motor		Problem Solving		Personal-Social	
	N	Fail, N (%)	P	Fail, N (%)	P	Fail, N (%)	P	Fail, N (%)	P	Fail, N (%)	P
Age at ART initiation (months)											
0 < 12	133	15 (11.28)	**0.005***	28 (21.05)	**< 0.001***	27 (20.3)	**0.003***	33 (24.81)	**0.03***	31 (23.31)	0.85
12-24	62	17 (27.42)		38 (61.29)		25 (40.32)		25 (40.32)		15 (24.59)	
CD4 percentage											
< 25	131	24 (18.32)	**0.12**	39 (37.4)	**0.03***	40 (30.53)	**0.02***	49 (37.4)	**<0.001***	35 (26.92)	0.11
≥ 25	46	4 (8.70)		9 (19.57)		6 (13.04)		5 (10.58)		7 (15.22)	
WAZ											
≤ -2SD	107	25 (23.36)	**0.004***	49 (45.79)	**<0.001***	38 (35.51)	**0.002***	44 (41.12)	**<0.001***	33 (31.13)	**0.008***
> -2SD	88	7 (7.95)		17 (19.32)		14 (15.91)		14 (15.91)		13 (14.77)	
HAZ											
≤ -3SD	119	25 (21.01)	**0.03***	45 (37.82)	0.14	36 (30.25)	0.16	42 (35.29)	**0.03***	35 (29.66)	**0.02***
> -3SD	76	7 (9.21)		21 (27.63)		16 (21.05)		16 (21.05)		11 (14.77)	
Age HIV infection first diagnosed (weeks)											
≤ 6	40	5 (12.5)	0.22	8 (20)	**0.01***	7 (17.8)	0.07	8 (20)	0.07	9 (22.5)	0.73
> 6	107	23 (21.5)		45 (42.06)		35 (32.71)		38 (35.51)		27 (25.23)	
WHO Stage											
≤ 2	44	2 (4.55)	**0.02***	3 (6.82)	**<0.001***	5 (11.36)	**0.009***	5 (11.36)	**0.002***	5 (11.36)	**0.03***
≥ 3	151	30 (19.87)		63 (41.72)		47 (31.13)		53 (35.1)		41 (27.33)	
HIV Encephalopathy											
Present	4 §	3 (75)	**0.02***	4 (100)	**0.01***	4 (100)	**0.005***	4 (100)	**0.007***	2 (50)	0.24
Absent	191	29 (15.18)		62 (32.46)		48 (25.13)		54 (28.27)		44 (23.16)	
Ever admitted to hospital											
Yes	140	30 (21.43)	**0.003***	56 (40)	**0.005***	45 (32.14)	**0.007***	49 (35)	**0.01***	38 (27.34)	0.07
No	54	2 (3.70)		10 (18.52)		7 (12.96)		9 (16.67)		8 (14.81)	

*Groups are compared using Chi-squared or Fisher's exact § tests**, *p-value <0.05***

4.4. Factors Associated with Failure of Specific ASQ Domains at Viral Suppression

At the time of viral suppression there were fewer significant differences between those failing and passing each domain, than pre-ART initiation. Children with WAZ ≥ 2 SD below the mean, at the time of viral suppression, were more likely to fail the fine motor and problem solving domains (TABLE 4.7).

No associations with failing specific domains were found with poverty (recorded as the lack of basic amenities such as water and electricity); parental employment; and family members living with HIV.

TABLE 4.7 Risk factors associated with failing specific domains of the ASQ in 108 virally suppressed children

