An Evaluation of Factors Associated with Early Infant HIV Acquisition, Infant Outcomes, and 9-12 Month Infant HIV Seroreversion in the Context of PMTCT Option B+: Prospective Data from an HIV Exposed Birth Cohort.

By

NICOLETTE MARIÉ DU PLESSIS

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SUPERVISOR: MICHAEL PEPPER

CO-SUPERVISORS: AMEENA GOGA, THEUNIS AVENANT

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SUMMARY

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NICOLETTE MARIÉ DU PLESSIS

Supervisor: Prof. M.S Pepper

Co-supervisor: Proff AE Goga and T Avenant

Department: Department of Paediatrics

University: University of Pretoria

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This PhD dissertation reports data from the very early infant diagnosis (VEID) study that was conceptualized when universal birth HIV PCR testing at Kalafong Provincial Tertiary Hospital was mandated. Three research questions were addressed: (1) What factors are associated with early infant HIV acquisition?; (2) What are the growth outcomes of HIV exposed, uninfected (HEU) infants?; and (3) What is the 9-month infant HIV seroreversion rate in the context of PMTCT Option B+ when using different rapid HIV tests?

The first research chapter demonstrates that maternal HIV viral load detectable in the perinatal period, maternal combination antiretroviral therapy (cART) with a duration <1 month, and having a symptomatic infant at birth are significant predictors of early infant HIV acquisition. Small-for-gestational-age was included with the above three characteristics in multivariate analyses. Two-risk (maternal cART duration and viral load), three-risk (maternal cART duration, maternal viral load and symptomatic newborn), and four-risk (maternal cART duration, maternal viral load, symptomatic and SGA newborn) models for HIV acquisition were developed with a predictive probability score of a newborn PCR

positive test of 0.28, 0.498, and 0.57 respectively. These findings could guide a targeted approach to infant HIV-testing at birth. However, using the three- and four-risk scores at a probability of 0.02 and 0.04, 20% and 24% of HIV infected infants will be missed respectively at birth compared with universal testing. Therefore, we support universal birth PCR testing within the South African PMTCT context.

The second research chapter describes growth outcomes of HEU infants in relation to maternal and infant birth characteristics. Mothers were mostly breastfeeding at birth and on universal lifelong cART. Maternal to child transmission of HIV after birth were less than 1% (0.31%) and occurred mostly by 6 weeks of age. HIV infection was associated with symptoms and signs of HIV-associated immunosuppression, such as growth faltering. The hospitalization rate was 41.3/1000 person-years. Longitudinal growth trends illustrated lower weight-for-age (WAZ) and length-for-age (LAZ) in males. HEU newborns that had one or more symptoms suggestive of HIV-associated immunosuppression had lower weight trends and newborns with a birth weight <2.5kg had significantly lower WAZ, LAZ and head circumference (HC) trends. The significance of poor LAZ trends, especially in male HEU infants, remains a concern. The impact on final height and body mass index (BMI) need careful follow up to ascertain if these growth trends will have a negative impact such as higher BMI values and possibly more obesity among HEU male adolescents/young adults.

Lastly, we describe seroreversion at 9 months amongst HEU infants whose mothers received cART. We observed that different rapid assays vary in performance, with specificity ranging between 45-97%. HIV ELISA testing did not document any seroreversion at nine months in our cohort. This finding highlights the need for further research to determine the age at seroreversion within the context of the PMTCT option B+ / universal maternal access to lifelong cART. It is possible that uninfected infants will remain seropositive beyond 18 months of age. Hence, future recommendations might include HIV PCR testing in infants up to 24 month of age.

RESEARCH OUTPUTS

Journal articles

Du Plessis N.M, Muller C.J.B, Avenant T, Pepper M.S, Goga A.E. An early-infant HIV-risk score for targeted testing of HIV infection at birth: results from the birth cohort of the Very Early Infant Diagnosis of HIV (VEID) study, South Africa. *PlosMedicine (under review)*

Du Plessis N.M, Haeri Mazanderani A, Sherman G, Muller C.J.B, Avenant T, Pepper M.S, Goga A.E. Seroreversion in a birth PCR tested, HIV exposed PCR negative PMTCT option B/B+ cohort at 9 months of age using rapid HIV tests kits and HIV ELISA: follow-up of infants from the Very Early Infant Diagnosis of HIV (VEID) study, Gauteng, South Africa. *WHO Bulletin (submission in progress)*

Du Plessis N.M, Muller C.J.B, Avenant T, Pepper M.S, Goga A.E. Later growth outcomes of birth PCR tested, HIV exposed PCR negative infants with universal maternal cART exposure: follow-up cohort from the Very Early Infant Diagnosis of HIV (VEID) study, Gauteng, South Africa. *JAIDS (under review)*

Conference presentations

Du Plessis NM, Muller CJB, Avenant T, Goga AE, Pepper MS.
Predictive probability models for targeted birth HIV PCR testing.
e-poster presentation. International Workshop on HIV Paediatrics, Amsterdam, The Netherlands, 20-21 July 2018.

Du Plessis NM, Muller CJB, Avenant T, Goga AE, Pepper MS.
Predictive probability models for targeted birth HIV PCR testing.
Research Cafe e-poster presentation. Faculty Day 2018, Faculty of Health Sciences, University of Pretoria, 21-22 August 2018.

LIST OF ABBREVIATIONS

3TC	Lamivudine
AIDS	Acquired Immunodeficiency Syndrome
ANC	Antenatal care
ARV	Antiretroviral
BF	Breastfeeding
cART	Combination antiretroviral therapy
CD4	Cluster of differentiation 4
CDC	Centers for Disease Control and Prevention
DoH	Department of Health
EFV	Efavirenz
EID	Early infant diagnosis of HIV
ELISA	Enzyme-linked immunosorbent assay
EMTCT	Elimination of mother-to-child-transmission of HIV
HC	Head circumference
HEI	HIV exposed and infected
HEU	HIV exposed and uninfected
HIV-1	Human immunodeficiency virus type 1
HUU	HIV unexposed and uninfected
IPOPD	Paediatric immunology outpatient department
KPTH	Kalafong Provincial Tertiary Hospital
LAZ	Length-for-age z score
LBW	Low birthweight
LPV/r	Ritonavir-boosted lopinavir
MTCT	Mother-to-child-transmission of HIV
NHLS	National Health Laboratory Services
NVP	Nevirapine

PCR	Polymerase chain reaction
PEP	Post-exposure prophylaxis
PEPFAR	President's Emergency Plan for AIDS Relief
PMTCT	Prevention of mother-to-child-transmission of HIV
REDCAP	Research Electronic Data Capture
RTHC	Road-to-Health Chart
RTK	Rapid test kit
SAMRC	South African Medical Research Council
SGA	Small-for-gestational age
TasP	Treatment as prevention
TAT	Turnaround time
TB	Tuberculosis
UNAIDS	The Joint United Nations Programme on HIV/AIDS
UNICEF	United Nations Children's Fund
UP	University of Pretoria
UTT	Universal test-and-treat
VEID	Very early infant diagnosis
VL	HIV viral load
WAZ	Weight-for-age z score
WHO	World Health Organization
ZDV	Zidovudine

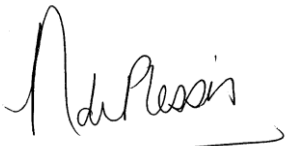
DECLARATION

I declare that the dissertation/thesis, which I hereby submit for the degree Doctor of Philosophy in Paediatrics at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

ETHICS STATEMENT

The author, whose name appears on the title page of this dissertation/thesis, has obtained, for the research described in this work, the applicable research ethics approval.

The author declares that s/he has observed the ethical standards required in terms of the University of Pretoria's Code of Ethics for Researchers and the Policy guidelines for responsible research.

Signature 

Student name *Nicolette Marie du Plessis*

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"The greatest challenge to any thinker is stating the problem in a way that will allow a solution"

Bertrand Russell

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CHAPTER 1

INFANTS AFFECTED BY HIV: PROVIDING A FULL PACKAGE OF CARE

The first child with AIDS was described in 1982, followed in 1983 with both the definition of acquired immunodeficiency syndrome (AIDS) and the the discovery of the virus responsible for this disease, subsequently named the human immunodeficiency virus (HIV).[1]

Women mostly acquire HIV infection by horizontal transfer as a sexually transmitted disease. Infants, however, are generally exposed to and infected with HIV via a vertical route through mother-to-child transmission. When developing prevention and care packages for infants affected by HIV, it is important to consider both the mode and timing of the vertical HIV transmission event(s) and the associated factors that increase the risk for mother-to-child transmission. This literature review aims to describe past and future concepts and challenges in infant HIV prevention and management as a forerunner to the experimental work that will be presented.

1.1 HIV ACQUISITION IN INFANTS

Vertical transmission of HIV from mother to child can take place antepartum, during labour and delivery, or postpartum via breastfeeding, and is known as mother-to-child transmission of HIV (MTCT). In the era before combination antiretroviral therapy (cART) was administered as standard treatment for HIV infected mothers, MTCT varied from 10-30% in non-breastfed infants, to 25-45% among breastfeeding populations.[2]

The exact time and mechanism of the HIV transmission event is still speculative.[3] The proportion of transmission events differs between breastfeeding and non-breastfeeding infants.[3,2] MTCT prevention trials during the last few decades have provided some insight into the timing of transmission of HIV by describing reduction rates linked to specific timed interventions.[3] (see Table 1). Early trials estimating transmission rates from 36 weeks of gestation through to delivery showed late gestation and the peripartum period to be high HIV transmission periods.

Table 1: Estimates of transmission events (%) of MTCT in non-breastfeeding and breastfeeding populations.

Timing of exposure	Transmission rate (%) Non-breastfeeding	Transmission rate (%) Breastfeeding
< 14 weeks gestation	3	2-4
14-36 weeks gestation	17	10-13
36 weeks through labour	50	29-39
Delivery	30	20-26
Breastfeeding ≤ 6months		39*
Breastfeeding for 18-24 months		18*

Adapted from Kourtis, 2006 [3]

**Breastfeeding transmission risk varies widely between studies*

Transmission of HIV through breastfeeding is complex. Whilst it was initially thought that MTCT was highest during the first few months postpartum, the Breastfeeding and HIV International Transmission Study (BHITS) meta-analysis showed a constant rate of 8.9 transmissions per 100 child-years of breastfeeding after the neonatal period for the duration of breastfeeding.[4]

Other maternal and pregnancy-associated risk factors for MTCT that have been identified include a low maternal cluster of differentiation 4 (CD4) cell count, primary HIV type 1 (HIV-1) infection, advanced maternal HIV disease, vaginal delivery, invasive obstetric procedures, chorioamnionitis, pre-labour rupture of membranes >4 hours before delivery, and preterm labour.[2] Low maternal vitamin A levels have been linked to MTCT, but African trials show no benefit of vitamin A supplementation in reducing MTCT rates.[5]

The mechanism of HIV integration and the establishment of HIV infection in the foetus and infant remain an important research focus. Through the use of molecular techniques, studies have demonstrated that unintegrated HIV-1 genomes were found in peripheral blood mononuclear cells in as many as 18% of HIV exposed newborns.[6] In the absence of activated lymphocytes, the unintegrated viral genome decays over time and does not cause an established HIV infection in the exposed infant. Immune activation therefore seems to be an integral part of HIV acquisition in infants.[6]

1.2 INFANT-HIV PREVENTION: MTCT STRATEGIES; EXAMINING THE EVIDENCE

The key factor for prevention of MTCT is the prevention of maternal HIV infection. In HIV infected women, strategies to prevent MTCT are directed at either a specific pregnancy-related phase, such as the intrapartum or postpartum period, or the whole period of vertical transmission risk (from pre-conception through to cessation of breastfeeding). During the first decade of the HIV pandemic, breastfeeding avoidance was the only intervention proposed to reduce MTCT. However, this only reduced postnatal MTCT. By the year 2000, numerous trials had been published that evaluated other interventions targeted at prevention of mother-to-child transmission of HIV (PMTCT) during the antepartum, intrapartum and postpartum periods. These interventions can be broadly categorised into antiretroviral and non-antiretroviral prevention strategies.

Table 2 summarises prominent PMTCT antiretroviral and non-antiretroviral intervention trials over the past few decades conducted in both developing and developed world settings. The different antiretroviral regimens were analysed in terms of percentage reduction in HIV transmission in breastfeeding and non-breastfeeding maternal-infant groups.

Table 2: Summary of prominent interventional trials that studied percentage reduction in HIV transmission of different interventions for PMTCT in breastfeeding and non-breastfeeding infants.

Antiretroviral intervention	Trial name (ARV agent/s)	ARV regimen (mother; infant)	Transmission reduction (%)
Non-breastfed infants	PACTG 076	Maternal ZDV ≥14–34 weeks of gestation + IVI ZDV during labour; infant ZDV 6 weeks	67
	Thai-CDC	Maternal ZDV ≥36 weeks gestation and during labour; no infant treatment	50
	ANRS*	Maternal ZDV+3TC ≥32–36 weeks of gestation + sdNVP at delivery; infant sdNVP+7-day ZDV	78
	PHPT	Maternal ZDV 28 weeks' gestation; infant ZDV 6 weeks	80
	PACTG 316	Maternal NVP at labour onset with routine cART; infant sdNVP	12.5
Breastfed infants	Ivory Coast studies*	Maternal ZDV ≥36-38 weeks of gestation + delivery	37-44
	PETRA trial*	4-armed study comparing use of maternal and infant ZDV+3TC	5-52 (at 6 weeks)
	HIVNET 012*	Maternal sdNVP at onset of labour; infant sdNVP	44 (6-8 weeks)
	SAINT*	Maternal sdNVP labour + postpartum; infant sdNVP vs maternal ZDV+3TC labour and 1 week postpartum; infant ZDV+3TC 1 week	23 (8 weeks)
	DITRAME studies*	Maternal ZDV(+- 3TC) ≥32-36 weeks gestation + 1 week postpartum; infant sdNVP	72-76 (6 weeks)
	Mashi*	Maternal ZDV ≥34 weeks gestation + sdNVP; infant sdNVP + ZDV 1 month	15
Non-ARV intervention	Intervention type	Intervention timing	Transmission reduction (%)
Other interventions	Vaginal disinfection*	Maternal vaginal suppository of 1% benzalkonium chloride >36 weeks; infant bathed with 1% benzalkonium chloride solution within 30 minutes of birth.	No reduction illustrated (Malawi)

	Vitamin A and micronutrients	Maternal vitamin A ≥12-27weeks + intrapartum	No reduction benefits
	Elective caesarean section	Elective caesarean section at term before onset of labour	50-87% (no ARV or ZDV only)

PACTG (Pediatric AIDS Clinical Trials Group); ANRS (Agence Nationale de Recherches sur le SIDA); PHPT (Perinatal HIV Prevention Trial); PETRA (Perinatal Transmission Study); HIVNET (HIV Network National Institute of Health); SAINT (South African Intrapartum Nevirapine Trial); DITRAME (DIminution de la Transmission Mère-Enfant)

ARV: antiretroviral; ZDV: zidovudine; 3TC: lamivudine; sdNVP: single-dose nevirapine

**Trials conducted in Africa.*

Table adapted from Kourtis el al, Mofenson et al, and Dabis et al [3, 7, 8]

PMTCT antiretroviral interventions

The most successful strategy for PMTCT has been antiretroviral therapy, both as treatment for maternal HIV and as post-exposure-prophylaxis for the infant. The exact mechanism of action for this reduction in MTCT is probably a combination of maternal HIV-1 viral load reduction, and/or prevention of establishment of HIV infection in the foetus and infant.[5]

Over the past two decades, different PMTCT drug-strategies have been studied, including single and multiple antiretroviral drug combinations administered to the mother and/or infant as short- or long-course therapy. The feasibility of these antiretroviral treatment (ARV) regimens has been considered following numerous trials conducted in low-middle income communities, particularly in relation to financial implications.[7]

Initial PMTCT studies such as the Pediatric AIDS Clinical Trials Group (PACTG) 076 trial (1994) showed a 67% reduction in the risk of MTCT (from 25.5% to 8.3%) in non-breastfed infants by 6 weeks of age with the use of a combination of maternal antiretroviral drug therapy (3-part regimen of zidovudine (ZDV) given orally to pregnant HIV-1-infected women starting between 14 and 34 weeks of gestation, intravenously during labour, pre- and intrapartum, and short course infant oral prophylaxis, as ZDV for 6 weeks).[9] After critical review of these and other studies, and considering the risk of MTCT at different gestational and infant ages, data suggests that two-thirds of early infant HIV transmission events occur after the first trimester of pregnancy.

The Thai Centers for Disease Control and Prevention (CDC) study demonstrated a 50% reduction in anticipated transmission events using a short course maternal ARV regimen

from 36 weeks gestation in a non-breastfeeding population. When comparing the reduction rate with studies such as the Perinatal HIV Prevention Trial (PHPT) study (also from Thailand), it was estimated that late antepartum and intrapartum transmission events contribute to 80% of transmission events in non-breastfed infants (illustrated in Table 1).

Several studies in Thailand and sub-Saharan Africa showed efficacy of shorter, less expensive ARV combinations of ZDV, ZDV/lamivudine (3TC), or single dose nevirapine (NVP) in mother-infant pairs in breastfed and non-breastfed cohorts. A randomized trial in Thailand in 1998 demonstrated that maternal short course therapy of oral ZDV from 36 weeks gestation and intrapartum, without any infant prophylaxis, reduced transmission rates at 6 months of age by 50% in non-breastfed infants. [10, 11] The efficacy of the maternal short course oral ZDV regimen was also illustrated in two subsequent West African trials at 3 (37%) months and 6 (38%) months.[12]

Data from the Perinatal Transmission (PETRA) trial showed a lack of efficacy in reducing MTCT with the use of only intrapartum maternal ARV treatment. However, there was a 63% reduction in HIV transmission when combining pregnancy, intrapartum and one-week postpartum ZDV/3TC therapy to maternal-infant pairs; and a 42% reduction if this combination was given intrapartum-postpartum only.[13]

Ultrashort NVP prophylaxis is a cost-effective and feasible PMTCT strategy in resource-limited settings. The HIV Network National Institute of Health (HIVNET) 012 trial conducted in Uganda demonstrated a MTCT reduction of 47% at 14-16 weeks, and 42% by 18 months, in breastfed infants when their mothers received a single dose of NVP during labour, followed by a single dose of NVP to their infants at 48-72 hours of life. [14] The risk reduction at 8 weeks of age in maternal-infant pairs enrolled in the HIV Network National Institute of Health SAINT trial was similar in both the intrapartum-postpartum short course NVP and ZDV/3TC arms. Although short term ARV use during pregnancy and postpartum to infants is well tolerated, ARV resistance including NVP-resistant viral mutations has been detected, and remains a concern in HIV infected infants after failed PMTCT.[2]

Recently, HIV treatment as prevention (TasP) has been posed as the strategy that may offer both a solution to the progression of clinical disease and early death among HIV infected patients, as well as a reduction in HIV transmission by reducing HIV exposure per event. The PMTCT program was the first to introduce the concept of treatment as prevention by offering pregnant and lactating women lifelong cART as a PMTCT strategy, in the form of PMTCT Option B+. All 22 global plan priority countries where 90% of pregnant HIV

positive women reside have adopted PMTCT option B+. Sustainability and cost-effectiveness of TasP has been demonstrated in an upper-middle-income country such as British Colombia.[15] TasP further impacts positively to reduce morbidity, mortality, and new HIV diagnoses. This has opened the door to global HIV TasP practices, and has substantiated the use of TasP in all PMTCT programmes.[15]

PMTCT non-antiretroviral interventions

Apart from elective caesarean section and avoiding breastfeeding, no other non-antiretroviral interventions have proven to be as effective as ARVs.

Possible mechanisms for MTCT during labour include transfusion of the mother's blood to the foetus during uterine contractions, infection after membrane rupture, and direct contact of the foetus with infected secretions and blood from the maternal genital tract. On the one hand, trials of elective caesarean sections before membrane rupture compared to vaginal deliveries, demonstrate a 50-87% decrease in the risk of MTCT in women without any ARV coverage or when receiving ZDV only, even with detectable plasma HIV viral loads.[5, 7] On the other hand, sepsis, haemorrhage, anaemia, etc. are expected to be greater with caesarean sections in HIV-infected women. Additionally, research amongst the general population demonstrates that even with accurate assessment of gestational age, the relative risk of neonatal respiratory morbidity with elective caesarean section at 38 weeks of gestation is greater than during the 39th week of gestation.[16] Furthermore, concerns about both maternal and neonatal complications following caesarean section are greater in developing countries, where facilities for both maternal and neonatal care are poor, and manpower is in short supply. Consequently, the role of mode of delivery in the management of HIV-infected women requires evaluation in light of the risks as well as the benefits. The current (2016) Guidelines for Maternity Care in South Africa advocates for safe vaginal delivery techniques and that caesarean sections are done for the same indications as for HIV negative women. These recommendations are made in the background of universal TasP (PMTCT Option B+) as the main PMTCT strategy in South Africa.[17]

Breastfeeding practices in HIV-infected mothers have been the focus of the updated 2016 World Health Organisation (WHO) infant feeding guidelines, whose purpose is to support and give guidance on breastfeeding duration. Breastfeeding whilst adhering to maternal

cART, irrespective of type of breastfeeding, is supported for at least 12 months, and may be continued for up to 24 months or longer providing the mother is receiving cART and is virally suppressed. Although exclusive breastfeeding is advised, the practice of mixed feeding should not be a reason to discontinue breastfeeding in HIV exposed infants, as long as the mother is receiving cART and is virally suppressed.[18-21]

1.3 GLOBAL AND LOCAL PMTCT POLICY FRAMEWORKS

Global PMTCT frameworks

There are four main components to these strategies: (i) primary prevention of HIV infection among women of childbearing age; (ii) prevention of unintended pregnancies among women living with HIV; (iii) prevention of HIV transmission from HIV-infected women to their infants; and (iv) providing appropriate treatment, care and support to mothers living with HIV, their children and their families.(WHO 2010) These components have since been incorporated into, and supported by, comprehensive prevention programs worldwide.