		Communication		Gross Motor		Fine Motor		Problem Solving		Personal-Social	
	N	Fail, N (%)	P	Fail, N (%)	P	Fail, N (%)	P	Fail, N (%)	P	Fail, N (%)	P
Age at ART initiation (months)											
6-24	79	10 (12.66)	0.74	8 (10.13)	0.15	7 (8.86)	0.45	6 (7.59)	0.33	4 (5.06)	0.13
≥ 24	29	3 (10.34)		6 (20.69)		4 (13.79)		4 (13.79)		4 (13.79)	
CD4 percentage											
< 25	28	5 (17.86)	0.28	5 (17.86)	0.38	4 (14.29)	0.42	4 (14.29)	0.30	2 (7.14)	0.98
≥ 25	79	8 (10.13)		9 (11.39)		7 (8.86)		6 (7.59)		6 (7.59)	
WAZ											
≤ -2SD	10 *§*	3 (30)	0.10	3 (30)	0.12	4 (40)	**0.009***	4 (40)	**0.006***	2 (20)	0.16
> -2SD	98	10 (10.2)		11 (11.22)		7 (7.14)		6 (6.12)		6 (6.12)	
HAZ											
≤ -3SD	58	4 (6.9)	0.08	6 (10.34)	0.38	5 (8.62)	0.56	5 (8.62)	0.81	3 (5.17)	0.34
> -3SD	50	9 (18)		8 (16)		6 (12)		5 (10)		5 (10)	
WHO Stage											
≤ 2	28	2 (7.14)	0.36	2 (7.14)	0.29	3 (10.71)	0.91	2 (7.14)	0.65	2 (7.14)	0.95
≥ 3	80	11 (13.75)		12 (15)		8 (10)		8 (10)		6 (7.50)	
Ever admitted to hospital											
Yes	77	12 (15.58)	0.08	11 (14.29)	0.56	9 (11.69)	0.44	7 (9.09)	0.89	6 (7.79)	0.84
No	30	1 (3.33)		3 (10)		2 (6.67)		3 (10)		2 (6.67)	

Groups are compared using Chi-squared or Fisher's exact § tests, ****p-value <0.05***

4.5. Children Dying Prior to Viral Suppression

Children who died (n=21) prior to attaining viral suppression had a median age of 11.47 months (IQR: 8.47 - 13.02) and had been on ART for a median time of 2.79 months (IQR: 1.25 - 5.7) prior to death. Although not statistically significant (apart from in the personal-social domain, p=0.04), when compared to the 108 children who attained viral suppression, pre-ART the children who died performed less well in all domains of the ASQ. Children who died differed significantly from those reaching the second analysis time point in that they presented with lower WAZ, HAZ, and absolute CD4 counts, pre-ART (TABLE 4.8).

TABLE 4.8 Pre-ART characteristics of children who died prior to attaining viral suppression compared to those achieving viral suppression

	Death prior to viral suppression	Attaining viral suppression	P
	21	108	
Male, N (%)	16 (76.19)	57 (52.78)	0.06
Mean age (months)	8.16	10.49	0.07
Mean WAZ	-3.58	-2.12	**<0.001**
Mean HAZ	-4.32	-3.1	**0.001**
Mean WHZ	-0.91	-0.26	0.17
Mean CD4 count (cells/mm^3)	486.7	1072	**0.002**
Mean CD4 percentage	13.72	18.47	0.07
Mean viral load	682 509	561 423	0.10
WHO Stage ≥ 3, N (%)	19 (90.48)	80 (74.07)	0.16
Developmental delay present, N (%)	12 (57.16)	41 (37.96)	0.07
Failing specific domains, N (%)			
Communication	4 (19.05)	16 (14.81)	0.74
Gross Motor	7 (33.3)	34 (31.48)	0.87
Fine Motor	7 (33.3)	23 (32.1)	0.23
Problem Solving	7 (33.3)	29 (26.85)	0.55
Personal-Social	8 (38.1)	19 (17.59)	**0.04**

4.6. Children Not Attaining Viral Suppression

Similarly, children failing to reach viral suppression (n=26) within the required timeframe, obtained more failed scores on all the ASQ domains pre-ART when compared to those attaining viral suppression, although, only differences in the gross motor scores showed statistical significance. The non-suppressed group had significantly lower WAZ, HAZ, absolute CD4 count, and were more likely to be assessed by the attending physician as having developmental delay pre-ART initiation (TABLE 4.9).

TABLE 4.9 Pre-ART characteristics of children who failed to attain viral suppression compared to those achieving viral suppression