In 2010, the WHO updated the 2006 PMTCT guidelines, following the emergence of new evidence.[22] The guidelines provided two PMTCT options for women who were not receiving cART for their own health (Option A and Option B). In April 2012, a third option emerged (Option B+), supporting lifelong cART administration to all HIV-infected pregnant and lactating women, regardless of CD4 cell count and clinical disease staging (Table 3).[23]

Table 3: WHO PMTCT ARV options and recommendations

Option	Maternal treatment (CD4≤350 cells/mm3)	Maternal prophylaxis (CD4>350 cells/mm3)	Infant prophylaxis
Option A	cART (triple ARV) starting at diagnosis, continue lifelong	Antepartum: AZT from 14 weeks Intrapartum: sdNVP at onset of labour and AZT/3TC Postpartum: AZT/3TC for 7 days	NVP from birth until 1 week after breastfeeding cessation, or 4-6 weeks if non-breastfeeding
Option B	cART (triple ARV) starting at diagnosis, continue lifelong	Triple ARVs from 14 weeks until 1 week after breastmilk exposure has ended	NVP or AZT from birth until 4-6 weeks (regardless of infant feeding method)
Option B+	Lifelong cART (Triple ARV) regardless of CD4 count		NVP or AZT from birth until 4-6 weeks (regardless of infant feeding method)

Adapted from WHO, 2012[23] CD4: cluster of differentiation; cART: combined antiretroviral therapy; ARV: antiretroviral; AZT: zidovudine; sdNVP single-dose nevirapine; 3TC: lamivudine

The WHO PMTCT guidelines included these different options in an attempt to unify international PMTCT strategies while benefitting patients by creating operational simplicity, avoiding ARV drug interruptions, protecting infants against MTCT in future pregnancies, and preventing sexual transmission in sero-discordant couples. High-income regions have achieved near-universal ARV-for-PMTCT coverage. PMTCT ARV coverage in sub-Saharan regions has increased from 53% in 2009 to 79% in WHO African regions by 2015. At the end of 2016 most low- and middle-income countries have implemented Options B+ either in small pilot areas or at a national level.[24]

Other international bodies such as the United Nations Children's Fund (UNICEF), and the Joint United Nations Programme on HIV/AIDS (UNAIDS) have also committed to a comprehensive approach to PMTCT. UNAIDS, the U.S. President's Emergency Plan for AIDS Relief (PEPFAR), and other partners launched the Global Plan towards the Elimination of New HIV Infections among Children by 2015 and Keeping Their Mothers Alive, in 2011.[25] The aim of this Plan was to eliminate new paediatric HIV infections by 2015, and improve maternal, newborn and child survival and health in the context of HIV.[26] Over the course of the Global Plan, a 60% reduction in annual paediatric HIV infections was seen in 21 high-burden countries in sub-Saharan Africa from 2009 to 2015, translating into 1.2 million new paediatric infections averted.[27, 28]

Following the 2007 WHO-led initiative for the elimination of congenital syphilis, the first edition of the Global Guidance on Criteria and Processes for Validation: Elimination of Mother-to-child Transmission of HIV and Syphilis was launched in 2014 as a dual initiative for elimination of mother-to-child transmission of HIV (EMTCT) and congenital syphilis.[29] This initiative aims to ensure quality maternal-child services to decrease child mortality and improve maternal health, and thus to achieve the 4th and 5th Millennium Development Goals (MDGs) and the health-related Sustainable Development Goals (SDG) which were released in 2015.[30] WHO released the second edition of the EMTCT global validation guidance document in 2017.[24]

In May 2016, the World Health Assembly endorsed three new WHO global health strategies on HIV, sexually transmitted infections and hepatitis. These strategies call for Member States and WHO to work together towards the goals of zero new HIV infections in infants by 2020, elimination of congenital syphilis as a public health threat by 2030, and less than 0.1% prevalence of hepatitis B surface antigen (HBsAg) among children by 2030.[24] Another strategy, the Start Free Stay Free AIDS Free Super Fast -Track framework, was established in 2016 after the successes of the Global Plan. These global strategies all focus on prevention of new HIV infections in children and their mothers, as well as testing and treating children, adolescents, and mothers living with HIV.[28, 31] The Global Validation Advisory Committee for EMTCT was established in 2015. Impact targets for validating EMTCT are bulleted below. In order to be valid, these impact targets should be achieved and maintained for one year:[24]

- population case risk of new paediatric HIV infections of ≤50 per 100 000 live births
- HIV MTCT risk of <5% (breastfeeding countries) OR <2% (non-breastfeeding countries)

Knowledge of the number of parturient HIV-infected women, the percentage who are taking cART, and their infants' HIV results, is required to measure these HIV EMTCT impact indicators.[24] In addition to the impact criteria, the WHO has introduced process criteria for EMTCT that must be achieved and sustained for at least two years. These process indicators include antenatal care (ANC) coverage and a minimum of one ANC visit in ≥95% of pregnancies, HIV testing uptake during pregnancy ≥95%, and cART treatment coverage in HIV-positive pregnant women of ≥95%.[24]

Cuba was the first country to receive WHO certification for EMTCT, followed by Thailand, Belarus, Republic of Moldova (syphilis only) and Armenia (HIV only), as well as Anguilla, Montserrat, Cayman Islands, Bermuda, Antigua and Barbuda, and St Christopher and Nevis.[24]

In 2013, a review by Gourley et al. described many factors specific to sub-Saharan Africa that contribute to the low ARV-as-PMTCT uptake, on an individual, community and health-system level.[32] Solution packages specific to low-to middle- income countries focussing on these three levels/tiers are needed if EMTCT goals are to be reached within vulnerable communities.[33] Novel PMTCT strategies that can be used especially in low-to middle-income areas where breastfeeding is promoted, include: development of novel long-acting formulations for prophylaxis of mothers and children, and increasing the inclusion of mothers and infants in care programs to more effectively prevent vertical transmission.[34]

South African PMTCT framework: history and progress

December 1981 and January 1982 saw the first AIDS-related mortalities in South Africa. However, very little attention was given to the disease over the ensuing decade. With the new democracy in 1994, again, HIV was not regarded as a major concern although a national AIDS plan was accepted in late 1994. It was only a decade later, on 1 April 2004, that ARV therapy was made available at health care facilities.[35]

The South Africa National PMTCT programme started in 2002 with maternal and infant single-dose nevirapine (sdNVP), after a Constitutional Court judgement against the Government. The timeline of SA PMTCT guidelines that followed is summarised in Table 4.[36]

Table 4: Timeline for SA PMTCT ARV and infant feeding guideline changes and National ARV guideline modifications (Adapted from Burton et al[36])

Year	SA PMTCT guideline for pregnant women		National ARV guideline for adults
	ARV for PMTCT	Feeding guidelines	
2002	Maternal: sdNVP during labour Infant: sdNVP within 72hrs	Formula feeding was promoted *Free formula milk provided for 6 months if mothers breastfed they were advised to do so for 3-4 months only with abrupt cessation thereafter*	
2004			cART if CD4<200 cells/mm^3 or WHO stage 4
2008	Maternal: AZT from 28wks gestation + sdNVP during labour Infant: sdNVP within 72hrs		
2010	Maternal: AZT from 14wks gestation + sdNVP and tenofovir/3TC during labour Infant: NVP for 6wks (lifelong cART or non-BF) or until breastfeeding cessation if mother not on cART		cART if CD4 < 350 cells/mm^3 + TB disease (any CD4 count)
2011		Exclusive breastfeeding for 6 months with continued breastfeeding to 12 months *Provision of free formula milk discontinued by September 2012*	
2013	Maternal: All pregnant women cART* but women with CD4<350 stop one week after breastfeeding cessation Infant: NVP for 6wks		
2015	All pregnant and breastfeeding women lifelong cART Infant: NVP for 6wks (dual NVP+AZT or extended NVP for infants of mothers that were non-compliant or newly/late diagnosed, respectively)**		Eligibility for cART includes CD4 < 500 cells/mm^3
2017		Any breastfeeding promoted for up to 2 years as long as mother on cART and virally suppressed	Test-and-treat universal lifelong cART

*SA PMTCT: South African Prevention of Mother-to-Child-Transmission of HIV; ART: antiretroviral therapy; sdNVP: single-dose nevirapine; CD4: cluster of differentiation; WHO: World Health Organization; AZT: zidovudine; 3TC: lamivudine; cART: combined antiretroviral therapy; TB: tuberculosis; ARV: antiretroviral *WHO PMTCT ARV Option B: Women initiating cART with CD4 < 350 and no other indication for cART, stop treatment after breastfeeding cessation **WHO PMTCT ARV Option B+*

The South African PMTCT (SAPMTCT) guidelines were published separately from the National HIV management guidelines in 2010,[37] and updated in March 2013.[38] The March 2013 guidelines provided cART as PMTCT for pregnant women, in line with the WHO PMTCT ARV Option B strategy. This recommended lifelong cART for all women with CD4<350 or cART until one week post breastfeeding cessation in women with higher CD4 cell counts. The first national consolidated guidelines for the prevention of mother-to-child transmission of HIV (PMTCT), and the management of HIV in children, adolescents and adults, was adopted in January 2015.[39] The updated guidelines aimed to provide continued guidance towards a reduction in the vertical transmission of HIV, building on research undertaken since the inception of the program, and on the 2010 and 2013 Policy and Guidelines documents.[33]

The 2015 SAPMTCT guidelines adopted WHO PMTCT Option B+ strategy.[39] Clinical management changes included initiation of lifelong cART for all HIV-positive women who are pregnant, breastfeeding or are within 1 year postpartum, regardless of CD4 cell count; efavirenz (EFV) as part of the first-line regimen regardless of the gestation of the pregnancy; HIV viral load testing for women on cART for ≥3 months duration at confirmation of pregnancy; repeat HIV testing for HIV negative women 3-monthly during pregnancy, at labour/delivery, at the 6 week Expanded Programme on Immunisation (EPI) visit, and 3-monthly throughout breastfeeding. Maternal HIV testing would be offered to mothers during routine antenatal care, postnatal care and EPI/child health follow-up visits. Furthermore, the 2015 guidelines made provision for birth HIV-PCR testing for all neonates.[39]

The WHO PMTCT Option B+ policy set the standard for cART initiation in adults regardless of HIV staging or level of immunodeficiency. Recent WHO recommendations support universal test-and-treat (UTT) for all children and adults affected by HIV. UTT supports the Joint United Nations Programme of HIV/AIDS (UNAIDS) 90-90-90 goals of: 90% of all HIV-infected patients should be aware of their HIV status, 90% of HIV-infected patients should receive cART, and 90% of HIV patients on cART should be virally suppressed.[40]

The UTT recommendation has led to new South African HIV guidelines that were adopted on 1 September 2016. South Africa was one of the first African countries to implement UTT.[41] The guidelines recommend that all HIV-positive children, adolescents, and adults should be offered cART treatment, prioritising those with a CD4 count of ≤350.[42]

The ANRS 12249 TasP trial in rural South Africa reported good cART uptake within a UTT setting, even among patients with high CD4 counts. However, staff shortages and healthcare professional practices that were previously focused on the clinically ill and immunologically suppressed individuals, could be reasons for perceived selection bias in initiating cART in patients with the lowest CD4 counts.[43,44] Disappointingly, the impact of TasP on HIV transmission during this trial showed no reduction in HIV incidence.[45] As with Option B+, there were some concerns regarding long-term follow-up and adherence. Poor adherence and follow-up practices could negate the potential benefit of cART in reducing transmission risk and improving maternal health.[46] HIV programmes need to consider three important principles to ensure long-term adherence to cART: correct treatment initiation (prescribing practices), enhancing pill-taking (adherence), and continuation of therapy (retention in ARV services).[45,47]

Infant feeding recommendations within the SAPMTCT programme have been complex and, at times, controversial. Formula feeding has been the recommended feeding practice for HIV-infected women living in developed countries since 1985. In 1992, the WHO recommended that HIV-infected women living in developing countries continue to breastfeed their infants. This recommendation was initially criticised. During the early years (2002 – 2011), South Africa followed the feeding guidelines of resource-rich countries, and advocated formula feeding as part of the SAPMTCT strategy (see Table 4). Several studies in sub-Saharan countries studied HIV-free survival in formula-fed infants compared to breastfed infants of HIV-infected mothers receiving cART or infants on PMTCT prophylaxis. Formula feeding in the setting of a low-income country led to more infant deaths from respiratory and gastrointestinal disease, irrespective of the infant's HIV status. Consequently, the WHO amended its infant feeding recommendations in 2010 in support of breastfeeding for HIV-positive mothers living in resource-limited areas whilst receiving maternal or infant antiretroviral interventions.[22,48] In the same year, WHO recommended a public health approach to infant feeding that promoted one feeding choice, based on what would be best for that setting, to all HIV-infected women accessing public health facilities. The 2010 PMTCT guidelines incorporated the evidence of poor outcomes in formula-fed

infants in low-income settings and supported breastfeeding as a feeding option in HIV-infected women. August 2011 saw The Tshwane Declaration that promoted, protected and supported breastfeeding, and adopted it as the preferred infant feeding method for HIV exposed infants.[49]

1.4 INFANT-HIV DIAGNOSTIC TOOLS AND ALGORITHMS

Infant diagnosis of HIV

Early infant diagnosis and treatment of HIV remains a public health priority globally. During the Children with HIV Early Antiretroviral Therapy (CHER) trial, infants were randomly assigned to receive deferred therapy versus early combined 3-drug antiretroviral (cART) therapy at a median age of 7.4 weeks. The study concluded that early HIV diagnosis and early antiretroviral therapy, irrespective of HIV clinical stage or CD4 cell counts, reduced early infant mortality by 76%, and HIV progression by 75%.[50]

The WHO estimates that, in 2014, only 50% of HIV exposed infants received an early infant diagnosis (EID) test before the age of 2 months. Improvements to EID should be sought in the milieu of revised WHO testing guidelines, global 90-90-90 targets, and the UNAIDS9 2020 Fast-Track goals (cART access to 90% of HIV-infected children, and less than 20 000 new paediatric HIV infections annually), as well as new technologies that can potentially improve access to testing.[51]

Accurate infant diagnosis of HIV requires the use of a suitable HIV laboratory assay at the specific infant age. The Centers for Disease Control and Prevention (CDC) first published guidelines for the diagnosis of HIV-1 infections in 1989, using serological testing. HIV-1 Western blot or HIV-1 indirect immunofluorescence assay (IFA) were then used to confirm positive results. These guidelines were followed by recommendations for HIV-2 antibody testing in 1992, and procedures for confirmatory laboratory testing of positive HIV rapid antibody tests in 2004. At that time, the recommendations and availability were for HIV antibody testing only.[52]

The kinetics of HIV-1 viremia after HIV infection in adults, and the appearance of different laboratory markers are demonstrated in Figure 1.

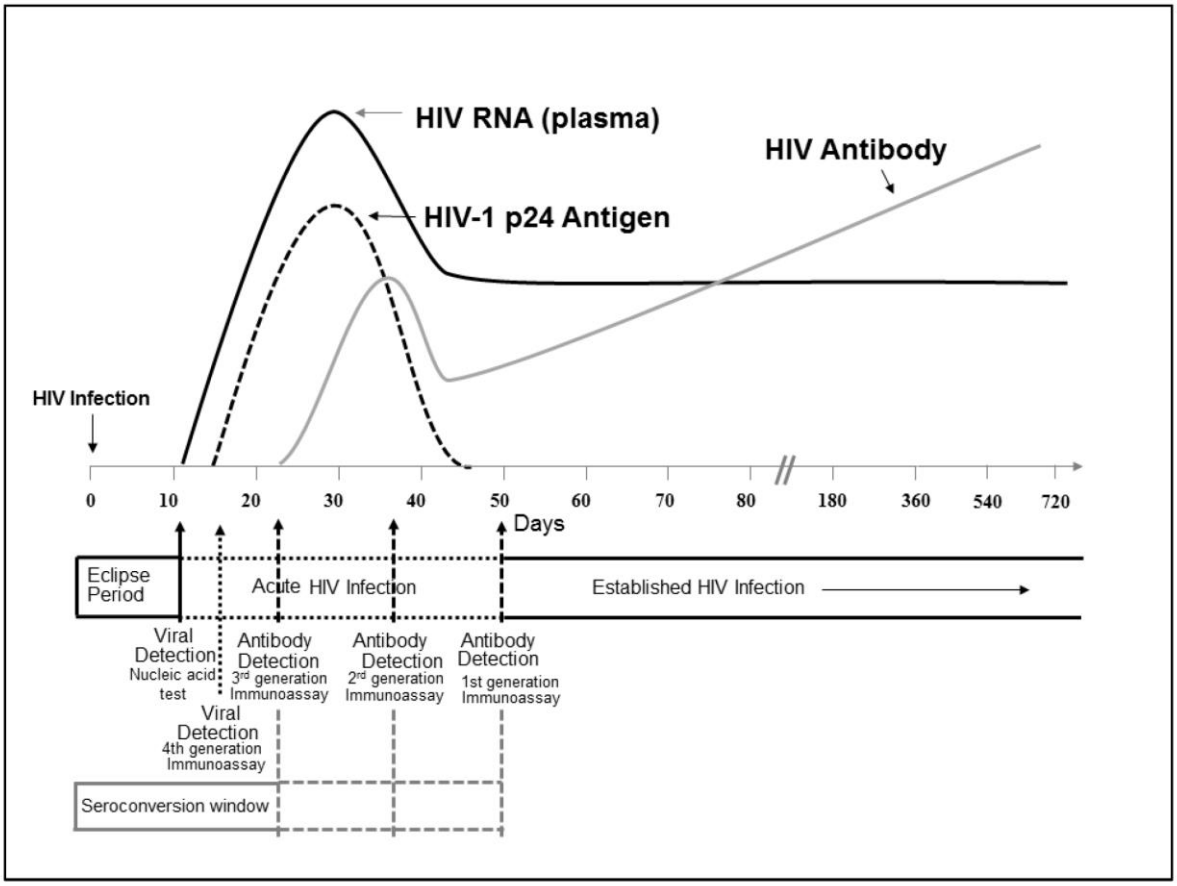

Figure 1: HIV-viremia in relation to different laboratory HIV-specific tests.[52]

There has been an improvement in HIV antibody detection based on different design principles and combinations of detecting anti-HIV IgM and IgG antibodies, as well as monoclonal antibodies against the p24 antigen. The currently used 4th generation immunoassay has allowed detection of HIV before seroconversion.[52]

A definitive diagnosis of infant HIV requires more than immunoassay testing. The presence of passively acquired maternal anti-HIV antibodies in HIV exposed infants and toddlers for up to 18 months necessitates different diagnostic algorithms from those used in older children and adults.[53] In 1989, Rogers et al. showed that the polymerase chain reaction (PCR) was successful in detecting HIV proviral sequences in infants.[53] HIV-PCR testing would become the gold standard in diagnosing HIV infection in HIV exposed children under the age of 18 months.[54,55]

The first nucleic acid testing service implemented in public health programmes was for early infant diagnosis of HIV. Because of the high cost required for PCR technology and infrastructure, this testing remains limited in low-resource countries. Only a few HIV-endemic countries, such as Botswana and South Africa, have national EID programmes.

Some programmes only test exposed infants with serological tests after 18 months of age. Further challenges include tracking of mother-infant pairs who are lost to follow up, and laboratory challenges including reagent availability, sample collection, and turnaround times (TAT) for results.[51]

The WHO recommends testing from 4 to 6 weeks of age with a viral detection assay that, under ideal conditions, has a minimum sensitivity of 95% and specificity of 98%. Drug pressure from both maternal treatment regimens and infant PEP drugs can contribute to decreased sensitivity of PCR assays to diagnose perinatal HIV infection at 6 weeks of age.[56] There is evidence that a single dose of NVP as infant PEP can decrease the HIV viral load (VL) in infected infants to below the limit of detection within 5 days (38%) and 2 weeks (17%).[57, 58] Repeated indeterminate assay results and false negative results have also been reported, making some HIV diagnoses difficult.[59,60]

The new, improved total nucleic acid (TNA) PCR assays such as the COBAS AmpliPrep/COBAS TaqMan (CAP/CTM) HIV-1 qualitative test v1.0 & 2.0 (Roche Molecular Systems, USA), or CAP/CTM v1.0 & 2.0 (Roche) and RealTime HIV-1 (Abbott Molecular, USA), show improved sensitivity and specificity, but have not been studied in the context of the lifelong maternal cART and increased infant ARV exposure/pressure.

Although the global focus of EID has been on the testing of exposed infants between the ages of 4 and 6 weeks, more recently birth testing has been considered feasible.[51] PCR testing at birth detects in utero infection, but cannot detect intrapartum HIV acquisition. Consequent to PMTCT and birth preventative measures relatively more intra uterine infections are expected compared to intrapartum transmission. Studies from both the early years of the HIV pandemic and more recent years therefore support infant HIV testing at birth to detect intra uterine infections early, as infants infected intrauterine have a rapidly deteriorating course and are thus at greater risk of mortality and morbidity. This would ensure that more HIV-infected infants are identified and treated earlier.[58, 61, 62] Improved PMTCT prophylaxis reduces intrapartum infection which, in turn, causes a relative increase in intrauterine infections detectable at birth.[62,63]

Infant testing protocols between 9 and 18 months of age

Current testing algorithms in the older infant rely on HIV-PCR testing of HIV exposed infants. Maternal antibodies, more specifically anti-HIV IgG, are transferred transplacentally to infants. HIV exposed but uninfected (HEU) infants will lose these maternal antibodies and revert to HIV-seronegative (seroreversion) status. HIV serology can therefore not be used in infancy to confirm HIV infection until maternal antibodies have disappeared. Although the maternal anti-HIV IgG antibodies are usually not detected after 9 months of age, they can occasionally (1-2%) be detected in infants up to 18 months of age.

Several factors may affect time to seroreversion. Postulated causes for prolonged (past 18 months) HIV seroreversion times are higher maternal antibody levels in communities with longer HIV endemicity, maternal ARV exposure and changes in transplacental HIV antibody transfer.[64] Factors such as gender, gestational age, malnutrition, and breastfeeding in infants have not been demonstrated to significantly change the time to HIV seroreversion. Gutierrez *et al* published a review of available seroreversion data in 2012 and illustrated that seroreversion in HEU infants happens at a later age than previously reported. Whereas infants born by vaginal delivery were more likely to serorevert at a younger age, maternal ARV exposure, low maternal HIV viral loads, and low maternal CD4 cell counts were associated with later seroreversion. The mechanisms of clearance of HIV-specific immunoglobulins are not fully understood. Some theories include a possible link between seroreversion and a decrease in maternal hypergammaglobulinemia and/or maternal immune reconstitution induced by ARV.[65]

The documentation of seroreversion to confirm the HIV status of a child under 18 months of age is seen as unreliable. Because of a lack of documentation by health care practitioners, some researchers advise that seroreversion should be documented and interpreted by HIV experts.[66]

The use of rapid HIV tests to detect HIV-exposure and seroreversion was studied by Sherman et al. Rapid tests do not all perform equally during infancy. The ideal rapid test should detect HIV exposure early in infancy, and HIV seroreversion from as early as 7 months of age. The ability of an HIV rapid test to detect HIV exposed infants up to 3 months of age was similar to an HIV ELISA test. After 7 months of age, rapid tests show increased specificity; they are thus useful to exclude HIV infection. Different rapid test kits have proven to have differing sensitivities and specificities at different times in infancy. The

influence of prolonged HIV exposure in breastfed infants should be taken into account when using rapid test kits (Alere DetermineTM HIV-1/2 Ag/Ab Combo is advisable in these instances).[67]

Clinically-guided diagnostic algorithms

The use of clinical parameters to diagnose HIV infection amongst infants has previously been studied. The WHO Integrated Management of Childhood Illness (IMCI) HIV algorithm is widely utilised in resource-limited settings to screen for HIV in children. Although it is a valid tool when implemented correctly, it was reported to have a low sensitivity during the first month of life when early identification and initiation of cART are crucial.[68] IMCI training is important for the successful implementation of this tool.[69] The use of clinical parameters as a tool to diagnose HIV infection in infants shows inconsistent results in other studies. Recommendations from these studies suggest that the use of a combination of physical findings may be helpful in identifying infants likely to be HIV-infected, although this needs further validation.[70-72]

New and updated testing algorithms were adopted in the 2013 and 2014 SAPMTCT guidelines in order to identify all HIV exposed infants as early as possible. These include HIV-PCR testing of all HIV exposed symptomatic or high-risk infants any time after birth, and asymptomatic infants at 6 weeks; previous guidelines only suggested HIV PCR testing at the EPI visit at 6 weeks of age. The 2013 guidelines advised that early (birth to <6 weeks) HIV-PCR testing should be done on symptomatic infants. Additional testing algorithms were added in 2014 to include high-risk targeted birth PCR testing. The two recommendations are listed in Table 5.[38,73]

Table 5: The list of symptoms and risk factors that are used to guide early (birth to <6 weeks) infant HIV-PCR testing

DoH 2013	**DoH 2014**
Symptomatic infants:	At birth (targeted):
Failure to thrive (including low birth weight) Haematological abnormalities such as anaemia or thrombocytopenia Congenital pneumonia Pneumonia Hepatosplenomegaly Extensive oral candidiasis Significant lymphadenopathy Any opportunistic infections	Low birth weight <2.5kg Premature infants Infants of mothers who used anti-tuberculous medication for active TB at any point during pregnancy Infants born to mothers with a VL >1000 copies/µl. Infants of HIV-positive mothers who were initiated on ART <4 weeks prior to delivery Infants of mothers who were unbooked, or diagnosed HIV positive in labour or shortly after delivery Breastfed infant of a newly diagnosed HIV-positive breastfeeding mother Infants who are symptomatic at birth

DoH: Department of Health; HIV: Human Immunodeficiency virus; PCR: polymerase chain reaction; ART: antiretroviral therapy

The risk of vertical transmission of HIV varies according to the timing and duration of maternal cART, and the maternal HIV viral load during delivery and breastfeeding. Newly diagnosed maternal HIV at or shortly after delivery carries a high risk of HIV transmission until maternal cART takes effect.[33]

The implementation of birth PCR testing based on clinical characteristics, as per the 2014 SAPMTCT guidelines, can lead to both operational and diagnostic challenges. The practicalities of birth PCR testing in a busy maternity unit, and timeous provision of the results to the patients, are new considerations that will require both training and careful implementation. It is therefore essential to investigate the feasibility and public health impact

of universal versus targeted birth testing (within 48-72 hours) for very early infant HIV diagnosis. In a targeted testing approach, it is important to develop the most sensitive predictor, or combination of predictors, for early identification of infant HIV infection. In addition to the infant characteristics listed above, maternal characteristics considered by HIV clinicians to be important risk factors for vertical HIV transmission also need to be taken into account. These include maternal virological failure, which has been defined as viral load of >1000 copies /ml; short duration (usually less than 12 weeks) of maternal antenatal cART; maternal HIV-positive status diagnosed at or after delivery; non-compliance / irregular compliance with maternal antenatal cART; unbooked mother at delivery; no repeat HIV result in mother's chart after 32 weeks gestation; prolonged rupture of membranes, and maternal TB infection during pregnancy.[3,74,75] Although the maternal risk factors were incorporated in the 2014 SAPMTCT guidelines, regular VL testing of mothers was previously not integrated completely into PMTCT-related card. Additionally the cut-off at >1000 copies/*ul* needs to be reviewed as mothers with VL between 0-1000 may still transmit HIV. In the absence of new research, predictive values for any indicators/risk scores to identify HIV-infected newborns and young infants are undefined. Thus, the introduction of such a set of infant and maternal characteristics into a risk score that may be used to identify high-risk (HIV infected) infants, allowing for earlier, targeted HIV-PCR testing, will be valuable to aid future guidelines.

The South African early infant diagnosis of HIV (EID) programme

South Africa has one of the longest-standing, leading EID programmes globally, and has been providing EID to HIV exposed infants and children since 2000. The South African National Health Laboratory System (NHLS) established a network of molecular testing facilities supported by 10 accredited referral laboratories. These PCR laboratories are linked to a network of referral facilities, making EID accessible to 4000 health services nationally. The sampling method in the South Africa EID programme, dried blood spot (DBS) sampling, led to this method becoming the international EID standard. The centralised model of EID services through the NHLS has permitted data collation and analysis of EID service performance.[51]

HIV-PCR testing at 6 weeks postpartum coincides with the EPI visit to the clinic. This is, however, too late for rapid intervention and early treatment of HIV-infected infants (before

the age of 7.4 weeks) as demonstrated in the CHER trial.[50] Furthermore, treatment initiation is often further delayed or missed as a result of loss to follow-up, which is of particular concern in many PMTCT programs in developing countries.[76]

Modeling the ideal timing of PCR tests in early infant diagnosis for South Africa, by considering birth, 6-, 10- and 14- week EPI visits, demonstrated that when using one PCR test the same number of HIV-positive infants is identified at birth or at 6 weeks of age. When using two PCR tests, the greatest number of HIV-infected infants can be identified at birth and 10 weeks of age.[58] The SAPMTCT programme adopted the birth- and 10-week testing strategy in 2015.

Although EID services were available in most (>95%) South African health care and EPI service centers, a review of these services in 2010 reported many missed opportunities. Poor documentation of maternal and infant HIV test results in the infant's Road-to-Health Chart (RTHC), maternal non-reporting, poor adherence to cART, inadequate maternal knowledge about MTCT, fear of discrimination, and lack of provider-initiated HIV retesting were some of the factors that needed to be addressed.[77]

1.5 INFANT-HIV TREATMENT CONSIDERATIONS AND CHALLENGES

A case report published in 2013 of an HIV-infected child in Mississippi who achieved viral control despite interruption of antiretroviral therapy, raised many challenging questions, and also afforded research opportunities.[78] This case report started the concept of a 'functional cure' in infants if they started on cART "hours" after birth. Shiau and Kuhn reviewed important research questions that need to be addressed around the clinical management of HIV-infected infants and young children.[79] These topics include timing of treatment initiation, indication and expected outcome of ARV therapy, type and combination ARV, and accurate and timely HIV diagnosis in infants. The traditional goal of early cART initiation for children was to reduce mortality and morbidity, as well as to achieve normal growth and development and improve quality of life. Birth PCR-testing in the window period from < 30 hours is an important new strategy, requiring further research to guide protocol change if a "functional cure" is to be considered.[80]

Managing HIV-infected neonates is complex. Circumstances that have led to poor maternal antenatal care and access to PMTCT strategies are likely to continue, and will complicate

the infant's management plan. Follow-up rates vary due to different service locations for maternal-infant postpartum care, and migratory patterns.[81]

The choice of newborn cART drugs is limited. There is a lack of formulations that can be used and given at small-weighted-dosages. Drugs that are currently used for 1st line cART in young children in South Africa are abacavir (ABC), lamivudine (3TC) and ritonavir-boosted lopinavir (LPV/r).[82] Abacavir is only labelled for use after 3 months of age and LPV/r is not recommended for use before 42 weeks postmenstrual age, due to its high ethanol and propylene glycol content in the liquid formulation that can lead to propylene glycol-associated adverse events, especially in preterm infants.[83] Current 1st line regimens are therefore appropriate for use in infants where the HIV diagnosis is confirmed after 6 weeks of age. Due to limited pharmacokinetic data for newborns, the optimal dosing to reach adequate therapeutic drug levels is also not well established for the first 4 weeks of life. Zidovudine (AZT) has dosing recommendations for both term and preterm infants. Lamivudine and AZT requires regular dose adjustments when prescribed during the first weeks of life. Although nevirapine is extensively used in PMTCT programmes and can be used in treatment regimens of newborns, the correct therapeutic dose and need for an induction dosage are less clear.[81,83]

Drug resistance is another factor that should be taken into account. Children exposed to non-nucleoside reverse transcriptase inhibitors (NNRTIs) for PMTCT have a risk of both viral resistance and virological failure on a nevirapine-containing regimen.[84,85] Phylogenetic reconstruction and drug resistance profiles of mother-infant pairs at baseline can provide evidence of either transmitted or acquired drug resistance to the infant, and should be requested if available.[86] In the 2014 South African guidelines, all positive HIV-PCR results require urgent action. Confirmation of the HIV status on a new blood sample and immediate initiation of cART should be carried out.[39] This requires good communication between health care facilities and the laboratory to access positive results within 2-7 days. Patient follow-up is vital to ensure all HIV-PCR-positive patients are appropriately managed as soon as possible.[56] It is further recommended in the guidelines that cART initiation and treatment regimens should be discussed with experts in the field of infant HIV.[39] Treatment guidelines are being developed at leading tertiary centres in South Africa, and include the Neonatal ART Guidance Document of Rahima Moosa Mother and Child Hospital[87] and, following a meeting by the South African HIV Society, Neonatal Diagnosis and Treatment Consultation Group, neonatal treatment guidelines were reviewed and published.[83]

Other clinical management concerns in HIV infected newborns and infants are:

- Growth: Specific cART drugs have been associated with abnormal bone density. The influence of exposure to these drugs through maternal cART needs to be carefully monitored.[88]
- Cognitive development: HIV infected individuals with established neurodevelopmental delay can catch up with their uninfected peers, especially once development is supported by the provision of early cART.[88]
- Co-infections: Although cART leads to improved response to TB therapy, TB therapy can lead to decreased cART effectiveness and increased toxicity due to drug interactions related to induction of the cytochrome p450 system. The effect of HIV on malarial infection in childhood is not clear. Limited paediatric data exists on interactions between cART and antimalarial drugs.[88] Co-morbidities such as co-infection with cytomegalovirus and syphilis add complexities of timing, drug pressure and interactions in newborn HIV management.[81]
- Malnutrition: Malnutrition may be complicated by poor gut absorption of cART drugs. Data suggest that children with malnutrition have worse outcomes after cART initiation. Optimal cART/nutritional combined strategies are needed for malnourished children.[88]
- Metabolic complications: Children on cART have complications such as fat wasting, dyslipidaemias and hyperlactatemia. Lipid management options are limited in children where high cholesterol levels due to prolonged cART may lead to cardiovascular disease.[88]

1.6 SPECIAL CONSIDERATIONS IN HIV EXPOSED UNINFECTED INFANTS

Early infant virological testing is imperative to identify and treat HIV-positive infants who are at risk of early mortality and morbidity. In 2015, only 51% of HIV exposed infants from the 21 Global Plan priority countries received early infant (< 2months of age) testing.[31] Apart from early infant testing, postpartum follow-up of HIV exposed infants remains a challenge. Community health workers are integral to facilitating recognition of HIV exposed infants, providing testing and counselling services to mother-infant pairs throughout breastfeeding and tracing patients who are lost to follow-up. Operational needs include ensuring that birth test results are returned to the patient, testing algorithms are adhered to, testing continues

during the breastfeeding period, and HIV infected infants are initiated on or referred for cART.

Research suggests that high rates of mother-infant pairs are lost to follow-up, and that low retention rates occur in women who are initiated on cART in Option B+ programmes when compared to other adults initiated on cART.[89-91] There are also reports of low retention rates for HIV- exposed infants throughout the entire exposure period until the determination of the final HIV outcome.[90] The major challenge with monitoring the growing cohort of HIV exposed infants, together with their HIV-infected mothers, remains the high burden of work especially for health care workers in clinical facilities.[31]

Children's health, both physically and mentally, is affected by the caregiver's mental capacity, violence, separation, death, poor nutrition, and repeated hospitalisation. Social support and stable interpersonal relationships lead to protection and prevention of short- and long-term mental and physical hardship.[92] These factors are an important consideration when managing a child in a house / environment affected by HIV.

The decreasing rate of vertical HIV transmission results in a large number of HIV exposed but uninfected (HEU) children. Crucial to the management of this growing group of children in health and social programmes is an understanding of the health and economic difficulties that they face.[93] One of the largest cohorts of HEU infants to date was recruited during The Zimbabwe Vitamin A for Mothers and Babies (ZVITAMBO) trial. A HEU group of 3135 infants were followed up for 1-2 years and was compared to an HIV unexposed group (n=9510) between 1997 and 2000. The study demonstrated that HEU infants had an increase morbidity, more admissions to hospital (50%) especially during the neonatal period, and frequent sick clinic visits (30% greater) during infancy than the unexposed infants. Skin infections, lower respiratory tract infections, and oral thrush were more frequent in the HEU group. Mortality amongst the HEU infants was also higher; 3.9-fold and 2.0-fold higher during the first and second years of life respectively. Acute respiratory infections, diarrhoea and/or dysentery, malnutrition, sepsis, and meningitis were frequent causes of death. HEU infants had higher documented growth failure after birth.[94]

Data support concerns that the HEU infant carries a higher mortality risk than an unexposed infant. Results from a prospective Ugandan study showed high background mortality (165.5 per 1000 children) in uninfected children of HIV-infected mothers.[95] Irrespective of infant HIV status, pooled mortality data in Africa show that all children of mothers with advanced HIV disease, or infants affected by maternal death, were at risk of death compared to infants

whose mothers survived or had less severe disease.[96] Previous studies identified various risk factors unique to HEU infants that can contribute to increased mortality and morbidity: severe maternal HIV disease, poor transplacental antibody transfer, replacement feeds instead of breastfeeding, perinatal antiretroviral drug exposure, and an increase in exposure to opportunistic infections from household HIV-infected members.[97]

Growth is a sensitive predictor of overall child health and has been studied in HEU infants. Although growth does not seem to be adversely affected in HEU infants, infants of HIV infected mothers often have lower birth weights when compared to HIV unexposed infants, and they show slower catch-up growth. Feeding choices are also important in considering different growth velocities between breastfed and formula-fed infants.[98]

Earlier reports suggested that up to 44% of HEU infants can present with transient clinical signs of HIV infection.[99] Infections of the skin, mucous membranes, and respiratory tract seem to be common. Severe opportunistic infections such as *Pneumocystis jirovecii* pneumonia (PCP) have also been documented in HEU infants.[97] Chougnet showed that both HIV-infected and HEU infants have reduced interleukin-12 (IL-12) production in cord blood, which can persist for at least 6 months and potentially lead to immunosuppression in these infants.[100] Abnormalities in cell-mediated immunity and T-cell development of HEU infants has also been investigated, with some of the abnormalities found to persist into childhood.[101] It is unclear whether severe infections in some HEU children are related to these immune abnormalities, or are simply due to the increased exposure to pathogens from an immune-suppressed household.[97]

There is little evidence concerning neurodevelopmental outcomes of HEU children in developing countries. The use of infant PEP such as zidovudine as well as maternal ARV during pregnancy has raised concerns in the past about adverse outcomes in infants born to HIV-infected mothers. Most studies are reassuring in that no significant adverse neurodevelopment outcomes are observed in HEU infants. Most detectable neurodevelopmental delays may be as a result of environmental stressors rather than in utero or postpartum ARV exposures.[93, 102]

Early weaning (cessation of breastfeeding at 6 months of age) of HIV exposed infants, especially in low-resource settings, has been demonstrated to significantly increase the risk of serious gastroenteritis, associated hospitalisations and overall gastroenteritis-associated mortality. The presumed reduction in HIV acquisition through early cessation of

breastfeeding is thus counteracted by an inability to provide safe alternative feeds, with a high gastroenteritis-associated morbidity and mortality in HEU infants.[103, 104]

Tuberculosis is endemic in sub-Saharan Africa and is fuelled by the on-going HIV epidemic. The TB and HIV epidemics have important consequences that affect the risk in children of contracting TB.[105] The HIV epidemic has caused a significant increase in the incidence of sputum smear-positive TB, and has led to a decrease in the peak age prevalence, with this now being in young adults of 20–35 years who are often parents of young children.[106, 107]TB infection in infants and young children under the age of 2 years, carries a 43% risk of progression to disease during the subsequent 12 months. Bacille Calmette-Guérin (BCG) vaccination is currently the only preventive strategy in high-burden countries. Isoniazid, although effective in disease prevention if used as post-exposure chemoprophylaxis, has not shown benefit as pre-exposure prophylaxis or in disease-free survival in HEU children.[108]

To date there is little published data on the risk of TB acquisition in HEU infants. In a short communication by Cotton et al. for the PACTG 1041 team, the authors estimated the maximum incidence of TB exposure in the HEU cohort as 10,026/100,000, predicting a possible infection risk of 5013/100,000 and a risk of disease of 2005/100,000. The background incidence of TB in the Western Cape Province was 916 per 100,000 and in children less than 2 years of age approximates 600/100,000.[109]

It is now standard practice to prescribe cART routinely to pregnant and breastfeeding mothers. This has consequently led to a large number of ARV exposed HEU children. The drug exposure usually starts in utero and can last up to 2 years or to breastfeeding cessation.[110] Although some concerns remain regarding the true safety of these drugs, standard drug combinations are now recommended to pregnant women.[39] There is no association between antenatal tenofovir (TDF) exposure and administration up to 12 months of breastfeeding in HEU infants, and poor linear growth in these infants.[110] There is, however, a need to recognise the consequences of prolonged exposure to HIV and antiretroviral drugs in HIV uninfected children.[111]

The Protocol AIDS Clinical Trial Group (PACTG) 076 study confirmed the efficacy of zidovudine in prevention of MTCT. Congenital abnormalities, immune status, growth parameters, and neurodevelopmental outcomes were not adversely affected in HEU infants during this trial.[112] However, an alert by the French Perinatal Cohort reported that antiretroviral nucleoside analogue exposure (such as AZT or 3TC) during the perinatal period could be associated with persistent infant mitochondrial disease. The mitochondrial

dysfunction can manifest as neurological symptoms, abnormal magnetic resonance imaging (MRI) findings, and hyperlactataemia.[113] Although these symptoms are mostly transient, they can persist for up to 6 months in some infants, and should be monitored carefully.[93]

Haematological abnormalities relating to haemoglobin, neutrophil, lymphocyte, and CD4 cell count values are described during the early neonatal period in HIV- exposed infants with intrauterine cART exposure. However, clinically significant differences between the haematological parameters in HEU and HIV unexposed uninfected (HUU) infants are rare.[114] Anaemia has been the primary concern in a prolonged AZT PEP regimen for infants, especially premature or anaemic newborns at birth. NVP used in infant PEP has demonstrated clear effectiveness in PMTCT programmes, but the frequency of resistance mutations in infants who subsequently become infected warrants careful consideration for future treatment regimens in this group.[115]

The NNRTI efavirenz was of particular interest because primate studies and human case reports indicated a potential for congenital neural tube defects.[116, 117] A systematic data review published in 2010 could only rule out a very large increase in risk of neural tube defects with first-trimester EFV exposure. With a limited number of reports the authors were unable to make definitive conclusions regarding the risk of rare outcomes such as neural tube defects.[116] A follow up review by the same group in July 2011 confirmed no increased risk of overall birth defects in infants with first-trimester EFV exposure.[118] Dolutegravir-containing cART regimens has recently been identified to replace EFV-containing regimens. Potential safety concerns in women of childbearing age was reported by the WHO in May 2018 following preliminary analysis of a Botswana study with a high reported rate of neural tube defects during pregnancies of mothers on dolutegravir-regimens.[119]

It is difficult to prove causality between ARV exposure during pregnancy and prematurity or low birth weight. Results of pregnancy outcomes are mixed due to the large number of confounding factors, such as maternal HIV disease staging, smoking, other drug use, maternal viral load and CD4 cell count, and timing of maternal ARV initiation. Research on other potential long-term effects of in utero antiretroviral drug exposure such as growth, development, and possible malignancies is limited, and follow-up time is relatively brief. Data regarding the risk of malignancies in HEU infants exposed to ARV is reassuring. Both the PACTG 219/219C study and the French Perinatal Cohort found that the overall incidence of cancer in HEU children did not differ significantly from that expected for the general population.[120, 121]

Future studies should further dissect the causes of infection susceptibility and growth failure and determine the impact of ART and cotrimoxazole on outcomes of this vulnerable group of infants in the current era.[94]

1.7 SUMMARY AND KEY QUESTIONS RELATING TO INFANTS AFFECTED BY HIV

The literature review highlights important concepts relating to infants affected by HIV. We know that vertical HIV transmission is complex and, although the exact time and mechanism of HIV integration remains speculative, certain high-risk factors such as primary maternal HIV infection during pregnancy have been identified. Maternal cART remain the most effective PMTCT strategy leading to reduction in maternal HIV viral load and possibly prevention of establishment of HIV infection in the foetus and infant. PMTCT strategies, including WHO Option B+, are now focused on providing lifelong maternal cART. Accurate and timeous infant HIV diagnosis is paramount to ensure early HIV treatment. Infant HIV diagnosis relies on PCR technology that comes at a high cost and remains limited in low-resource settings. Targeted PCR testing in HIV exposed symptomatic and/or high-risk infants is not a new concept – this was adopted in the 2013 and 2014 SAPMTCT guidelines – albeit the list of symptoms and risks are long and difficult to implement within busy obstetric and neonatal services. Whilst early infant testing should be PCR-based, older HEU infants will lose maternal antibodies and serorevert to HIV-seronegative. Serological testing (rapid assays and ELISA tests) to detect HIV exposure early in infants and seroreversion as early as 7 months would be a useful and cost-effective diagnostic tool in infant diagnostic algorithms. Infants that are affected by HIV remain a vulnerable group, even after adequate measures to ensure they remain exposed but uninfected. HEU infants have specific health concerns including higher mortality rates, more frequent hospitalisations, and higher rates of growth faltering. The influence of PMTCT programmes that provide lifelong maternal cART and supports prolonged breastfeeding practices need ongoing evaluation to determine the benefits and/or risks to the HEU infant.

Some of the key questions in the area of very early infant diagnosis and treatment were recently summarised by Davies:[122] Intrapartum HIV transmission prevention strategies remain an important research focus. EID algorithms that delineate optimal HIV PCR test timing should be specific to a country or community's resources. With this in mind, universal

or targeted testing algorithms should be considered together with the background HIV-burden-rate in a given population. Follow up and retention in care are important future focus areas in both HIV infected and HEU infants. With new ARV drug availability infant ARV combinations and drug safety are focus areas for research. Ideal cART timing in the newborn period remain unanswered.

This PhD is part of a larger very early infant diagnosis (VEID) study that was conceptualized with the start of birth HIV PCR testing at Kalafong Provincial Tertiary Hospital. The VEID study was designed to answer research questions related to targeted vs universal birth PCR testing, HEU growth outcomes and infant testing algorithms at 9 months of age. Results outlined in the first research chapter (chapter 2) enumerate the number of additional early infant HIV infections identified using a universal HIV testing approach for all HIV exposed infants, compared with a targeted HIV testing approach for mother-infant pairs who meet specified criteria. Additionally, we have investigated the predictive values of individual or combined clinical and laboratory characteristics in identifying very early infant HIV infection. Chapter 3 describes health outcomes, focusing on growth-related outcomes, up to 9 months of age in an HIV exposed birth cohort managed according to current SAPMTCT guidelines. HIV seroreversion rates at 9 months are described in chapter 4 in relation to rapid serological assays and laboratory ELISA testing in an attempt to guide future infant HIV-testing algorithms.

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CONCEPTUAL FRAMEWORK

After reviewing the literature, a conceptual framework was developed to understand the main drivers of infant HIV infection and outcomes of HIV exposed infants. As illustrated in Figure 2, many factors are thought to increase the risk of HIV acquisition in HIV exposed newborn at birth and the exposed infant up to 9-12 months of age. These factors / risks are illustrated in the blue text boxes. Maternal factors include HIV disease staging as evidenced by the maternal viral load, CD4 cell count, opportunistic infections (TB), late seroreversion during pregnancy, maternal cART duration and compliance. Newborn clinical signs that were found to indicate high risk infants (HIV infected newborns) include low birth weight (LBW), small-for-gestational age (SGA), preterm, and symptomatic newborns. If HIV acquisition is not diagnosed at birth, the infants remain HIV exposed uninfected (HEU). There are, again, maternal and infant factors that may increase the risk of HIV acquisition during infancy, poor growth and seroreversion. These factors, illustrated in the blue text boxes, include maternal viral load, CD4 cell count, breastfeeding, cART duration and compliance, low infant birthweight (LBW) and gestational age (small for gestational age (SGA) and preterm).