	Virological Failure	Attaining viral suppression	P
	26	108	
Male, N (%)	14 (53.85)	57 (52.78)	1
Mean age (months)	12.7	10.49	0.08
Mean WAZ	-3.09	-2.12	**0.01**
Mean HAZ	-4.44	-3.1	**<0.001**
Mean WHZ	-0.63	-0.26	0.49
Mean CD4 count (cells/mm^3)	704.7	1072	**0.003**
Mean CD4 percentage	15.25	18.47	**0.005**
Mean viral load	598 324	561 423	0.84
WHO Stage ≥ 3, N (%)	23 (88.46)	80 (74.07)	0.19
Developmental delay present, N (%)	16 (61.54)	41 (37.96)	**0.03**
Failing specific domains, N (%)			
Communication	7 (26.72)	16 (14.81)	0.14
Gross Motor	16 (61.54)	34 (31.48)	**0.004**
Fine Motor	11 (42.31)	23 (32.1)	**0.03**
Problem Solving	10 (38.46)	29 (26.85)	0.24
Personal-Social	9 (34.62)	19 (17.59)	0.06

CHAPTER 5

DISCUSSION

Study results will be discussed in this chapter. Challenges encountered will be described, and recommendations for clinical practice will also be made.

5.1. Neurodevelopmental Delay

Standardised screening tests are preferred when determining the presence or absence of developmental delay, as physician-based assessments have been shown to be poorer in identifying children with delay.(59) In our study, based on history and physical examination findings, 39.9% of the children were classified by the physician as having developmental delay pre-ART. This figure corresponds with published data reporting similar pre-ART findings in children infected with HIV.(6, 7) Physician detected global developmental delay, however, cannot be directly compared to the domain-specific delay assessed in the ASQ. Children showed improvement on ART with significantly fewer being classified by the physician as having delay when attaining viral suppression (9.26%, $p<0.001$). However, of the children initially determined as delayed, 17.1% did not show improvement on ART. Neurodevelopment is a complex process influenced by multiple interactive factors namely, genetic, health, disease, treatment and psychosocial. Studies have shown that ART alone has limited beneficial effects on neurodevelopment in the first years of life, (35, 60) and these children may also benefit from programmes aimed at enhancing early childhood development. Children found presenting with developmental delay were appropriately

referred to hospital-based allied medical practitioners, such as occupational therapy, physiotherapy and speech therapy.

5.2. Communication

Language, has been shown to be delayed in infants infected with HIV.(11, 61) Our results, assessed as performance in the communication domain, showed communication to be the least affected domain pre-ART, with 16.4% of the children failing this domain. This may have been due to the young age of the cohort at baseline (median 8.83 months, range: 2.24 - 24.9), which made elucidation of language delay more difficult. Children showed no significant improvement in this domain post-ART initiation, with 43.8% continuing to demonstrate communication delay when re-assessed at viral suppression. This may be more reflective of the true extent of the delay, as a more comprehensive assessment of language is possible with the older child. Published data also reports a high prevalence of language delay in young ART-naïve children, greater than 75% in some instances. (11, 61)

5.3. Gross Motor

HIV-1 infection has been shown to be associated with an increased risk of abnormal motor development.(13) Gross motor function is the most affected domain in terms of severity and persistence of delay.(62) Gross motor delay may be related to the loss of muscle strength as well as to HIV-related CNS involvement.(63) Similarly, our results showed gross motor to be the domain most affected, with 33.9% failing the domain at baseline. Other South African studies have reported higher numbers, 72% and 85%.(11, 43) Although significant improvement was shown at viral suppression, gross motor was still the most affected domain

with 29.4% showing continued failure. In our analysis, factors associated with failure of the gross motor domain pre-ART included: children who initiated ART at an older age; had more severe disease pre-ART; and had been diagnosed as having HIV encephalopathy. This confirms findings in other studies where motor developmental delay has been reported to be associated with disease severity, CD4 count and growth.(14, 62, 64)

5.4. Fine Motor

Fewer studies discriminate between gross and fine motor skills, and these are more often assessed together under the term motor development. The ASQ specifically screens for delay in fine motor development. Pre-ART, 26.7% failed the fine motor domain. Comparative South African data showed a lower percentage of fine motor fallout with 12.5% of an ART-naïve cohort, aged 18 - 30 months, delayed in terms of fine motor skills.(11) Fine motor function improved significantly post ART initiation, with only 10.2% failing the domain when virally suppressed. However, of those who failed this domain pre-ART, 26.1% showed no improvement at viral suppression. Lowick et al. report 30% of virally suppressed pre-school children on ART, from similar socioeconomic backgrounds as our cohort, as having severe delay in the hand-eye domain when assessed using the Griffiths Mental Development Scales-Extended Revised Version.(35) These results show that although there is an improvement in fine motor skills post-ART initiation, delays still exist and may require additional interventions in order to improve fine motor performance. Our results show that children with growth restriction well below the norm at the second time point were significantly more likely to fail this domain even when virally suppressed ($p=0.009$), suggesting multifactorial aetiologies for fine motor delay which need to be addressed in order to improve outcomes in this domain.