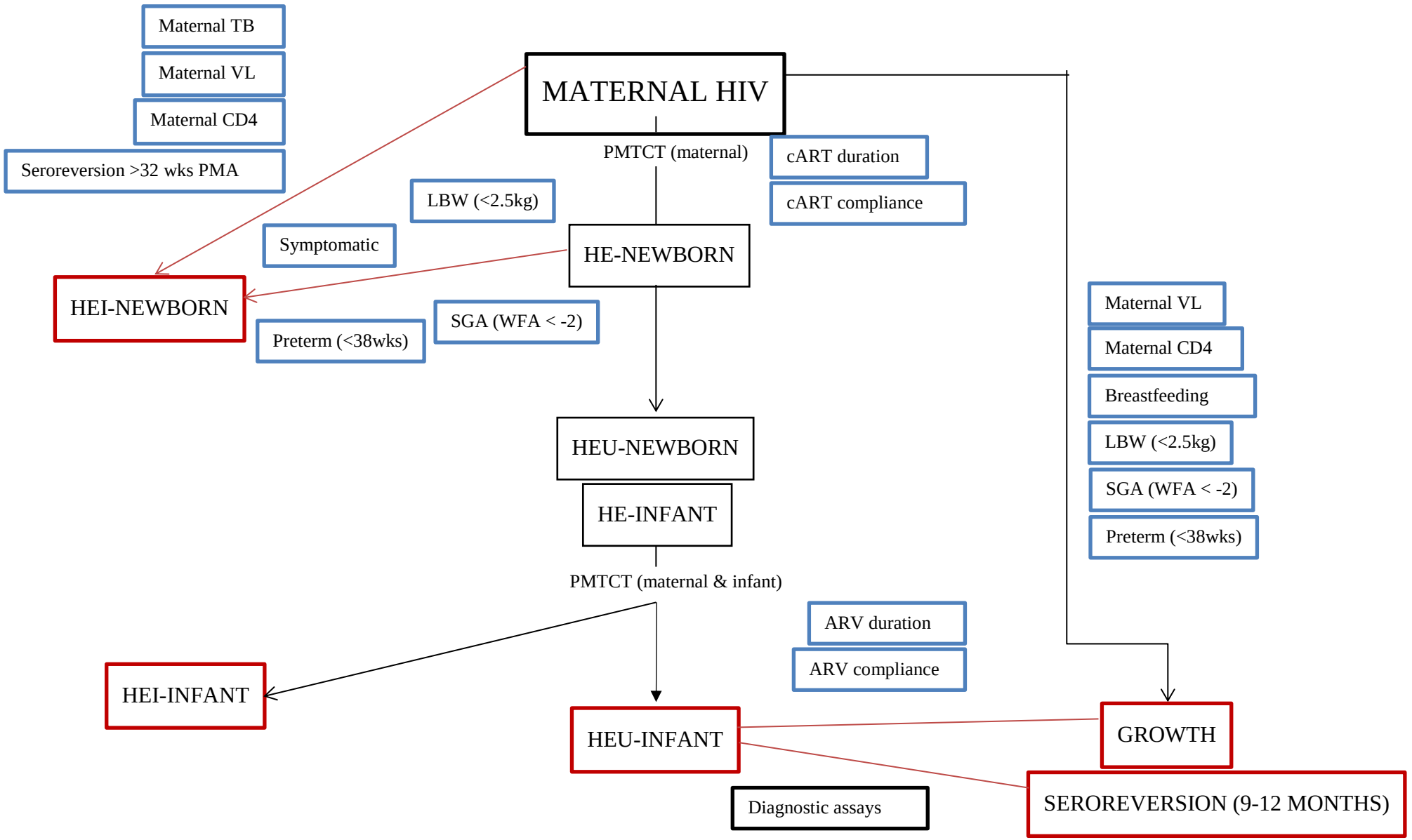

Figure 2: Conceptual framework of the PhD thesis looking at the main drivers of HIV acquisition at birth up to 9-12 months of age

HIV: Human Immunodeficiency virus; TB: tuberculosis: VL: viral load; CD4: cluster of differentiation; PMA: post-menstrual age; PMTCT: Prevention of Mother-to-Child-Transmission of HIV; HE: HIV exposed; HEI: HIV exposed and infected; HEU: HIV exposed but uninfected; LBW: low birthweight; SGA: small-for-gestational age; WFA: weight-for-age; cART: combined antiretroviral therapy; ARV: antiretroviral

PROBLEM STATEMENT

Several advances have been made to decrease infant HIV acquisition. The current global goals aim to eliminate infant HIV transmission by targeting suboptimal antenatal care, maternal treatment failure and primary HIV infection in pregnancy that lead to continued infant acquisition of the disease. Infants born to mothers with these high-risk scenarios are thought to contribute disproportionally to the infant HIV transmission risk. Infants affected by HIV need diagnostic and management considerations that includes appropriate and accurate early and late infant HIV diagnostic algorithms; follow up with specific attention to growth and developmental monitoring, and retention in care of HIV exposed uninfected infants; and a comprehensive health programme that will lead to effective management of the mother-infant unit.

BACKGROUND (VEID STUDY)

This PhD dissertation uses data and describes results from the very early infant diagnosis (VEID) research project.

The VEID project was initiated in August 2014 with the permission of the National Department of Health (NDOH), University of Pretoria Research Ethics Committee (protocol 285_2014) and Kalafong Provincial Tertiary Hospital. This study was part of a National Department of Health initiative to test the public health impact and feasibility of universal versus targeted birth PCR testing, and was implemented at two sites in Gauteng – Kalafong Provincial Tertiary Hospital (KPTH) and Rahima Moosa Mother and Child Hospital (RMMCH).

The study was undertaken at the paediatric and neonatal inpatient services at Kalafong Provincial Tertiary Hospital (KPTH), a tertiary hospital in Tshwane district, Gauteng. KPTH has approximately 6400 deliveries per annum, of which 34% are low birth weight (<2.5kg), 6.3% are very low birth weight (<1.5kg) and 28% are born to HIV exposed mothers. All deliveries are attended by a midwife or doctor. All infants are checked by a paediatric student intern, intern, medical officer or registrar. Adult and paediatric HIV services are provided on site at specialised clinics.

The VEID project aims to evaluate the additional benefit of universal versus targeted very early infant HIV testing and to investigate the use of maternal and infant characteristics in developing a targeted approach to very early infant HIV testing. The first study participant was enrolled on 12 August 2014. A second phase of the research project was initiated on 2 October 2014. Follow up of HIV exposed infants from the VEID project cohort was done at the KPTH Paediatric Immunology Clinic (IPOPD) at 6, 10, and 14 weeks, and 6 and 9 months. Clinical outcomes and infant HIV status were documented and investigated at these time points. Routine care was provided to all infants at these visits, including anthropometric measurements, growth and developmental assessment, and feeding/nutritional counselling; routine Expanded Programme on Immunisation (EPI) vaccines were also given. VEID study recruitment, follow up and management of the birth and follow up cohorts is illustrated in Figure 3. Seroreversion recruitment is shown in Figure 4.

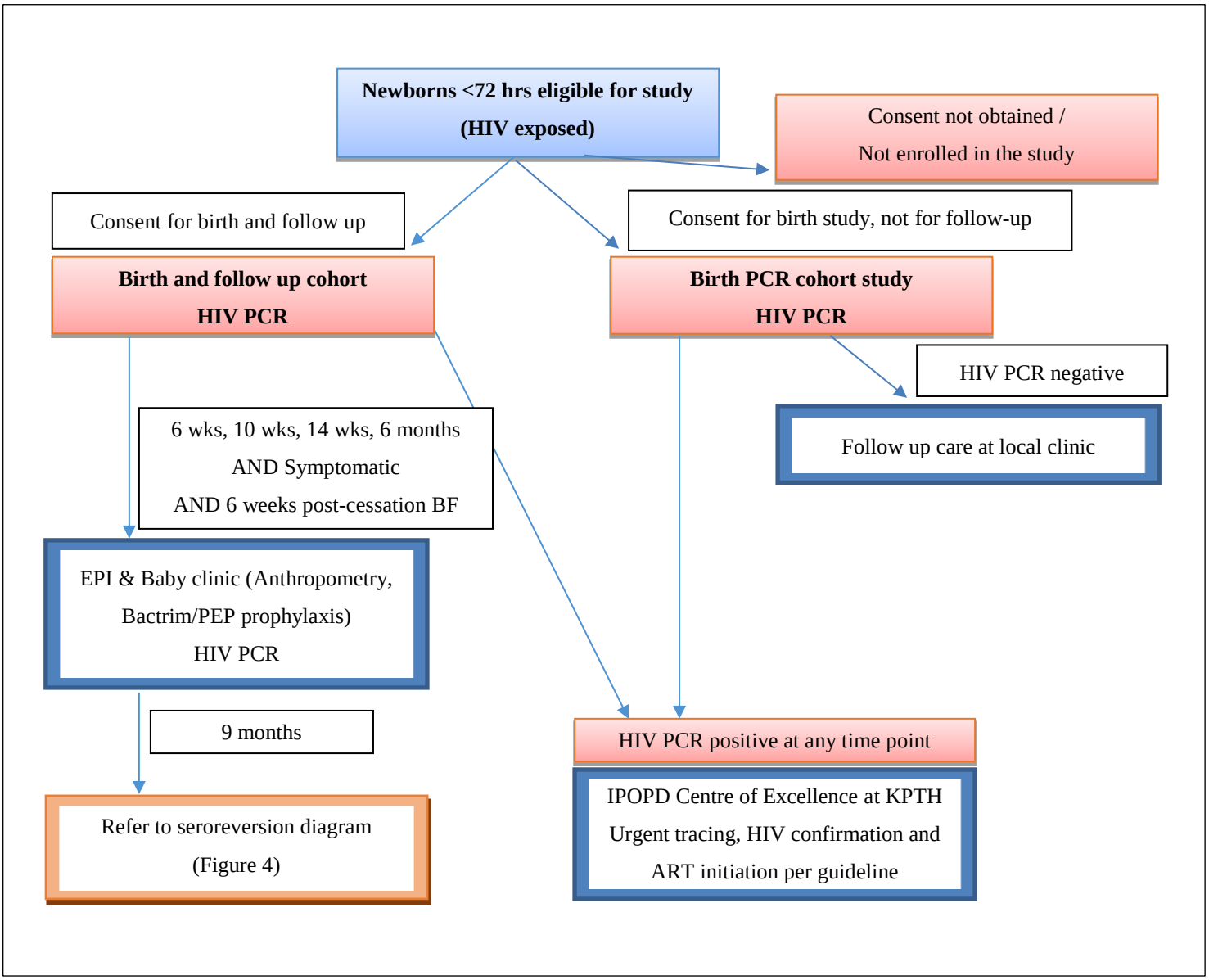

Figure 3: Enrolment, follow up, and management of the birth and follow up cohort

HIV: Human Immunodeficiency virus; TB: tuberculosis: VL: viral load; CD4: cluster of differentiation; PMA: post-menstrual age; PMTCT: Prevention of Mother-to-Child-Transmission of HIV; HE: HIV exposed; HEI: HIV exposed and infected; HEU: HIV exposed but uninfected; LBW: low birthweight; SGA: small-for-gestational age; WFA: weight-for-age; cART: combined antiretroviral therapy; ARV: antiretroviral, IPOPD: Pediatric Immunology Outpatient clinic

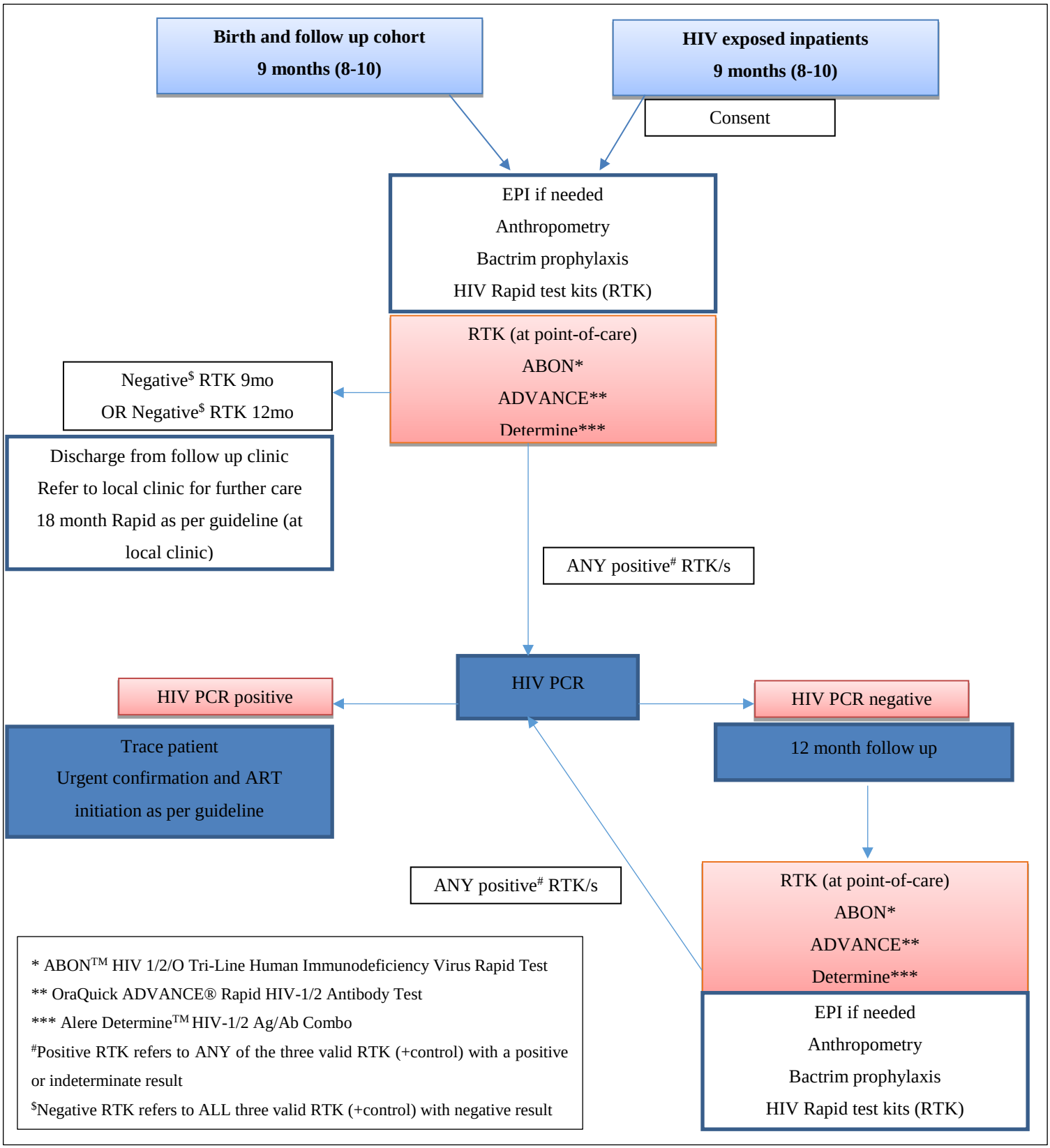

Figure 4: Seroreversion recruitment and testing

EPI: Expanded programme on Immunization; HIV: Human Immunodeficiency virus; PCR: polymerase chain reaction; ART: antiretroviral therapy

CHAPTER 2

Full title: An early-infant HIV-risk score for targeted HIV testing at birth: results from the Very Early Infant Diagnosis of HIV (VEID) study, South Africa.

Short title: An early-infant HIV-risk score for targeted HIV testing at birth.

Authors:
Nicolette M du Plessis (1), Chris JB Muller (2), Theunis Avenant (1), Michael S Pepper (3), Ameena E Goga (1)(4)

(Accepted for publication in Pediatrics 2018-3834: An early-infant HIV-risk score for targeted HIV testing at birth)

Affiliations:

(1) Department of Paediatrics, Faculty of Health Sciences, University of Pretoria, South Africa
(2) Department of Statistics and Actuarial Science, University of Stellenbosch, Stellenbosch, South Africa

(3) Institute for Cellular and Molecular Medicine, Department of Immunology, and SAMRC Extramural Unit for Stem Cell Research and Therapy, Faculty of Health Sciences, University of Pretoria, South Africa

(4) Health Systems Research Unit, South African Medical Research Council, Pretoria, South Africa

Corresponding author:

Nicolette M du Plessis

Department of Paediatrics, University of Pretoria, Pretoria, South Africa

email: nicolette.duplessis@up.ac.za

Author contributions

Nicolette M du Plessis: conceptualization, methodology, software, validation, formal analysis, investigation, data curation, original draft preparation, review and editing

Chris JB Muller: formal analysis, data curation, original draft review and editing

Theunis Avenant: conceptualization, methodology, formal analysis, investigation, original draft review and editing

Michael S Pepper: conceptualization, methodology, original draft review and editing

Ameena E Goga: conceptualization, methodology, validation, data curation, original draft preparation, review and editing

2.1 ABSTRACT

Background

Early HIV testing is needed to guarantee early HIV treatment success for very young infants, but universal testing is expensive. In this study, we examined the feasibility of using an early-infant HIV-risk score for targeted PCR testing and early diagnosis of HIV.

Methods and findings

We reviewed the maternal and infant characteristics of a cross-sectional sample of HIV exposed newborns at Kalafong Provincial Tertiary Hospital, Gauteng, South Africa. Infants were clinically evaluated and tested for HIV infection by PCR within 72 hours of birth. We quantified associations between HIV infection and individual parameters by fitting univariate and multivariate logistic regression models. We determined sensitivity and specificity for various cut-points of the derived risk scores.

From August 2014 to December 2016, of 15 175 live births, 3356 (22.12%) were born to HIV-infected mothers. We screened 1911 infants, and enrolled 1759 (92%) of these. Mothers who had no antenatal care visits (5.7% (97/1688)) were more likely to give birth to babies who tested PCR positive (p=0.0005). Most mothers (98.8%) knew their HIV status before delivery and were on cART (1626/1704, 95.4%). Virological control varied with HIV viral loads not detectable in 595 (60.15%) of mothers. One in five mothers (217/990, 21.9%) had viral loads greater than 1000 copies/μL. More than a quarter of babies (432/1655, 26.1%) were born at a gestational age <38weeks. Low birth weight (<2.5kg) was documented in 398/1598 (24.55%) and 13/31(40.63%) of the PCR negative and positive infants, respectively (p=0.0329). Fewer than 15% of babies were clinically symptomatic at birth. Growth restriction or small for gestational age were documented in 204/1689 (12.08%) babies, of whom six (6/37, 16.22%) were PCR positive. Symptomatic newborns more frequently tested HIV positive (p=0.0042). The newborn HIV PCR positivity rate was 1.8% (31/1759).

The most significant risk factors for HIV infection in very young infants were perinatally detectable maternal viral load, maternal cART <1 month duration, and an infant that was symptomatic at birth. Two-risk (maternal cART duration and viral load), three-risk (maternal cART duration, -viral load and symptomatic newborn), and four-risk (maternal cART duration,

-viral load, symptomatic and SGA newborn) models for HIV acquisition was developed with predictive probability scores of 0.28, 0.498, and 0.57 respectively. These findings could guide a targeted approach to infant HIV-testing at birth. However, using the three- and four-risk scores at a probability of 0.02 and 0.04, 20% and 24% of HIV infected infants will be missed at birth compared with universal testing, respectively.

Conclusion

Targeted PCR testing to diagnose HIV infection in very young infants requires access to maternal viral load testing. Even if risk models include other parameters such as maternal cART history, infant birthweight, gestation estimates and symptoms, one-in-five infected infants will not be targeted for testing. At present we support universal PCR testing at birth within the South African PMTCT context.

2.2 INTRODUCTION

Human immunodeficiency virus (HIV) can be vertically transmitted from mother to child (MTCT) antepartum, during labour and delivery, or postpartum via breastfeeding. Risk factors for MTCT include low maternal cluster of differentiation 4 (CD4) cell count, primary HIV type 1 (HIV-1) infection, advanced maternal HIV disease, vaginal delivery, invasive obstetric procedures, chorioamnionitis, pre-labour rupture of membranes >4 hours before delivery and preterm labour [1]. Despite the success of prevention of MTCT (PMTCT) programs in reducing intrapartum HIV infections [2, 3] in South Africa, proportionately more babies are being born with HIV, due to in-utero transmission [4].

Early infant diagnosis (EID) and treatment of HIV remains a global public health priority. During the Children with HIV Early Antiretroviral Therapy (CHER) trial, infants were randomly assigned to receive deferred therapy versus early combined 3-drug antiretroviral (cART) therapy at a median age of 7.4 weeks [5]. Early HIV diagnosis and early antiretroviral therapy, irrespective of HIV clinical stage or CD4 cell count, reduced early infant mortality by 76%, and HIV progression by 75% [5]. Whilst EID has focussed on testing exposed infants between the ages of 4 and 6 weeks, more recently birth testing has been considered feasible [6]. Birth testing, using polymerase chain reaction (PCR), can detect in-utero infection and allows for initiation of treatment at 14 weeks [7], which is vitally important for these infants who have rapid disease progression and double the mortality rates compared to infants who are infected intrapartum [8].

In 2013 and 2015, the South African Department of Health adopted updated guidelines for infant HIV testing to identify all HIV-infected infants as early as possible [3]. The guidelines included HIV-PCR testing at 6 weeks of age and testing of symptomatic or high-risk HIV exposed infants any time after birth [9]. Additional testing guidelines were added in January 2015 to include PCR testing at birth for high risk infants, then in June 2015, birth HIV-PCR testing of all HIV exposed infants together with 10-week PCR testing was recommended [9, 10].

In this study, we tested the feasibility of using a model to identify infants at high risk for intra-uterine HIV infection for targeted PCR testing. Parameters for the model were extracted from individual or combined clinical and laboratory data. We compare the number of early infant HIV infections identified using a universal and a targeted HIV testing approach at Kalafong Hospital in Pretoria, South Africa. This study forms part of the Very Early Infant Diagnosis of

HIV (VEID) study, a project which was part of a National Department of Health initiative to test the public health impact and feasibility of universal versus targeted birth PCR testing, and was implemented at two sites in Gauteng – Kalafong Provincial Tertiary Hospital (KPTH) and Rahima Moosa Mother and Child Hospital (RMMCH).

2.3 METHODS

A cross-sectional sample of HIV exposed neonates born at or referred, within 72 hours of birth, to Kalafong Provincial Tertiary Hospital (KPTH) were recruited to the study. Data abstraction and infant HIV testing occurred within 72 hours of birth and infants were followed up within seven days if the birth PCR test was positive. Seventy-two hours was chosen as a cut-off for testing as this was feasible in a South African public health setting.
Neonates were recruited from 12 August 2014 until 19 December 2016.

Data collection procedures

Trained research staff recruited and followed-up patients. Researchers identified mothers with known HIV positive or negative status, and mothers of unknown HIV status, daily, from Monday through Friday during working hours, in the obstetric unit pre- and post-delivery. Mothers who were HIV negative were sent for HIV counselling and testing if their last test was done before 32 weeks gestation or >3 months previously, in accordance with national protocols. Mothers with unknown HIV status received HIV counselling and testing.
Researchers interviewed mothers, and recorded data onto a case report form (CRF). Researchers documented self-reported maternal demographic and educational information, as well as maternal and infant characteristics known to be associated with infant HIV acquisition [10]. Researchers reviewed medical records for maternal HIV results, infant birth weight, duration of labour, and PMTCT interventions. The following infant parameters were documented: birth history; anthropometric data: weight, length, head circumference, mid-upper arm circumference (MUAC); and the presence or absence of symptoms (*see addendum A for definitions*) that included failing to thrive (includes LBW), birthweight $\leq$ 2.5 kg, haematological abnormalities like anaemia or thrombocytopenia, if available for infants, congenital pneumonia, hepatosplenomegaly, oral candidiasis, significant lymphadenopathy, and any opportunistic infections. Maternal characteristics documented included antenatal care, labour history, PMTCT interventions and the timing thereof, the last documented maternal viral

load, in accordance with the current SAPMTCT guideline, antiretroviral drug history, and compliance. Poor compliance was defined as <95% dose compliance using self-reported data or visual analogue scale.

Research nurses or qualified medical doctors working at KPTH drew blood from HIV exposed infants, which were tested for HIV infection using total nucleic acid PCR (TNA PCR).

The laboratory reported all HIV PCR positive results to study investigators on a daily basis. Newborns with positive or indeterminate HIV PCR results were referred to HIV clinicians for specialised care. Newborns with confirmed HIV infection were managed by the Immunology (HIV) clinic in accordance with national newborn HIV management guidelines. Mothers with high HIV viral loads or other medical problems were referred to the relevant adult services.

Ethical and legal considerations

Management at KPTH granted permission to conduct the study, and the University of Pretoria, Faculty of Health Sciences Research Ethics Committee gave ethical clearance (protocol 285_2014). Each mother gave written informed consent upon enrolment in the study.

Case report forms were initially paper-based but in November 2014 all CRFs were entered directly into the REDCap (Research Electronic Data Capture, https://www.project-redcap.org/) system hosted at the South Africa Medical Research Council (SAMRC).

Data analysis

From the REDCap system, data were analysed using statistical software SAS (version 9.4 TS1M5). Continuous data were expressed as means and standard deviations (SD) or as medians and interquartile ranges (IQR) for skewed distributions. Discrete or categorical data were summarised using frequencies and percentages. Normally distributed data were compared using independent t-tests, otherwise non-parametric alternatives were used.

The weight-for-age z-scores were calculated using WHO gestational-age-related formulas. We used univariate and multivariate logistic regression models to identify associations between HIV infection, and maternal and infant parameters. The following predictor variables were considered: preterm gestational age (yes = 1, no = 0), low birth weight < 2,5kg (yes = 1, no = 0), maternal HIV viral load (VL) value (LDL = 1, <1000 =2, ≥1000 =3), maternal HIV viral load (VL) value (LDL = 1, <1000 =2, 1000 – 10 000 =3, >10 000 = 4), maternal HIV seroconversion after 32 weeks gestation (yes = 1, no = 0), maternal cART duration at birth < 4weeks (yes = 1, no = 0), small-for-gestational age (yes = 1, no = 0), maternal tuberculosis (yes

= 1, no = 0), symptomatic (yes = 1, no = 0), maternal CD4 cell count value (<200 cells/mm3 = 1, 200-500 cells/mm3 = 2, >500 cells/mm3 = 3). Multivariate regression was initially done on a saturated model (full model) and thereafter reduced to 2-, 3-, and 4-risk models. Model significance was measured with p-values of 0.05 and 0.25.

After fitting logistic regression models, the variables with the highest predictive value were selected using Schwarz Bayesian Information Criterion (SBC or BIC). The performance of the selected models was evaluated using the C-index, or the area under the receiver-operating curve (ROC). We determined the derived risk scores, sensitivity and specificity, as well as false positive and false negative rates for various cut-off values. We developed probability models with the end-point of a positive birth HIV PCR using logistic regression of univariate and multivariate characteristics and risk factors. For modelling purposes, only positive and negative PCR test results were included.

2.4 RESULTS

Study population

Between August 2014 and December 2016, 15175 live babies were born at KPTH, 3356 (22.12%) of these to HIV-infected mothers. Researchers screened 1911 infants within 72 hours of birth (Figure 1). Informed consent was obtained from 1759 (92.05%) eligible patients. Patients with birth HIV PCR test results were included (n=1691, 96.13%).

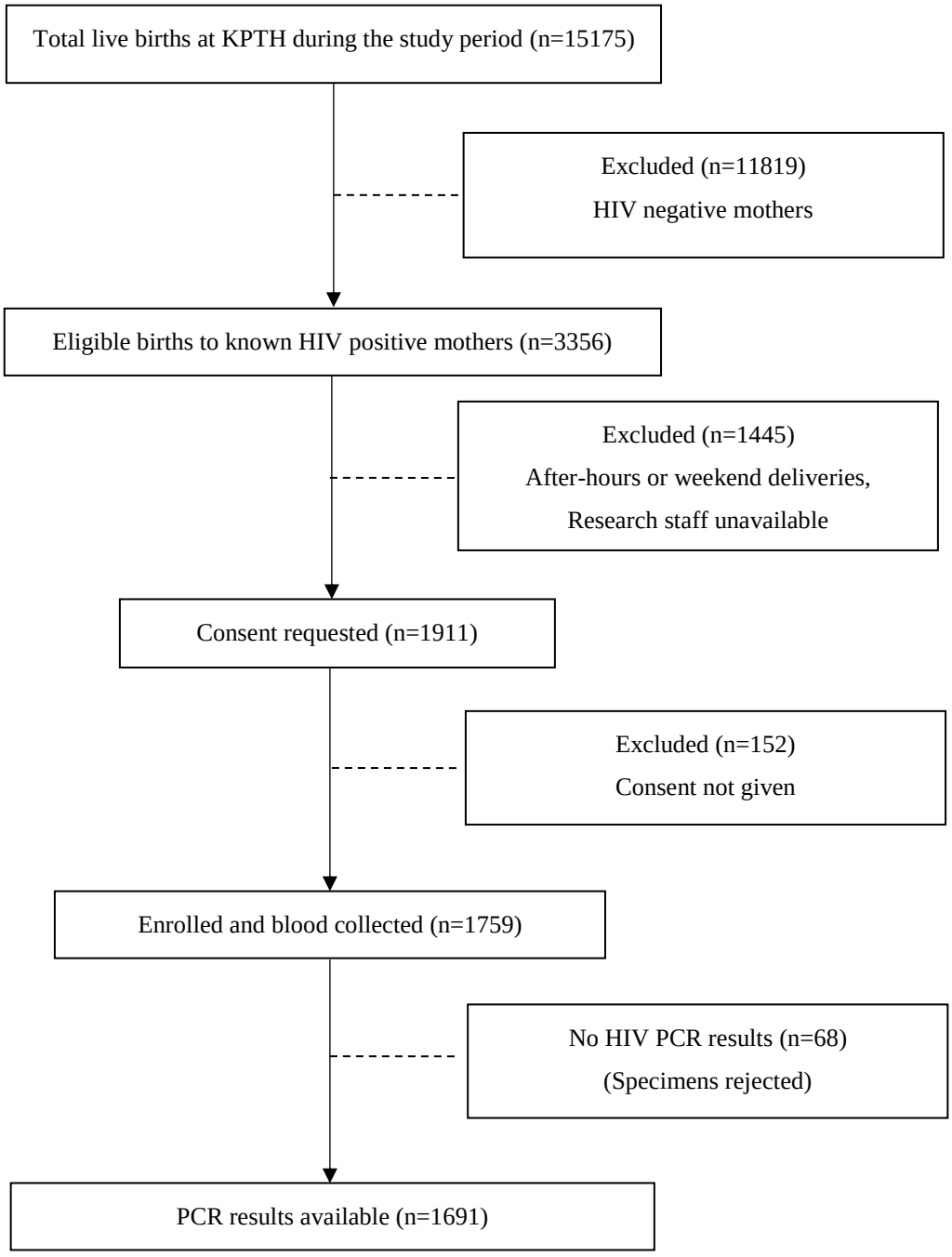

Figure 1: Consort diagram showing the selection of enrolled patients with HIV PCR test results.

PCR: polymerase chain reaction

Maternal characteristics

Almost half of HIV positive mothers were in their thirties (854/1759, 48.55%) and most had a secondary education (1557/1759, 88.52%). Newborns with positive HIV PCR tests were

associated with mothers who were 20 years and younger or between the ages 26 – 29 years. Although most women were not legally married (1370/1706, 80.30%) almost half received monetary support from a partner (809/1726, 46.87%) and just over a third were employed (599, 34.70%). Almost all mothers reported having access to a cell phone (1625/1720, 94.48%) and household assets such as a television (1415, 82.28%) and fridge (1252, 72.79%) (Table 1).

Most mothers knew their HIV status before delivery (98.77%) and were on combination antiretroviral medication (cART) (1626/1704, 95.29%) namely fixed dose combination (FDC) treatment (1473, 90.09%), with 105 (7.09%) having received FDC for less than one month before delivery. Close to a quarter of HIV positive mothers (376/1687, 22.25%) knew their HIV seroreversion towards the end of pregnancy (> 32 weeks' gestation). Using laboratory data, we could trace HIV viral loads within three months of delivery for 935 (53.16%) mothers. Similarly, we could trace CD4 cell counts for 1196 (67.99%) mothers for the preceding 6-months. We recorded 562 (60.11%) mothers with undetectable HIV viral load (LDL), indicating virological control. About 1 in 5 mothers (208, 22.24%) had an HIV viral load level of more than 1000 copies/mL documented within 3 months before delivery. We observed mild immunological suppression in 588 (49.16%) and 394 (32.94%) mothers who had CD4 counts between 200 and 500 cells/mm^3, and > 500 cells/mm^3, respectively. Maternal HIV viral load and maternal CD4 cell count values were negatively correlated -0.103 (p=0.0053).

Very few mothers had tuberculosis (21/1730, 1.21%), most of whom (13, 65%) were being treated for tuberculosis. Only 5.75% (97/1688) of mothers had no ANC visits, the median visits being 4 (IQR 3 – 5 visits). Babies with positive HIV PCR results were associated with mothers who had no ANC visits (p=0.0005) (Table 1). Babies with positive HIV PCR results were associated with mothers who had mean VLs of 53424.55 (SD = 116854.73) copies/mL, while babies with negative HIV PCR results were associated with mothers who had mean VLs of 11361.99 copies/mL (SD = 66185.65) (p=0.0511).

Pre-eclampsia accounted for two thirds of pregnancy complications (165/250, 66.00%) and the proportion of prelabour rupture of membranes (PROM), proven urinary tract infections (UTI), and documented vaginal discharge were all less than 5%, at 3.64% (63/1733), 3.75% (65) and 3.11% (54) respectively. Most mothers planned to breastfeed exclusively (1604/1715, 93.53%).

Table 1: Descriptive statistics of maternal, general and HIV-related variables of the cohort and of the mother-infant pairs with positive HIV PCR tests, Kalafong Provincial Tertiary Hospital, August 2014 – December 2016.

Variables	**Modalities**	**Birth cohort* n (%)**	**PCR positive n/N (%)**	**p-value** (pos vs neg)**
General maternal information				
Maternal age at delivery (yrs) (N=1729)	<= 20 21-25 26-29 30-39 >= 40	81 (4.68%) 295 (17.06%) 401 (23.19%) 854 (49.39%) 98 (5.67%)	5/81 (6.17%) 5/295 (1.69%) 11/401 (2.74%) 13/854 (1.52%) 0/98 (00.00%)	0.0202
Level of education (N=1638)	No education Primary Secondary Tertiary	20 (1.22%) 121 (7.39%) 1495 (91.27%) 2 (0.12%)	1/20 (5.00%) 3/121 (2.48%) 30/1495 (2.01%) 0/2 (0.00%)	0.4087*
Antenatal visits (N=1566)	No ANC visits 1-2 visits 3-5 >5	90 (5.75%) 300 (19.16%) 867 (55.36%) 341(21.78%)	7/90 (7.78%) 11/300 (3.67%) 11/867 (1.27%) 3/341 (0.88%)	0.0005
Maternal HIV diagnosis				
Maternal HIV status known at birth (N=1630)	Yes No	1610 (98.77%) 20 (1.22%)	30/1610 (1.86%) 3/20 (15.00%)	0.0068*
Seroreversion after 32 weeks of gestation (N=1618)	Yes No	360 (22.25%) 1258 (77.75%)	10/360 (2.78%) 22/1258 (1.75%)	0.2047*
Maternal HIV treatment and compliance				
On cART at birth (N=1635)	Yes No	1558 (95.29%)* 77 (4.71%) **1473 (90.09%) of cART were FDC*	27/1558 (1.73%) 6/77 (7.79%)	0.0036*
cART duration <4 weeks at birth (N=1481)	Yes No	105 (7.09%) 1377 (92.91%)	7/105 (6.67%) 20/1377 (1.45%)	0.0020*

Variables	Modalities	Birth cohort* n (%)	PCR positive n/N (%)	p-value** (pos vs neg)
Self-reported compliance to cART (%) (N=1290) Median = 96% IQR 1% – 98%	0-9 10-19 20-89 90-100	68 (5.27%) 7 (0.54%) 172 (13.33%) 1043 (80.85%)	3/68 (4.41%) 2/7 (28.57%) 0/172 (0.00%) 16/1043 (1.53%)	0.0312*
Maternal viral load and CD4 parameters				
Maternal HIV viral load ≤ 3 months of delivery (absolute value, copies/mL) (N=935)	LDL ≤ 1000 ≥ 1000	562 (60.11%) 165 (17.65%) 208 (22.24%)	1/562 (0.18%) 7/165 (4.24%) 27/208 (12.98%)	< 0.0001
Maternal CD4 count within 6 months of delivery (cells/mm3) (N=1196)	< 200 200-500 > 500	214 (17.89%) 588 (49.16%) 394 (32.94%)	12/214 (5.61%) 12/588 (2.04%) 7/394 (1.78%)	0.0089

**Birth cohort includes all enrolled patients. **p values were calculated between the PCR-positive and PCR-negative study groups of the cohort using either *Fisher or Chi-square calculations.*
PCR: polymerase chain reaction, ANC: antenatal care; PROM: preterm rupture of membranes; PET: pre-eclampsia; cART: combination antiretroviral therapy; LDL: lower than detectable levels; TB: tuberculosis

Infant characteristics

Of the infants enrolled in the study, 53.16% (935) were boys. More than a quarter of enrolled infants (432/1655, 26.10%) were born at less than 38 (37 completed) weeks, with a median gestation of 35 weeks (IQR 32-36 weeks) (Table 2).

Table 2: Descriptive statistics of infant characteristics of the cohort and PCR-positive babies born to mother that are HIV positive, Kalafong Tertiary Provincial Hospital, August 2014 – December 2016.

Variables	**Modalities**	**Birth cohort n (%)**	**PCR positive n/N (%)**	**p-value**
Gestational age at birth (weeks) (N=1588)	< 34 weeks	105 (6.61%)	3/105 (2.86%)	0.0015 (Fisher)
	34 – 37 weeks	209 (13.16%)	10/209 (4.78%)	
	>=38 weeks	1526 (96.10%)	19/1526 (1.25%)	
Any birth symptom/sign (N=1689)	IUGR/SGA	204 (12.08%)	6/204 (2.94%)	0.0042
	Pneumonia	28 (1.66%)	1/28 (3.57%)	
	Anaemia	5 (0.30%)	0 (0.00%)	
	Thrombocytopaenia	2 (0.12%)	1/2 (50.00%)	
	Hepatomegaly	2 (0.12%)	0 (0.00%)	
	Splenomegaly	1 (0.06%)	1/1 (100.00%)	

IUGR/SGA: intra-uterine growth restriction / small-for-gestational age

Babies had a mean birth weight of 2.83kg. Low birth weight (<2.5kg) was documented in 398/1598 (24.55%) of the HIV PCR-negative infants and 13/32 (40.63%) in the positive group (p=0.0329 (Chi)). The median z-score for weight was -0.33 (IQR -1.06 – 0.38).

Fewer than 15% (14.33%) of enrolled infants displayed clinical symptoms at birth. No newborns had generalised lymphadenopathy or extensive oral candidiasis. Evidence of growth restriction or small for gestational age (IUGR/SGA) were documented in 204/1689 (12.08%) of the enrolled infants, of whom six were PCR positive. Pneumonia and anaemia were documented in 1.7% and 0.3% of infants, respectively (Table 2). Newborns that were symptomatic more frequently tested HIV positive (p=0.0042).

HIV PCR results

Of the 1691 infants with birth PCR results, 31 (1.8%) were HIV positive, 1646 (97.3%) were negative, and 14 (0.83%) were indeterminate; 3.87% samples were rejected (68/1759). According to the National HIV testing protocol, the indeterminate HIV PCR results were repeated in 12 of the 14 patients, two infants could not be traced. Half of the repeated HIV PCR tests (6/12) tested positive and half negative, increasing the positivity rate to 2.2% (37/1703).

The mean turnaround times (TAT) for the PCR results was 68.27 hours (IQR 46.05 – 93.88 hours).

HIV-risk score models

Univariate (unweighted) model

We used univariate regression models to identify associations between HIV PCR positive outcome, and infant and maternal characteristics.

Newborns with positive PCR results were significantly associated with maternal viral load levels <1000 (OR = 26.53; 95% CI 1.353 to 520.310, p=0.002) and ≥1000 (OR = 123.67; 95% CI 7.385 to >999.99); maternal cART duration less than 4 weeks (OR = 0.146, 95% CI 0.057 to 0.373; p<0.0001); and symptomatic newborns (p = 0.237, 95% CI 0.100 to 0.561; p=0.0011).

Multivariate models

The saturated multivariate regression model only identified two significant risk factors (p<0.05), namely maternal viral load (p=0.0002) and symptomatic newborns (p=0.02). We retained variables with a p < 0.25 in further models to increase predictive value; therefore, cART < 4 weeks (p=0.17) and SGA (p=0.25) were added to risk models. These four parameters were combined in 2-, 3- and 4-risk regression models. Table 3 illustrates risk models, starting with the individual unweighted model, followed by two 2-risk models (model 1 & 2), a 3-risk (model 3) and a 4-risk (model 4) model and finally the full model that used nine parameters in one weighted model. Maternal HIV viral load and infant symptoms were the first risks in the 2-risk model, whilst the second 2-model score modelled 2 maternal parameters, maternal viral load and duration of maternal cART. The 3-risk model incorporated maternal viral load, cART duration, and infant symptomatology; the 4-risk model added infant size for gestational-age to the mentioned 3 risks.

Table 3: Associations between maternal and infant characteristics and weighted newborn HIV acquisition in HIV-exposed infants in the VEID study

Characteristics	**Unadjusted OR (95% CI)** ***P-value***	**Adjusted OR Model 1** ***P-value***	**Adjusted OR Model 2** ***P-value***	**Adjusted OR Model 3** ***P-value***	**Adjusted OR Model 4** ***P-value***	**Adjusted OR Full model** ***P-value***
Preterm gestational age	0.55 (0.23 to 1.30) *0.17*	-	-	-	-	0.51 (0.13 to 1.98) *0.33*
Low birth weight (LBW)	0.42 (0.18 to 1.01) *0.05*	-	-	-	--	2.11 (0.40 to 11.06) *0.38*
Maternal VL (1) (LDL/<1000/≥1000) Comparing values						
<1000 vs LDL	26.53 (1.35 to 520.31)	24.48 (1.27 to 472.49)	30.92 (1.62 to 590.21)	28.11 (1.50 to 527.33)	27.84 (1.55 to 501.43)	25.05 (1.74 to 359.98)
≥1000 vs LDL	123.67 (7.39 to >999.99) *0.0002*	103.17 (6.22 to >999.99) *0.0005*	141.50 (8.66 to >999.99) *0.0002*	117.69 (7.28 to >999.99) *0.0004*	113.82 (7.32 to >999.99) *0.0004*	100.45 (7.98 to >999.99) *0.0002*
Maternal HIV seroconversion >32 week's gestation	0.71 (0.28 to 1.79) *0.46*	-	-	-	-	0.81 (0.28 to 2.28) *0.68*
Maternal cART duration <4 weeks	0.15 (0.06 to 0.37) *<0.0001*	0.31 (0.12 to 0.83) *0.02*		0.45 (0.16 to 1.29) *0.14*	0.42 (0.14 to 1.24) *0.12*	0.46 (0.15 to 1.39) *0.17*
Small-for-gestational age	0.48 (0.19 to 1.23) *0.12*	-	-	-	0.66 (0.22 to 1.97) *0.46*	0.51 (0.16 to 1.60) *0.25*
Maternal TB	0.94 (0.05 to 18.32) *0.97*					1.20 (0.04 to 39.56) *0.92*
Symptomatic (No vs Yes)	0.24 (0.10 to 0.56) *0.001*		0.18 (0.07 to 0.46) *0.0004*	0.21 (0.08 to 0.57) *0.002*	0.24 (0.09 to 0.66) *0.006*	0.22 (0.06 to 0.82) *0.02*
Maternal CD4 cell count	0.35 (0.12 to 0.99)					0.83 (0.26 to 2.62)

(<200/200–500/>500 cells/mm^3)	0.31 (0.12 to 0.83)					0.71 (0.25 to 2.01)
Comparing values:	*0.06*					*0.81*
<200 vs >500						
200-500 vs >500						
Maternal VL (2) (LDL/<1000/1000-10000/>10000)						
Comparing values:						
<1000 vs LDL	26.528 (1.353 to 520.279)					
1000-10000 vs LDL	76.990 (4.177 to >999.999)					
>10000 vs LDL	166.890 (9.799 to >999.999)					
	0.0002					

1600 observations were read, 33 of these from PCR-positive patients, and 634 observations were used

AIC/BIC/R2 for model 1: 135.08/152.89/0.08; model 2: 128.56/146.37/0.09; model 3:126.11/148.37/0.09; model 4: 125.16/151.87/0.10; final model: 123.86/177.29/0.10

AIC: The Akaike information criterion (AIC) is an estimator of the relative quality of statistical models for a given set of data. Given a collection of models for the data, AIC estimates the quality of each model, relative to each of the other models.

BIC (SC): Bayesian information criterion (BIC) / Schwarz criterion (also SBC, SBIC) is a criterion for model selection among a finite set of models; the model with the lowest BIC is preferred. R^2: Variation in y explained by the model. (Cox-Snell measures)

Probability testing of each risk-model

Mothers with an HIV viral load of ≥1000 copies/mL who deliver a symptomatic newborn have a 38% chance of a positive PCR HIV test result at birth (model 1 – 2 risks, Figure 3a). Another 2-risk model, containing only maternal characteristics, maternal viral load and cART duration, showed a probability of 28% for a newborn PCR-positive test if maternal viral load is ≥1000 copies/mL and the mother has not received cART treatment for more than 4 weeks (model 2 – 2 risks, Figure 3b). In model 3, newborns have a probability of 0.49 of a positive PCR test if the newborn is symptomatic, the mother had received cART for less than 4 weeks before delivery and if the mother had a HIV viral load of ≥ 1000 copies/mL (Figure 3c). The four-risk model included maternal characteristics - viral load and cART duration - and infant characteristics - symptomatic newborn and SGA; in this model newborns have the highest probability of a positive HIV PCR if maternal viral load ≥1000 copies/mL, cART duration of less than 4 weeks before delivery, and an infant that is symptomatic and small-for-gestational age (model 4, Figure 3d).

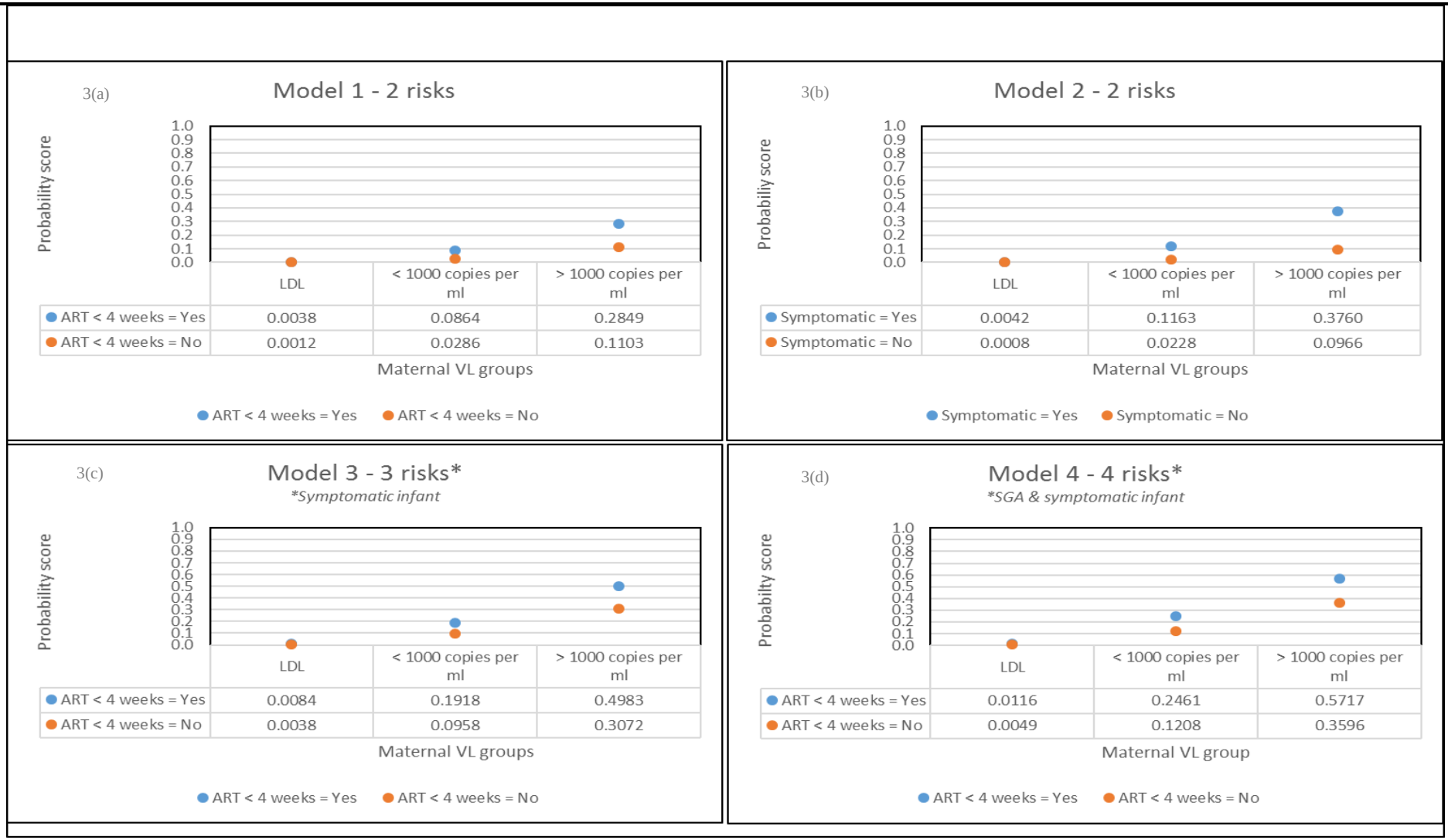

Figure 3: Probability of a HIV-infected newborn by risk factors using multivariable analysis as per models as illustrated in 3(a),3(b),3(c) and 3(d).