5.6. Problem Solving

Compared to HIV uninfected controls, children infected with HIV have been shown to score significantly lower on cognitive function tests.(13, 61, 65) In our cohort 29.7% failed the problem solving domain pre-ART. Children with more severe HIV disease, poor growth and a history of hospital admissions, were more likely to perform poorly. Our findings reflect published data reporting cognitive performance to be worse in children more severely affected by HIV.(10, 14, 64) Although there was significant improvement in the number of children passing this domain when virally suppressed, 20.7% did not improve on ART and those showing poor growth were significantly more likely to fail this domain (p=0.006). Similar persistent delays in cognitive performance post-ART initiation have been reported, (66, 67) indicating that viral suppression may not be a reliable predictor of improved cognitive outcomes.

5.7. Personal-Social

Personal-social development investigates the development of normal patterns of play, self-care, and interactions with others and with the environment. In the group assessed over two time points (n=108) a significant improvement in the number of children passing this domain pre-ART (82.4%) and at viral suppression (92.6%), was demonstrated (p=0.02). This domain was the least affected when the cohort had achieved viral suppression. Data from a South African pre-school cohort on ART, found 26.7% to be severely affected in this respect.(35) These results raise serious concerns regarding the developmental wellbeing of children on

ART and highlight the need for early ART and programmes to assist HIV positive children in personal and social interaction.

5.8. Growth

Childhood HIV infection is known to adversely affect growth parameters.(61, 62) In our cohort, pre-ART, the children's growth parameters fell below the norm with mean WAZ greater than -2 SD, and mean HAZ greater than -3 SD, below the norm. However, mean weight-for-height (WHZ) was only slightly decreased at -0.27 SD below the norm, which was not sufficient to classify the cohort as wasted. As expected, the children showed significant improvements in WAZ on ART. Mean WAZ was, however, still below the norm (-0.68) after a mean 9.4 months on ART. Mean HAZ also improved over this period, but the improvement was not as marked as that seen in the mean WAZ. The mean HAZ at viral suppression remained greater than -3 SD below the norm. As expected, weight is a more readily reversible parameter, with changes becoming evident sooner than changes in height due to the differences in the nature of these parameters. The growth parameter improvements shown in our cohort correspond with published data showing similar ART-related growth improvements.(14, 43, 68) ART may not be the only factor associated with improvements in growth. Other factors involved may include access to care, food supplementation received from the clinic and dietary advice from treating practitioners.

Poor growth has been associated with poor development.(43, 62) This finding was born out in our study, which showed that children whose pre-ART growth parameters were the most severely affected were more likely to fail all five domains on the ASQ. Children with stunting pre-ART were found to be more at risk of failing the three domains of communication, problem solving and personal-social. These findings suggest that early ART initiation, before

growth parameters are affected, could improve developmental outcomes. Improving the nutritional status of children pre-ART may have far-reaching consequences in terms of improving early childhood developmental outcomes.

5.9. Virologic and Immunologic response

As expected, in this cohort of children under the age of 24 months, initiation of ART was associated with improvements in virologic and immunologic markers. Among those initiating a PI-based ARV treatment regimen, 86.7% attained viral suppression of HIV RNA < 400 copies/mL within the required, pre-determined timeframe of 52 weeks. Rates of viral suppression correspond to those reported in other paediatric cohorts receiving PI-based ART.(69)

Median CD4 percentage at baseline was low (17.8%, range: 1.26 - 43), but showed significant improvement once viral suppression had been attained (29.9%, range: 11.6 - 55.7, $p<0.001$), consistent with reported findings.(68)

Children who failed to attain virological suppression were more likely to be developmentally delayed, as assessed clinically by the physician, than those who suppressed ($p=0.03$). This was borne out in the sub-analysis by domain, where both gross motor and fine motor skills were significantly delayed in virological failures versus suppressors ($p=0.004$ and $p=0.03$ respectively). It is possible that developmental delay could increase the risk of virological failure. The exact mechanism would need to be studied further, but one possible reason for this could be if gross motor coordination problems translated into difficulty in swallowing medication, with subsequent sub-adequate drug plasma levels. It is apparent that children

with developmental delay do warrant extra vigilance for virological failure and, that children who are failing virologically should be assessed for neurodevelopmental problems.