Mat_vl_grp = maternal viral load group (1 = lower than detectable, 2 = less than 1000 copies/mL, 3 = equal & more than 1000 copies/mL); sympt = symptomatic; art_less_4w_del = combination antiretroviral therapy less than 4 weeks' duration; SGA = small-for-gestational age

We used logistic regression to calculate the predicted probability of HIV-positivity for each patient using the 4-risk model. These results, together with the statistical equation for the 4-risk model, are shown in the addendum. Each model can be used, at a risk-score probability cut off, to determine targeted PCR testing algorithms. When using both the 3- and 4-risk model scores and considering a probability of 0.02 and 0.04 as an indication for targeted birth testing, the sensitivity is 80% and 76% respectively (Table 4).

Table 4: Sensitivity and specificity values of both 3- and 4-risk models as probability levels 0.02 and 0.04.

	Probability level	**True positive (Sensitivity)**	**True negative (Specificity)**
3- & 4-risk scores	0.02	20/25 (80%)	494/773 (64%)
	0.04	19/25 (76%)	603/773 (78%)

2.5 DISCUSSION

In this study, we identified associations between maternal and infant characteristics and positive HIV PCR tests for newborns. We combined significant variables to build predictive models for early-infant HIV-risk scores to detect HIV infection at birth. According to our models, newborns had the highest (0.57) probability for a positive HIV PCR positivity if newborns were symptomatic and SGA, and were born to a mother who received cART for <4 weeks and had a VL≥1000. Newborns had a 0.28 probability of a positive HIV PCR if only maternal VL≥1000 and cART<4 weeks were included in the model. Our study population had high HIV prevalence among mothers (22.12%) and overall PCR positivity in newborns was 2.2%, which is higher than the national average of 1.1% [3].

Mothers who gave birth to newborns with in-utero acquired HIV more frequently had either no (p=0.0023) or fewer than three ANC visits (p=0.02). At the time of the study, basic antenatal care guidelines in South Africa recommended an early ANC visit (<12 weeks gestation) followed by four follow-up ANC visits for low risk pregnancies. In mid-2016, eight follow-up visits were recommended, and mothers who test HIV positive during pregnancy should initiate cART a week later [11]. Mothers who are infected with HIV

should receive continuous education about pregnancy-related issues. Antenatal care should complement HIV monitoring and treatment in these mothers.

Almost all mothers knew their HIV status before delivery (99%) and were on cART (95%). Encouragingly, these values are line with two of the current UNAIDS 90-90-90 goals, namely, 90% of all HIV-infected patients should be aware of their HIV status and 90% of HIV-infected patients should receive cART [12]. Mothers who had been on cART for shorter than 4 weeks were significantly associated with newborns who had a positive PCR HIV test at birth. Despite most mothers knowing their status and receiving cART, only 60% had achieved viral suppression, far less than the target of 90% of HIV patients on cART who should be virally suppressed to reach the 90-90-90 goals.

In-utero HIV infection in newborns was significantly associated with a detectable maternal viral load. Both VL levels < 1000 and ≥ 1000 copies/mL proved significant risks for in-utero HIV acquisition ($p = 0.0002$ and $p < 0.0001$ respectively), in both univariate and multivariate analyses. Myer et al. emphasized the importance of diligent maternal viral load monitoring and management during pregnancy and breastfeeding. Viral load testing 4 weeks before or at delivery can provide valuable information to guide targeted interventions from birth, including birth-PCR testing [13, 14]. Healthcare facilities providing ANC services should focus on the importance of providing cART for mothers at least 4 weeks before delivery. Mothers who are HIV positive should be enrolled in PMTCT programmes, which should consider repeat HIV testing, treatment, and viral load monitoring at 34-36 weeks' gestation. In future, the value of maternal VL point-of-care testing should be assessed.

In our study, HIV infected newborns were more likely to be preterm (< 38 weeks gestation), low birth weight, and small-for-gestational age, although none of these parameters were significantly associated with a positive HIV PCR test in the univariate regression models. We included small-for-gestational age values in the 4-model multivariate analysis, which added predictive value to targeted testing models. We recommend that all healthcare facilities with maternity services have infant gestational age tables and infant-scales to determine weight-for-age measurements for HIV-exposed newborns.

The newborn HIV PCR positivity rate was 1.83% (31/1691) with less than 5% of samples rejected (68, 3.9%). After repeat testing of the indeterminate results, an additional six patients tested HIV positive, increasing the positivity rate to 2.2% (37/1703). With a

maternal HIV positivity percentage of 22% and 1.8% MTCT, 396 per 100 000 live births were HIV infected in this study. Goga et al. [15] looked at early (4-8 weeks) MTCT risk in South Africa from 2011-2012 and 2012-2013 in relation to the South African PMTCT strategy, which is in line with WHO Option A. In 2011-2012, MTCT was 2.7% and 2.6% in 2012 – 2013, varying between provinces (1.9-5.4%). Mothers who started cART during or before the first trimester of pregnancy had low risk of early MTCT (1.2%) [2]. We recorded a similar rate of MTCT, indicating that most MTCT events are therefore in-utero and intrapartum. The risk has not changed significantly in the past five to six years. The current South African MTCT case rate is not close to the elimination of MTCT (EMTCT) target of ≤50 per 100 000 live births [16].

In this study, we present models for targeted birth PCR testing which may be useful to diagnose newborn HIV infection in resource constrained settings. Additionally, risk models could be used to identify exposed uninfected infants that could benefit from a multiple drug (2- or 3-drug) post-exposure prophylaxis regimen compared to a single drug regimen. Our findings indicate that maternal VL testing is vital for a targeted birth PCR approach. Maternal cART history, infant birth weight, gestation estimates and symptoms can also be combined in two-, three-, and four-risk models. Our 3- and 4-risk models achieved a sensitivity of close to 80%, indicating that 1-in-5 HIV infected newborns will not be offered targeted birth testing. Globally, EMTCT and early infant diagnosis and treatment are prioritised, thus we support universal birth testing within the South African PMTCT programme.

2.6 ACKNOWLEDGEMENT

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2.8 ARTICLE ADDENDUM

S1 Appendix: Definitions for infant- and maternal characteristics captured during the very early infant diagnosis (VEID) study

S1 Table: Probability scores of infant HIV acquisition per infant risk model

Other PhD tables: Log regression models of individual and combined parameters

S1 Appendix: Definitions for infant- and maternal characteristics captured during the very early infant diagnosis (VEID) study

Infant characteristics

- Failing to thrive (includes LBW)
    - Less or equal to 2.5 kg
        - Preterm growth charts
    - WHO growth charts less than -2 z-scores
        - Z-score charts for male and female infants
- Haematological abnormality like anaemia or thrombocytopaenia (if available on infants)
    - Reference ranges for age (http://www.labcareplus.org/docs/REFERENCE_RANGES.pdf)
- Congenital pneumonia
    - Diagnosis of pneumonia in the first 28 days of life presumed to be secondary to congenital exposure / causes
    - Respiratory distress
        - Tachypnoea > 60 breaths per minute (bpm), retractions, grunting, apnoea, alar flare, cyanoses
    - Maternal risk factors for congenital pneumonia to be documented
        - Preterm rapture of membranes, maternal pyrexia, raised inflammatory markers in maternal blood, foul smelling liquor
- Pneumonia
    - According to the South African EDL guidelines any patient under the age of 60 days must be classified as having very severe pneumonia.
    - Non-severe
        - Cough and fast breathing > 60/bpm
    - Severe (Above plus one of the following)
        - Lower chest wall indrawing
        - Auscultatory signs i.e. decreased breath sounds, bronchial breathing, crackles, increased vocal resonance or pleural rub
        - Dullness on percussion
    - Very severe (Above plus at least one)
        - Central cyanosis
        - Inability to feed
        - Convulsions, lethargy or decreased level of consciousness
        - Grunting
        - Nasal flaring
        - < 60 days old
- Hepatosplenomegaly
    - Hepatomegaly defined as a palpable liver > 1 cm below the costal margin in the right mid-clavicular line.

  - Splenomegaly noted if the spleen is palpable.
- Extensive oral candidiasis
  - Candida confined to the tongue and buccal mucosa and/or extending into the pharynx and/or oesophagus. It must be painful and interferes with eating and swallowing to be extensive.
- Significant lymphadenopathy
  - Palpable lymph nodes greater than 1 cm
- Any opportunistic infections - as defined in the Guidelines for the Prevention and Treatment of Opportunistic Infections Among HIV-Exposed and HIV-Infected Children[20]

Maternal characteristics

- Detailed PMTCT interventions and timing thereof (poor characteristic will be defined as no repeat HIV result in mother's chart after 32 weeks gestation)
- Maternal HIV positive status, date of diagnosis (diagnosed at or after delivery)
- Maternal viral load ≥1000 copies /ml (date and value)
- CD4 T-cell count (date and value)
- Antiretroviral (ART) history, including duration of maternal ART
- Compliance with maternal antenatal ART (poor compliance will be defined as< 95% dose compliance using self-reported data or visual analogue scale)
- Antenatal care received at any facility (poor antenatal care will be defined as first antenatal visit only 4 weeks prior to delivery or no known HIV results at delivery or less than 4 antenatal visits in total)
- Birth gestation – as indicated on the ANC card, or documented by the attending obstetrician, or estimated by Ballard score at birth if gestation is unknown (prematurity defined as infant born before 37 completed weeks gestational age)
- Labour details (prolonged - >4 hours- - rupture of membranes)
- Other maternal comorbidities (tuberculosis, hypertension, documented urinary tract infection, documented vaginal discharge)

S1 Table: Probability scores of infant HIV acquisition per infant risk model

Probability values for model 1 (maternal cART duration & viral load group)

Probability	*Viral load group*	*cART duration < 4 weeks*
0.0008	1	No
0.0042	1	Yes
0.0228	2	No
0.1163	2	Yes
0.0966	3	No
0.3760	3	Yes

Probability values for model 2 (maternal VL group & newborn symptoms)

Probability	*Viral load group*	*Symptomatic*
0.0008	1	No
0.0042	1	Yes
0.0228	2	No
0.1163	2	Yes
0.0966	3	No
0.3760	3	Yes

Probability values for model 3 (maternal VL, cART duration & symptoms)

Probability	*Viral load group*	*cART duration < 4weeks*	*Symptomatic*
0.0008	1	No	No
0.0018	1	Yes	No
0.0038	1	No	Yes
0.0084	1	Yes	Yes
0.0221	2	No	No
0.0481	2	Yes	No
0.0863	3	No	No
0.0958	2	No	Yes
0.1746	3	Yes	No
0.1918	2	Yes	Yes
0.3072	3	No	Yes
0.4983	3	Yes	Yes

Probability values for model 4 (maternal VL, cART duration, SGA & symptoms)

Probability	*Viral load group*	*cART duration < 4weeks*	*SGA*	*Symptomatic*
0.0008	1	No	No	No

0.0012	1	No	Yes	No
0.0019	1	Yes	No	No
0.0028	1	Yes	Yes	No
0.0033	1	No	No	Yes
0.0049	1	No	Yes	Yes
0.0077	1	Yes	No	Yes
0.0116	1	Yes	Yes	Yes
0.0212	2	No	No	No
0.0318	2	No	Yes	No
0.0491	2	Yes	No	No
0.0724	2	Yes	Yes	No
0.0815	3	No	No	No
0.0833	2	No	No	Yes
0.1183	3	No	Yes	No
0.1208	2	No	Yes	Yes
0.1742	3	Yes	No	No
0.1777	2	Yes	No	Yes
0.2418	3	Yes	Yes	No
0.2461	2	Yes	Yes	Yes
0.2709	3	No	No	Yes
0.3596	3	No	Yes	Yes
0.4690	3	Yes	No	Yes
0.5717	3	Yes	Yes	Yes

Other PhD tables: Log regression models of individual and combined parameters

Model	Parameter	AIC	BIC	R2	p-value	OR	95% CI	Predictive probability (PCR positive)
1	Preterm gestational age	187.486	196.390	0.0025	0.1713	0.545	0.229 - 1.300	0.030 (No) vs 0.054 (Yes)
2	Low birth weight	185.928	194.832	0.0055	0.0523	0.422	0.176 - 1.009	0.029 (No) vs 0.065 (Yes)
3	Maternal HIV viral load (VL) value: LDL *(lower than detectable)* <1000 *(less than 1000 copies/mL)* ≥1000 *(equal and more than 1000 copies/mL)*	140.275	153.631	0.0746	0.0002			
	Comparing maternal VL <1000 to a VL LDL					26.529	1.353 - 520.310	0.001 (grp1) vs 0.033 (grp2) vs 0.137 (grp3)
	Comparing maternal VL ≥1000 to VL LDL					123.67	7.385 - >999.99	
4	Maternal HIV viral load (VL) value: LDL *(lower than detectable)* <1000 *(less than 1000 copies/mL)* 1000-10 000 *(between 1000 and 10000 copies/mL)*	138.008	155.816	0.0797	0.0002			

	>10 000 (*more than 10 000 copies/mL*)							
	Comparing maternal VL LDL with a VL of >10 000					0.006	<0.001 - 0.102	0.001 (grp1) vs 0.033 (grp2) vs 0.090 (grp3) vs 0.177 (grp4)
	Comparing maternal VL < 1000 to VL >10 000					0.159	0.047 - 0.535	
	Comparing maternal VL between 1000-10 000 to a VL >10 000					0.461	0.161 - 1.321	
5	Maternal HIV seroconversion after 32 weeks gestation	188.827	197.731	0.0008	0.4642	0.707	0.279 - 1.791	0.033 (No) vs 0.046 (Yes)
6	Maternal cART duration at birth < 4weeks	177.295	186.199	0.0202	<.0001	0.146	0.057 - 0.373	0.026 (No) vs 0.156 (Yes)
7	Small-for-gestational age	187.434	196.338	0.0034	0.1247	0.480	0.188 - 1.225	0.031 (No) vs 0.062 (Yes)
8	Maternal tuberculosis	191.558	200.462	0.0000	0.9659	0.937	0.048 - 18.323	0.036 (No) vs 0.038 (Yes)
9	Symptomatic	180.153	189.057	0.0148	0.0011	0.237	0.100 - 0.561	0.025 (No) vs 0.098 (Yes)
10	Maternal CD4 cell count value: <200 cells/mm^3 200-500 cells/mm^3 >500 cells/mm^3	183.548	196.904	0.0090	0.0578			

	Comparing maternal CD4 cell count of <200 cells/mm^3 to a count of >500 cells/mm^3					0.345	0.120 - 0.990	0.027 (grp1) vs 0.0300 (grp2) vs 0.082 (grp3)
	Comparing maternal CD4 cell count of between 200-500 cells/mm^3 to a cell count >500 cells/mm^3					0.312	0.117 - 0.831	

AIC: The Akaike information criterion (AIC) is an estimator of the relative quality of statistical models for a given set of data. Given a collection of models for the data, AIC estimates the quality of each model, relative to each of the other models.

BIC (SC): Bayesian information criterion (BIC) / Schwarz criterion (also SBC, SBIC) is a criterion for model selection among a finite set of models; the model with the lowest BIC is preferred. R^2: Variation in y explained by the model. (Cox-Snell measures)

Multivariate regression models – saturated (full), 4-, 3-, and 2-risk models

Model	AIC	BIC	R2	Variable	p-value	OR	95% CI
ALL	123.862	177.287	0.0961				
				Preterm gestational age No vs Yes	0.3319	0.511	0.132 - 1.983
				Low birth weight No vs Yes	0.3770	2.110	0.403 – 11.063
				Maternal HIV viral load (VL) value: LDL *(lower than detectable)* <1000 *(less than 1000 copies/mL)* ≥1000 *(more than 1000 copies/mL)*	0.0002		
				Comparing maternal VL <1000 to a VL LDL		25.052	1.743 – 359.979
				Comparing maternal VL ≥1000 to VL LDL		100.449	7.975 - >999.999

				Maternal HIV seroconversion after 32 weeks gestation No vs Yes	0.6824	0.805	0.284 – 2.278
				Maternal cART duration at birth < 4weeks No vs Yes	0.1693	0.462	0.153 – 1.390
				Small-for-gestational age No vs Yes	0.2462	0.507	0.161 – 1.597
				Maternal tuberculosis No vs Yes	0.9206	1.195	0.036 – 39.561
				Symptomatic No vs Yes	0.0240	0.219	0.059 – 0.819
				Maternal CD4 cell count value: <200 cells/mm^3 200-500 cells/mm^3 >500 cells/mm^3	0.8107		
				Comparing maternal CD4 cell count of <200 cells/mm^3 to a count of >500 cells/mm^3		0.826	0.260 – 2.624
				Comparing maternal CD4 cell count of between 200-500 cells/mm^3 to a cell count >500 cells/mm^3		0.708	0.250 – 2.011
4-RISK MODEL 1.Mat HIV VL group 2. Mat ART < 4weeks	125.157	151.870	0.0954				
				Maternal HIV viral load (VL) value: LDL *(lower than detectable)* <1000 *(less than 1000 copies/mL)*	0.0004		

3. Infant Symp 4. Infant SGA				≥1000 *(equal and more than 1000 copies/mL)*			
				Comparing maternal VL <1000 to a VL LDL		27.842	1.546 - 501.427
				Comparing maternal VL ≥1000 to VL LDL		113.818	7.315 - >999.999
				Maternal cART duration at birth < 4weeks No vs Yes	0.1158	0.421	0.143 – 1.238
				Small-for-gestational age No vs Yes	0.4579	0.662	0.222 – 1.969
				Symptomatic No vs Yes	0.0059	0.239	0.086 – 0.662
3-RISK MODEL 1.Mat HIV VL group 2. Mat ART < 4wks 3. Infant Symp	126.111	148.371	0.0947				
				Maternal HIV viral load (VL) value: LDL *(lower than detectable)* <1000 *(less than 1000 copies/mL)* ≥1000 *(equal and more than 1000 copies/mL)*	0.0004		
				Comparing maternal VL <1000 to a VL LDL		28.111	1.499 – 527.328
				Comparing maternal VL ≥1000 to VL LDL		117.691	7.275 - >999.999
				Maternal cART duration at birth < 4weeks No vs Yes	0.1356	0.446	0.155 – 1.288
				Symptomatic No vs Yes	0.0020	0.213	0.080 – 0.568
	128.558	146.366	0.0909				

2-RISK MODEL 1. Mat HIV VL group 2. Infant Symp				Maternal HIV viral load (VL) value: LDL *(lower than detectable)* <1000 *(less than 1000 copies/mL)* ≥1000 *(equal and more than 1000 copies/mL)*	0.0002		
				Comparing maternal VL <1000 to a VL LDL		30.919	1.620 - 590.211
				Comparing maternal VL ≥1000 to VL LDL		141.495	8.663 - >999.999
				Symptomatic No vs Yes	0.0004	0.177	0.069 - 0.459
2-RISK MODEL 1. Mat HIV VL group 2. cART duration	135.078	152.886	0.0825				
				Maternal HIV viral load (VL) value: LDL *(lower than detectable)* <1000 *(less than 1000 copies/mL)* ≥1000 *(equal and more than 1000 copies/mL)*	0.0005		
				Comparing maternal VL <1000 to a VL LDL		24.481	1.268-472.486
				Comparing maternal VL ≥1000 to VL LDL		103.168	6.228- >999.999
				Maternal cART duration at birth < 4weeks No vs Yes	0.0204	0.311	0.116-0.834

CHAPTER 3

Full title: Later outcomes of birth PCR tested, HIV exposed PCR negative infants with universal maternal cART exposure.

Authors:

Nicolette M du Plessis (1), Chris JB Muller (2), Theunis Avenant (1), Michael S Pepper (3), Ameena E Goga (1)(4)

(Submitted as Chapter 3 of the PhD dissertation. Manuscript submission planned for JAIDS or AIDS)

Affiliations:

(1) Department of Paediatrics, University Of Pretoria, Faculty of Health Science, Pretoria, South Africa
(2) Department of Statistics and Actuarial Science, University of Stellenbosch, Stellenbosch, South Africa
(3) Institute for Cellular and Molecular Medicine, Department of Immunology, and SAMRC Extramural Unit for Stem Cell Research and Therapy, Faculty of Health Sciences, University of Pretoria, South Africa
(4) South African Medical Research Council, Health Systems Research Unit, Pretoria, South Africa

Corresponding author:

Nicolette M du Plessis

Department of Paediatrics

University of Pretoria

Pretoria

South Africa

Email: nicolette.duplessis@up.ac.za

Authors contributions

Nicolette M du Plessis: conceptualization, methodology, software, validation, formal analysis, investigation, data curation, original draft preparation, review and editing

Chris JB Muller: formal analysis, data curation, original draft review and editing

Theunis Avenant: conceptualization, methodology, formal analysis, investigation, original draft review and editing

Michael S Pepper: conceptualization, methodology, original draft review and editing

Ameena E Goga: conceptualization, methodology, validation, data curation, original draft preparation, review and editing

3.1 ABSTRACT

Introduction

The global reduction in mother-to-child-transmission (MTCT) of HIV rate results in large numbers of HIV exposed uninfected (HEU) children. Crucial to the management of this group is an understanding of their health challenges. A prospective study at age-time points 6-, 10-, 14-weeks, 6- and 9-months was undertaken at Kalafong Provincial Tertiary Hospital, South Africa, to document growth outcomes in an HEU cohort.

Methods & Findings

Growth curves were developed for both sexes and in relation to maternal viral load, cART duration, newborn symptoms and low birth weight (LBW). Response profiles was used to evaluate the sequence of mean z scores for weight-for-age (WAZ), length-for-age (LAZ), and head circumference (HC). Linear mixed effects models were fitted.

PCR results, available for 2262/2393 (94.53%) patient-visits, indicated HIV infection in 7 (0.31%) (4 at 6 weeks (0.96%), and one each at 14 weeks (0.25%), 6 months (0.27%), and >1 year of age). Hospitalization rate was 41.3/100 person-years. 94/2013 (4.7%) had ≥ 1 symptom/sign of HIV-associated immunosuppression; significantly associated with a subsequent positive PCR.

Males had lower WAZ ($p=0.0048$) and LAZ ($p<0.05$). HEU infants with birth-documented HIV-associated symptoms had lower WAZ ($p<0.0001$). LBW was associated with decreased WAZ ($p<0.0001$), LAZ ($p=0.0006$) and HC ($p=0.0117$).

Discussion

HIV infection of 0.31%, mostly by age 6 weeks, was associated with HIV-like symptoms. Hospitalization rate was 41.3/1000 person-years. Longitudinal growth illustrated lower WAZ and LAZ in males. HEU newborn characteristics - HIV-associated symptoms/signs and LBW - was associated with lower growth curves.

3.2 INTRODUCTION

The current lower rate of vertical (mother to child) human immunodeficiency virus (HIV) transmission results in a large number of HIV exposed but uninfected (HEU) children. Crucial to the management of this growing group of children in health and social programmes is an understanding of the health difficulties that they face.[1] Several studies have demonstrated that HEU infants have an increased morbidity, more admissions to hospital, and frequent sick clinic visits during infancy compared to unexposed infants. Furthermore, infant mortality is higher during the first and second years of life. Acute respiratory infections, diarrhoea and/or dysentery, malnutrition, sepsis, invasive bacterial disease and meningitis are frequent causes of death.[2,3] Studies that have described foetal and postnatal growth in HEU infants before the availability of universal maternal combination antiretroviral therapy (cART) show differences in birth weight and length-for-age as significant findings between exposed and unexposed cohorts.[3]

Evans et al, in a 2016 review, identified key research areas pertaining to HEU infants. Future research areas include mortality and growth in the era of cART, causes of infection susceptibility, better characterization of HEU immune function, evaluation of the mechanism of immune activation and its impact on HEU mortality and morbidity, impact of cotrimoxazole prophylaxis, infections prophylaxis and vaccination strategies, and novel breastfeeding-transmission preventative measures.[3] Our study aimed to describe growth in HEU infants in the era of universal maternal cART and in relation to maternal and infant birth characteristics.

3.3 METHODS

The HEU cohort was a prospective cohort from the very early infant diagnosis (VEID) of HIV study.

Live-born infants to a known HIV infected mother were eligible for enrolment and birth HIV polymerase chain reaction (PCR) testing was performed within 72 hours at Kalafong Provincial Tertiary Hospital (KPTH). Mother-infants pairs with a negative birth PCR, that were able and willing to follow up at the Kalafong Hospital Paediatric Immunology

Outpatient (IPOPD) baby clinic, were included in the HEU study cohort. Newborn recruitment started 12 August 2014 and the last follow up clinic was 19 December 2016.

Research procedure

Trained research staff enrolled, examined and followed up patients. At each follow-up clinic, infants received the required Expanded Programme on Immunization (EPI) vaccinations and a clinical examination that include standardized anthropometric measurements. PCR testing was performed at 10 weeks, in symptomatic infants (as per prevention of mother-to-child transmission of HIV (PMTCT) guidelines) or after 6 weeks of breastfeeding cessation, and at 9 months if any of the HIV rapid test kit-assays (RTK) was reactive. Prophylactic infant-PMTCT treatment (nevirapine/zidovudine) and cotrimoxazole were provided at six weeks according to the national PMTCT programme at the time.[4,5] All HEU infants with a PCR positive and/or indeterminate result at any follow up visit were exited from the HEU cohort study and further managed at the Kalafong Paediatric Immunology clinic as per protocol. They were excluded from analysis from this point onwards. Mothers with HIV- or medically-related health problems were referred to the appropriate services within KPTH. After study completion patients were referred for further care to the appropriate primary healthcare facilities.

Research interviews by trained staff were documented on case report forms (CRF). Documented infant-parameters included anthropometry (weight, length, head circumference) and HIV-associated symptoms - failing to thrive, hepatosplenomegaly, oral candidiasis, significant lymphadenopathy, any opportunistic infections. Admissions or other healthcare visits were noted. Maternal characteristics included viral load (last documented value) and cART duration.

Ethical and legal considerations

Hospital management granted permission and ethical clearance was obtained at the Faculty of Health Sciences Research Ethics Committee, University of Pretoria (protocol 285_2014). Written informed consent was obtained from each mother upon enrolment.

CRF were paper-based until November 2014, thereafter data were directly entered into REDCap (Research Electronic Data Capture, https://www.project-redcap.org/) hosted at South Africa Medical Research Council (SAMRC).

Data analysis

From REDCap, data was analysed using statistical software SAS (version 9.4 TS1M5). Continuous data was expressed as means and standard deviations (SD) or as median and interquartile ranges (IQR) for skewed distributions. Discrete or categorical data was summarised using frequencies and percentages. The independent t-test was used for comparison of normally distributed data; otherwise non-parametric alternatives was used.

Growth curves, with smoothing parameters of 60, were developed. Growth trends of the cohort in relation to maternal viral load, cART duration, symptomatic newborn, and birth weight were determined. Male and female growth curves were developed for the cohort and compared with each other. Infant data points after HIV infection were not included in the trend analysis. Maternal viral load levels were recorded within 3 months of or at labour and defined during analysis as lower-than-detectable (LDL), less than 1000 copies/mL (1-1000) and more or equal to 1000 copies/mL (≥1000). Duration of cART was recorded at birth (< 1 month and longer than 1 month's duration). Symptoms suggestive of HIV-associated immunosuppression was documented at birth according to VEID study definitions (*see chapter 2, addendum A*). Prematurity was present if newborns were born before 37 completed weeks gestation. Low birth weight (LBW) was defined as birth weight < 2.5kg. Z-scores were calculated for weight-for-age (WAZ), length-for-age (LAZ) and head circumference for age (HC) using CDC 2000 Growth Charts adjusted for gestational age for preterm infants and World Health Organisation (WHO) 2006 growth charts from the Multicentre Growth Reference Study for term infants. Analysis of response profiles was used to evaluate the sequence of mean WAZ, LAZ, and HC by exposure-group over time. Linear mixed effects models were fitted separately for each birth characteristic, as well as, for all characteristics combined in one model. The models included random effects for intercept and an unstructured correlated covariance matrix was assumed for the random effects. In addition, use of the WHO's Child Growth Standards z-scores ensured that infant gender was also controlled for in the linear mixed effects model. All testing used a significance level of 0.05, with two-sided hypothesis testing and no corrections for multiple testing.

3.4 RESULTS

Study population

Between August 2014 and December 2016, 1759/1911(92%) of screened mother-infant pairs granted consent. Excluding 91/1759 (5%) of birth PCR results that were unavailable, 613/1668 (37%) HEU infants formed the HEU follow up cohort (Figure 1).

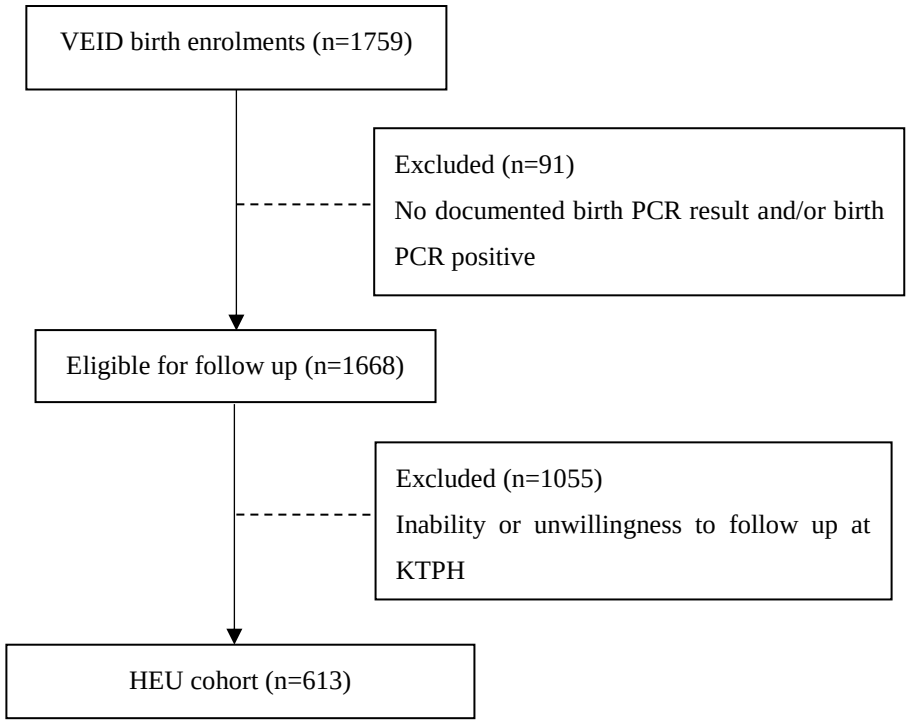

Figure 1: Cohort diagram of VEID birth and follow up cohorts

PCR: polymerase chain reaction, VEID: Very Early Infant Diagnosis of HIV study, KPTH: Kalafong Provincial Tertiary Hospital, HEU: HIV-exposed uninfected

Of 2393 follow up visits, 2074 were at time points 6/10/14 weeks, 6 months and 9 months. The absolute number of patients and percentage of expected patient-visits (patients seen/613) at each visit was 422 (68.84%) at 6 weeks, 423 (69.00%) at 10 weeks, 436 (71.13%) at 14 weeks, 412 (67.21%) at 6 months, and 381 (62.15%) at 9 months (Figure 2). Additional visits were ≥ 12 months (204/613, 33%) and at other time points (109/613, 17.78%).

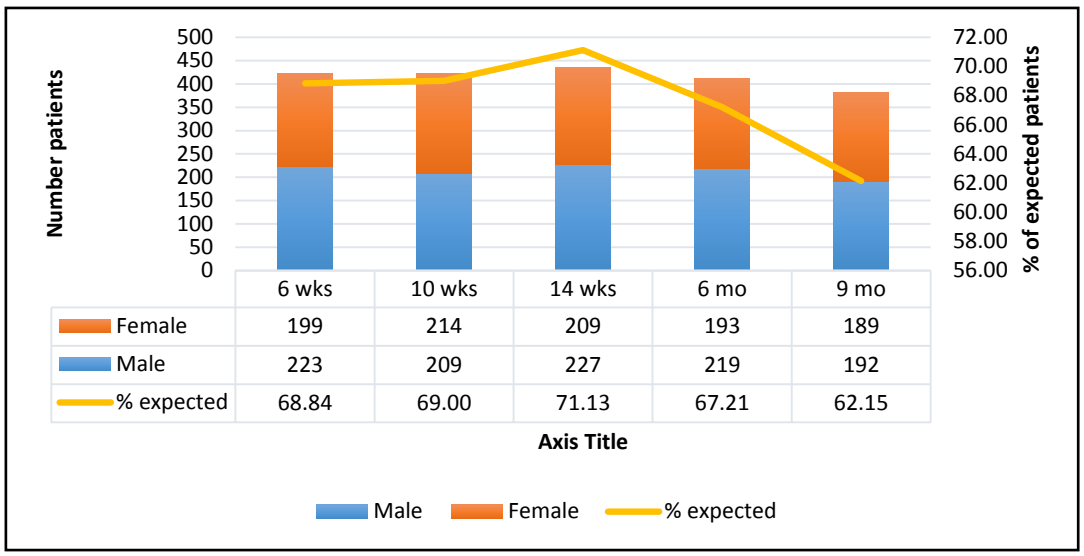

Figure 2: Patients, male and female, seen at each scheduled time point and the percentage of expected patient-visits per time point.

HIV infection

HIV PCR results were validated in 2262/2393 (94.53%). HIV infection was documented in 7 (0.31%) (4/415, 0.96% at 6 weeks, 1/397, 0.25% at 14 weeks, 1/373, 0.27% at 6 months, and one in a patient aged 15 months that came for an unscheduled visit after defaulting from 6 weeks of age). HIV infected infants had all received NVP as PMTCT, were breastfed, and all mothers were on cART (apart from one defaulter). Apart from two maternal viral loads that were > 1000 copies/mL (infants tested HIV positive at 6 weeks of age); mothers were virologically suppressed. More than half (4/7, 57%) of infected infants had symptoms/signs suggestive of HIV infection from birth, notably growth faltering. Two infants (29%) were born prematurely. These were identical twin brothers, born to a mother on cART 3 months (90 days) prior to delivery. Both infants tested HIV negative at birth, but seroconverted at 6 weeks and 6 months respectively. The mother was virologically suppressed at 6 weeks postpartum and reported good compliance; she was exclusively breastfeeding. Individual profiles of the HEU patients that seroconverted are tabulated in Supplemental Digital Content 1.

Infant follow up parameters

Nineteen HEU infant hospital admissions were documented, a hospitalization rate of 41.3/100 person-years. Less than 5% of study visits (94/2013, 4.7%) documented one or more symptoms/signs suggestive of HIV-associated immunosuppression; five infants subsequently tested PCR positive. The association between the presence of HIV-associated symptoms and testing PCR positive was statistically significant (p value < 0.0001 (Fisher's exact test)). Symptoms documented were failure to thrive (60/94, 65.2%), generalised lymphadenopathy (16/94, 17.4%), oral candidiasis outside of the neonatal period (14/94, 15.2%), pneumonia (12/94, 13.0%), and less frequently hepatomegaly (5/94, 5.4%) and splenomegaly (2/94, 2.2%).

Maternal follow up parameters

Most mothers (97.32%) self-reported good treatment compliance and continued cART. Fifty-five (55/613, 8.97%%) mothers either defaulted treatment or needed urgent referral to adult HIV services for treatment-related problems identified during a follow up visit.

Exclusive breastfeeding was the feeding choice in 93.5% (1539/1646) of mothers directly post-delivery. Forty (6.53%) indicated they prefer formula feeds from birth. The majority of feeding change occurred between birth and 6 weeks of age (from 6.53% (40/613) to 25.64% (107/422) non-exclusive BF). At 9 months, more than two-thirds (291/381, 76.38%) were not receiving breastmilk as the main daily milk-feed. Feeding options at each time point and percentage change are tabulated in supplemental digital content 2.

Longitudinal growth

Median growth curves differed between sexes, males had lower weight (p=0.0048), length (p=0.0488) and HC (p=0.0373) growth. (Figure 2).

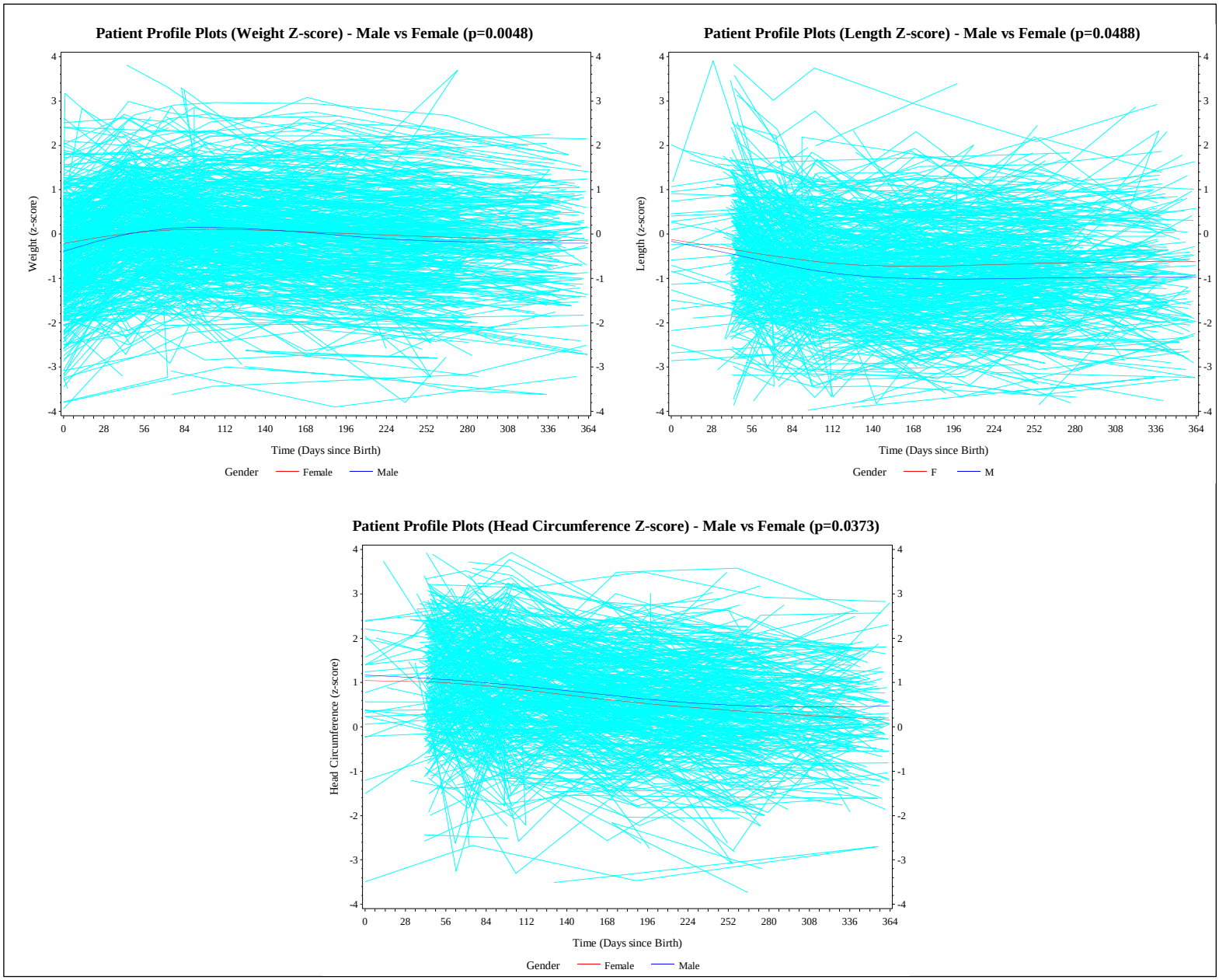

Figure 2: Longitudinal growth for weight-for-age, length-for-age and HC-for-age, with male and female median growth lines.

Longitudinal growth curves were generated in relation to maternal and infant birth characteristics. Maternal viral load and cART duration was weighted against infant growth. Viral load did not show a significant influence on growth. cART duration > 1 month antenatally was associated with lower WAZ, LAZ and HC trends by the end of the first year, though not statistically significant. (Figure 3)

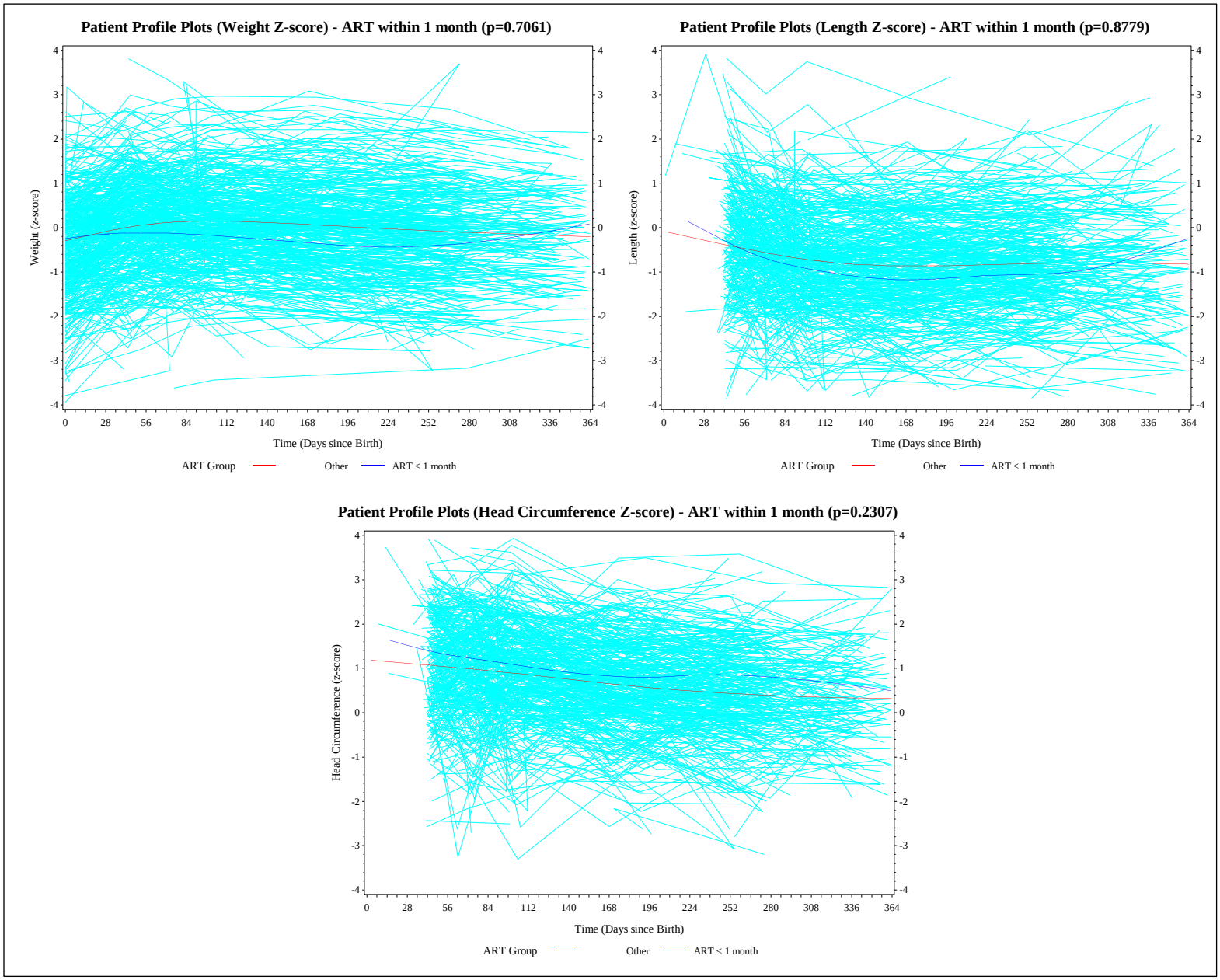

Figure 3: Longitudinal growth in relation to maternal cART duration shorter or longer than 1-month duration antenatally.