5.10. Mortality

When assessed clinically for developmental delay by the examining physician, no significant difference was found in the presence of neurodevelopmental delay between the children who died prior to virologic suppression (n=21, 10.8%), and those who attained suppression (p=0.07). Children who died performed less well in the personal-social domain (p=0.04), but did not display significant differences in the other ASQ domains.

As shown in published data, children more severely affected by their HIV disease have a higher risk of dying.(70) Children in our cohort who died prior to attaining viral suppression showed more weight and height growth failure, and had lower mean CD4 cell counts when compared to those attaining viral suppression. Children showing more severe manifestations of HIV disease at ART initiation need to be vigilantly monitored to prevent adverse outcomes.

5.11. Challenges

- The ASQ has not been normed on South African children, nor has the test been validated on ill children affected by HIV.
- Possible inter-observer variability, as the questionnaires were conducted by different trained staff members at the two time points.

- The retrospective study design based on data collected for the Neverest 2 trial possibly excluded children who may have attained viral suppression beyond the 52 weeks required by the design of the original study.
- Missing data.
- Although many parameters showed improvements after viral suppression on ART, longer follow-up time would be of interest.
- Even though extensive attempts are made to follow-up patients enrolled on a clinical trial, loss to follow-up after initial study enrolment is problematic.

5.12. Clinical Recommendations Based on Findings

- Prompt initiation of ART at a young age is warranted in order to prevent detrimental effects of HIV on neurodevelopmental.
- Health care practitioners need to be made aware of the importance of developmental screening for all children presenting to HIV treatment services.
- Considering the large number of paediatric patients infected with HIV, and affected with associated neurodevelopmental delays, regular developmental screening should constitute an important component of comprehensive health care provision so as to identify infants and young children with developmental delays. Parent-completed questionnaires may be a means of screening this vulnerable population without placing additional burdens onto busy clinic staff.
- Practitioners need to be trained in the use of basic developmental screening tools in order to facilitate early detection, and referral, of children in which developmental delay is detected.

- Health practitioners should be made aware that children more severely affected by their HIV disease are at increased risk of developmental delay.
- Although many children show improvement in all spheres of development after initiating ART, some children do not improve and others show deterioration. Referral to appropriate therapeutic services as well as home-based stimulation programmes is important in improving outcomes.

CHAPTER 6

CONCLUSION

The main aim of this study was to determine the prevalence of neurodevelopmental delay; describe the facets of development affected; and explore risk factors associated with delay, in a cohort of young HIV-1 infected children before and after ART initiation. Information was gathered using a simple, inexpensive developmental screening tool, namely the Ages and Stages Questionnaire.

The conclusions are summarised below:

- HIV infected, ART-naïve children had a high prevalence of neurodevelopmental delay in the domains of communication, gross motor, fine motor, problem solving and personal-social, as measured by the ASQ.
- The prevalence of neurodevelopmental delay improved post ART-initiation, but even when viral suppression had been attained, delays persisted. The reasons for this are likely multifactorial. It is important to note that some HIV-associated neurodevelopmental delay is irreversible, and early ART initiation in young children is a strategy that can be used to minimise potential delay.
- Children with growth faltering and more severe disease pre-ART, were at highest risk for neurodevelopmental delay, which again highlights the need for early ART, before growth and immune status is compromised.
- Improved anthropometric, immunologic and virologic measures were seen post ART initiation.

Our findings confirm that children with untreated HIV infection are at high risk of neurodevelopmental delay. Children receiving a PI-based ART regimen showed improved scores on a neurodevelopmental screening test conducted pre-ART and again once viral suppression had been attained. Careful longitudinal monitoring of neurodevelopment is an essential component of comprehensive care for all children with HIV whether or not they are receiving ART. There is a need for basic developmental screening tools to be incorporated into paediatric HIV clinics. This would facilitate timely and judicious referral to appropriate services and could further improve neurodevelopmental outcomes of HIV-infected children.

CHAPTER 7

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APPENDICES

Appendix I - Ages and Stages Questionnaire, example

Appendix II - Ethical Clearance Certificate