Infants with symptoms at birth suggestive of HIV-associated immunosuppression had lower growth trends for WAZ (p<0.0001), LAZ (p=0.0724) and HC (p=0.0812). Children with IUGR/SGA at birth did not demonstrate catch up growth during infancy. Low birth weight newborns had significantly lower growth trends for weight (p<0.0001), length (p=0.0006) and HC (p=0.0117). (Figure 4&5)

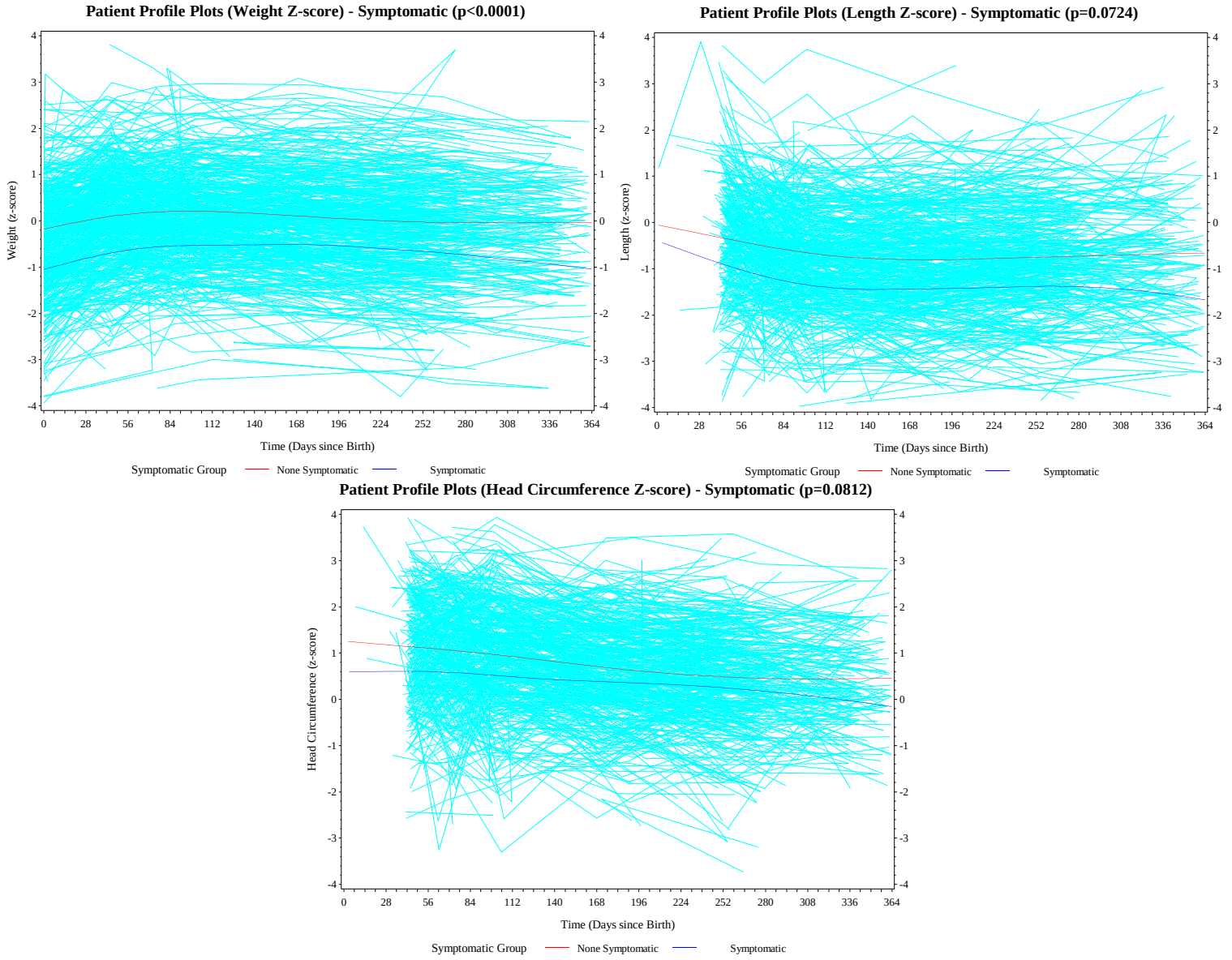

Figure 4: Growth curves in relation to symptoms at birth suggestive of HIV-associated immunosuppression

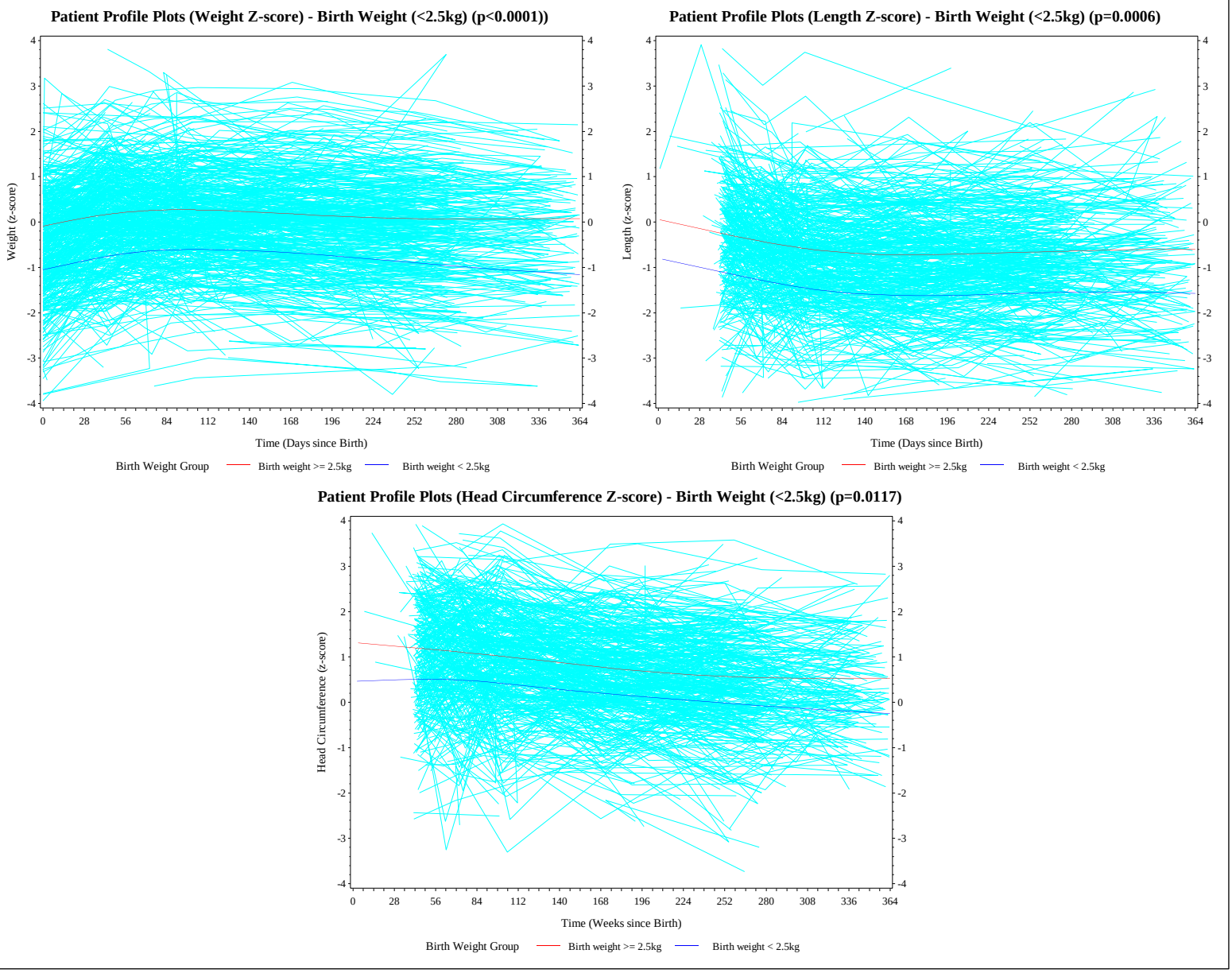

Figure 6: Longitudinal growth trends illustrated in relation to birth weight

3.5 DISCUSSION

This study examined growth trends in HEU infants as part of the VEID study. Mothers were mostly breastfeeding at birth and on universal lifelong cART. MTCT after birth were less than 1% (0.31%) and occurred mostly by 6 weeks of age. HIV infection was associated with symptoms and signs of HIV-associated immunosuppression, such as growth faltering. The hospitalization rate was 41.3/1000 person-years. Longitudinal growth trends illustrated lower WAZ and LAZ in males. HEU newborns that had one or more symptoms suggestive of HIV-associated immunosuppression had lower weight trends and newborns with a birth weight <2.5kg had significantly lower WAZ, LAZ and HC trends.

HIV infection was < 1.00% (0.31% overall) and documented more frequently at the 6 week follow up visits (0.96%).These infant-mother pairs were breastfeeding and had mostly undetectable maternal viral loads at birth, but 50% of them had evidence of symptoms from birth (growth faltering). HEU infants that are symptomatic at birth, develop new symptoms of HIV-like immunosuppression, and/or have poor growth trends need frequent HIV testing to identify HIV infection timeously. This finding support an earlier PCR test than 10 weeks to identify infected infants after birth.

Co-morbidities were frequently documented at follow up of the HEU infants during the first 9 months of life. The lower infant hospitalization rate in this cohort of 41.3/1000 persons year when compared with previous HEU cohorts is encouraging. The European Collaborative Study observed a hospitalization rate of 264/1000 child-years from 1985 to 2002. [6,7] The HEU infant vulnerability to infectious diseases and increased morbidity and mortality is not well understood. One reason could include improved health and monitoring of mother-infant pairs under WHO option B/B+ with universal maternal cART. The immune mechanisms and reconstitution from lifelong maternal cART remains unclear. Close to 5 % of the cohort had new evidence suggestive of HIV-associated immunosuppression, frequently growth faltering, and had a significantly higher chance compared to asymptomatic HEU infants of HIV infection. The pathophysiology of HIV-like symptoms or signs in uninfected but exposed infants can possibly be related to phenotypical and functional immunological factors.[8] The immunological consequence of prolonged in-utero cART exposure remain an important unanswered question.[7,8] We support repeat HIV testing

in HEU infants that develop symptoms suggestive of HIV-associated immunosuppression within the national PMTCT programme.[5]

Maternal cART compliance during breastfeeding remain important. Although less than 3% (2.68%) of mothers defaulted lifelong cART in this cohort, this translated to 55 HEU-cohort infants that were exposed to a non-compliant mother and, in turn, possibly inadequate PMTCT. Feeding practices change frequently and early during the infant's first year of life. Although most HIV infected mothers indicated that they would exclusively breastfeed, close to a quarter (25.36%) of mothers changed feeding practices by 6 weeks postpartum and almost half (48.62%) by 14 weeks. Our data demonstrate that frontline health practitioners need to support the WHO recommendation that any breastfeeding is better than no breastfeeding, proviso that mothers are compliant on cART. [9,10]

This study illustrated a decrease in length (LAZ) growth trends over the first year of life, especially in males. LBW HEU infants did not show catch up growth during the first year. This supports findings published in 2011 and 2016 from the Mashi and Mma Bana studies conducted in Botswana of lower LAZ in cART exposed infants. WAZ in our cohort was not significantly decreased after 12 months, though reports of poor WAZ trends were reported over 24 months in the Botswana cohorts in 2016.[11,12] In the doctoral thesis by Ramokolo (2017) growth trends in HIV unexposed South African children were evaluated, related to early infant feeding practices. They demonstrated that children who were not breastfed at 12 weeks had higher mean WAZ between 12 and 24 weeks, higher BMI-for-age Z-scores at 2 years and were more likely to be overweight or obese. Although most of the children were initiated on breastmilk early, the proportion of breastfed children decreased in the first 12 weeks of life while the frequency of formula feeding increased. It was also demonstrated that HEU children had similar attained growth and growth velocities compared to HU children in the absence of maternal ART, but demonstrated that cART-exposed HEU children had poorer birth and early attained growth outcomes than HU children.[13,14] The significance of poor LAZ trends, especially in male HEU infants, remain a concern. The impact on final height and body mass index (BMI) need careful follow up to ascertain if these growth trends will have a negative impact such as higher BMI values and possibly more obesity among HEU male adolescents/young adults.

Maternal cART exposure, especially for longer than 1 month prior to birth, was associated with a smaller head circumference growth trend. This is similar to reports from the Pediatric HIVAIDS Cohort Study (PHACS).[15,16] Neurodevelopmental outcomes in HEU infants is of great interest and paramount to this, the influence of exposure to maternal cART. If an association between HC and neurodevelopmental outcomes are demonstrated in HEU infants, HC measurements could be used as a surrogate for developmental delay.

3.6 FUNDING

Two dedicated research nurses were funded by the South African Medical Research Council. Additional PCR testing was supported and funded by National Department of Health. Rapid test kit-assays were funded by the Paediatric Infectious Diseases Division of the University of Pretoria.

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Supplemental Digital Content 1: Profiles of the HEU cohort that seroconverted after birth.

VEID number	**Visit**	**Age (days)**	**Previous tests done on patient**	**Birthweight & Gestation**	**Feeding choice**	**Maternal viral load**	**Antenatal cART duration (days)**	**Comments**
48	6 wk	41	Birth PCR negative	2.09kg Term	EBF	171 353 (at 6 week visit)	102	Symptomatic at birth (IUGR) FTT by 6 weeks of age
236	6 wk	46	Birth PCR negative	3.7kg Term	EBF	LDL (at 6 weeks visit)	117	Asymptomatic infant
*300**	6 wk	52	Birth PCR negative	2.12kg Term	EBF	LDL (6 weeks post-delivery)	90	Asymptomatic
424	6 wk	45	Birth PCR negative	2.7kg Term	EBF	48 843 (at 6 week visit)	452	Asymptomatic birth Oral Candidiasis at 6 weeks
1090	14 wk	119	Birth PCR negative	1.05kg 26 weeks	PEBM in neonatal unit, EBF thereafter.	LDL (2 weeks pre-delivery)	1783	Symptomatic at birth (IUGR, anemia, pneumonia); BPD 32 days O2 dependent.
*301**	6 mo	269	Birth, 113, 126, 231 days PCR negative tests	1.92kg Term	EBF	LDL (6 weeks post-delivery)	90	SGA, growth continued on the -3 z-score; F/U with twin that tested PCR positive at 6 weeks of age.
410	15 mo	447**	Birth & 6 weeks PCR negative;	2.2kg	EBF	LDL (6 weeks	1117, but stopped cART 2	IUGR birth FTT at 6 weeks

			3xRTK positive at 447 days	35 weeks gestation		post-delivery)	months post-delivery (defaulted)	Mom reported 67% compliance to medication at birth Defaulted F/U

**Twin brothers*

VEID: very early infant diagnosis, wk: weeks; mo: months; PCR: polymerase chain reaction; RTK: rapid test kit-assays; EBF: exclusive breastfeeding; PEBM: pasteurized expressed breastmilk; LDL: lower than detectable; cART combination antiretroviral therapy; IUGR: intra-uterine growth restriction; SGA: small-for-gestational age; BPD: bronchopulmonary dysplasia; F/U: follow up; FTT: failure to thrive

Supplemental Digital Content 2: Feeding practices

Time point	**Birth**	**6 weeks**	**10 weeks**	**14 weeks**	**6 months**	**9 months**
Total visits (N)	613	422	423	436	412	381
Feeding change		67	48	57	52	27
Non-EBF (n)	40* **Non-BF*	107	155	212	264	291
(n/N) %	6.53%	25.36%	36.64%	48.62%	64.01%	76.38%

CHAPTER 4

Seroreversion in a birth PCR tested, HIV exposed PCR negative PMTCT option B/B+ cohort at 9 months of age using rapid HIV tests kits and HIV ELISA: follow-up of infants from the Very Early Infant Diagnosis of HIV (VEID) study, Gauteng, South Africa.

Authors:

Nicolette M du Plessis (1), Ahmad Haeri Mazanderani (2)(3), Gayle Sherman (2)(4), Chris JB Muller (5), Theunis Avenant (1), Michael S Pepper (6), Ameena E Goga (1)(7)

(Submitted as Chapter 4 of the PhD dissertation. Manuscript submission planned for WHO Bulletin or Journal of Clinical Virology)

Affiliations:

(1) Department of Paediatrics, University Of Pretoria, Faculty of Health Science, Pretoria, South Africa

(2) Centre for HIV & STIs, National Institute for Communicable Diseases, National Health Laboratory Service, Johannesburg, South Africa

(3) Department of Medical Virology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa Centre for HIV & STIs, National Institute for Communicable Diseases, National Health Laboratory Service, Johannesburg, South Africa

(4) Department of Paediatrics & Child Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

(5) Department of Statistics and Actuarial Science, University of Stellenbosch, Stellenbosch, South Africa

(6) Institute for Cellular and Molecular Medicine, Department of Immunology, and SAMRC Extramural Unit for Stem Cell Research and Therapy, Faculty of Health Sciences, University of Pretoria, South Africa

(7) South African Medical Research Council, Health Systems Research Unit, Pretoria, South Africa

Corresponding author:

Nicolette M du Plessis

Department of Paediatrics

University of Pretoria

Pretoria

South Africa

Email: nicolette.duplessis@up.ac.za

Author contributions: (conceptualization, methodology, software, validation, formal analysis, investigation, data curation, original draft preparation, review and editing)

Nicolette M du Plessis: conceptualization, methodology, software, validation, formal analysis, investigation, data curation, original draft preparation, review and editing

Ahmad Haeri Mazanderani: conceptualization, methodology, original draft review and editing

Gayle Sherman: conceptualization, methodology, original draft review and editing

Chris JB Muller: formal analysis, data curation, original draft review and editing

Theunis Avenant: conceptualization, methodology, formal analysis, investigation, original draft review and editing

Michael S Pepper: conceptualization, methodology, original draft review and editing

Ameena E Goga: conceptualization, methodology, validation, data curation, original draft preparation, review and editing

4.1 ABSTRACT

Introduction

Accurate infant diagnosis of HIV requires suitable laboratory assays at specific ages. The presence of passively acquired maternal anti-HIV IgG in exposed infants, for up to 18 months, necessitates different diagnostic algorithms from older children and adults. This study aims to determine seroreversion with different serological assays in an HIV exposed PCR negative cohort whose mothers received PMTCT option B/B+ and breastfeeding.

Methods & Findings

Seroreversion at 9 months-age was determined using three HIV rapid test kit-assays (RTK); ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test, OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test, and Alere Determine™ HIV-1/2 Ag/Ab Combo. A 4th generation ELISA test was done on a subset of samples (74) using the ARCHITECT HIV-1/2 Ag/Ab Combo assay (Abbott Laboratories, Wiesbaden, Germany). All seropositive or indeterminate results were submitted for HIV PCR testing.

At 9-months, 381 infants were seen at mean age 265 days (SD ± 20 days). RTK in 368 infants showed 9 seropositive results on all 3 RTK and 131 seronegative on all assays. ADVANCE had the highest specificity (96.99%) in HIV exposed uninfected infants followed by ABON (74.47%). Determine was the least specific (45.05%). Seroreversion by negative ELISA was not demonstrated.

Discussion

Our study shows different serology assays vary in performance regarding identifying seroreversion at 9 months among HIV exposed infants. RTK differed in specificity (45 – 97%). No seroreversion was documented with HIV ELISA. We support the current recommendation of repeat PCR testing during infancy. Delayed seroreversion beyond 18 months with future consideration for PCR testing up to 24 month of age should be considered.

4.2 INTRODUCTION

Accurate infant diagnosis of HIV requires the use of a suitable HIV laboratory assay at the specific infant age. In 1989, the Centers for Disease Control and Prevention (CDC) first published guidelines for the diagnosis of HIV-1 infections using serological testing. HIV-1 Western blot or HIV-1 indirect immunofluorescence assays (IFA) were initially used to confirm positive results. These guidelines were expanded with recommendations for HIV-2 antibody testing in 1992, and procedures for confirmatory laboratory testing of positive HIV rapid antibody tests in 2004. At that time, the recommendations and availability were for HIV antibody testing only.[1] There have been significant improvements in HIV antibody detection since the first generation immunoassays based on different design principles and combinations of detecting anti-HIV IgM and IgG antibodies, as well as monoclonal antibodies against the p24 antigen. The currently used 4th generation immunoassay allows for detection of HIV before HIV infection.[1]

A definitive diagnosis of infant HIV requires more than immunoassay testing. The presence of passively acquired maternal anti-HIV IgG antibodies (transferred transplacentally) in HIV exposed infants and toddlers for up to 18 months necessitates different diagnostic algorithms from those used in older children and adults. 2 In 1989, Rogers et al. showed that the polymerase chain reaction (PCR) was successful in detecting HIV proviral sequences in infants.[2] HIV-PCR testing would become the gold standard in diagnosing HIV infection in HIV exposed children under the age of 18 months.[3,4]

HIV exposed but uninfected (HEU) infants will lose maternal anti-HIV IgG antibodies and revert to HIV seronegative (seroreversion) status. Although the maternal anti-HIV IgG antibodies are usually not detected after 9 months of age, they can occasionally (1-2%) be detected in infants up to 18 months of age. Several factors may have an effect on the time to seroreversion. Postulated causes for prolonged (past 18 months) HIV seroreversion times are higher maternal antibody levels in communities with longer HIV endemicity, ARV exposure, and changes in transplacental HIV antibody transfer.[5] Factors such as gender, gestational age, malnutrition, and breastfeeding in infants have not been demonstrated to significantly change the time to HIV seroreversion. Gutierrez et al. published a review of available seroreversion data in 2012 and illustrated that seroreversion in HEU infants

happens at a later age than previously reported. Whereas infants born by vaginal delivery were more likely to serorevert at a younger age, maternal antiretroviral (ARV) exposure, low maternal HIV viral loads and low maternal CD4 cell counts were associated with later seroreversion. The mechanisms of clearance of HIV-specific immunoglobulins are not fully understood. Some theories include a possible link between seroreversion and a decrease in maternal hypergammaglobulinemia and/or maternal immune reconstitution induced by ARVs.[6]

HIV testing algorithms, as guided by the World Health Organization (WHO), in children > 18 months and adults consist of 2-3 rapid tests with high clinical sensitivity (>99%) and specificity (≥98%). However, HIV rapid test-assays has been reported to have varying performance even within a single African country. This was related to gender, patient comorbidities, and other factors associated to geographic location.[7] The use of rapid HIV tests to detect HIV exposure and seroreversion in infants was studied by Sherman et al. (2008). Rapid tests do not all perform equally well during infancy. The ideal rapid assay should detect HIV exposure early in infancy, and HIV seroreversion from as early as 7 months of age. The ability of an HIV rapid test to detect HIV exposed infants up to 3 months of age was similar to an HIV enzyme-linked immunosorbent assay (ELISA) test. After 7 months of age, rapid tests show increased specificity; they are thus useful to exclude HIV infection. Different rapid test kits have proven to have differing sensitivities and specificities at different times in infancy.[8] The World Health Organization (WHO) recommended in 2015 that the selection of an HIV testing approach should be done once the correct testing strategy and validated testing algorithm have been selected in each setting, and that HIV confirmation is done in patients diagnosed with HIV before combination antiretroviral therapy (cART) initiation.[9]

Our study aims to determine seroreversion with different serological assays in an HIV exposed PCR-negative infant cohort whose mothers received on the background of prevention of mother-to-child-transmission of HIV (PMTCT) care in accordance with option B/B+ (with universal maternal cART access and breastfeeding).

4.3 METHODS

The HIV exposed uninfected (HEU) study cohort is part of the Very Early Infant Diagnosis of HIV (VEID) study. This was a prospective cohort study with birth enrolment and follow up visits at age-time points of 6-, 10-, 14-weeks, 6- and 9-months.

Patients were eligible for enrolment in the birth cohort of the study if they were live-born to a known HIV infected mother. Birth HIV PCR testing was done within 72 hours of birth at Kalafong Provincial Tertiary Hospital. Infants enrolled in the birth cohort of the VEID study with a negative birth HIV PCR result, and able to be followed up at scheduled visits (6 weeks, 10 weeks, 14 weeks, 6 months, and 9 months) at the Kalafong Hospital Paediatric Immunology Outpatient (IPOPD) baby clinic, were included in the HEU study cohort.

Recruitment started on 12 August 2014 and the last study HEU follow up clinic was on 19 December 2016 (total study period of 861 days - 2 years 4 months 8 days).

The sample size for the seroreversion cohort was determined by the number of VEID study participants that attended their 9-months study visit, as well as the availability of the HIV Rapid test kits assays at IPOPD.

Research procedure

Infants enrolled in the VEID birth cohort and eligible for the HEU cohort, with birth-PCR negative results, were given a follow-up appointment for the immunology / VEID outpatient clinic, Kalafong Provincial Tertiary Hospital, at six weeks post-delivery. Each infant's follow dates at 6 weeks, 10 weeks, 14 weeks, 6 months and 9 months of age were calculated from their date of birth. Seroreversion at 9 months of age was determined with the use of three HIV Rapid test kit-assays (RTK); the ABON^{TM} HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test, OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test, and the Alere Determine^{TM} HIV-1/2 Ag/Ab Combo. Tests were done at the IPOPD baby clinic during the 9 month follow up visit on whole blood collected for study purposes. Study participants with a seropositive or indeterminate assay at nine months had an HIV PCR test done and were followed up at 12 months of age for repeat rapid testing as per study protocol. Seronegative results (all three kits) were documented in the patient-held Road to

Health Booklet (RTHB) and the caregiver was advised to repeat the test at 18 months of age as per current national protocol. A 4th generation ELISA assay was performed on a subset of serum samples submitted for HIV PCR testing (100 samples) using the ARCHITECT HIV-1/2 Ag/Ab Combo assay (Abbott Laboratories, Wiesbaden, Germany).

Maternal and infant characteristics were captured on case report forms (CRF). Maternal characteristics included viral load (VL) (last documented value), cART duration and infant-feeding choice (breastfeeding or formula feeds).

Ethical and legal considerations

Management at KPTH granted permission to conduct the study, and the University of Pretoria, Faculty of Health Sciences Research Ethics Committee gave ethical clearance (protocol 285_2014). Each mother gave written informed consent upon enrolment in the study.

Case report forms were initially paper-based but in November 2014 all CRFs were entered directly into the REDCap (Research Electronic Data Capture, https://www.project-redcap.org/) system hosted at the South Africa Medical Research Council (SAMRC).

Data analysis

Data was analysed using statistical software SAS (version 9.4 TS1M5) by accessing the REDCap (Research Electronic Data Capture) system.

Continuous data was expressed as means and standard deviations (SD) or as median and interquartile ranges (IQR) for skewed distributions. Discrete or categorical data was summarised using frequencies and percentages. The independent t-test was used for comparison of normally distributed data; otherwise non-parametric alternatives was used.

4.4 RESULTS

Between 12 August 2014 to 19 December 2016, 1759 (92%) of 1911 screened birth-patients were enrolled in the VEID study. Negative birth HIV PCR participant (613) indicated their ability and willingness to follow up. Figure 1 illustrates the HEU clinic attendees at each time point between birth and 9 months (absolute numbers and percentage expected). Figure 2 demonstrates the consort diagram of patients seen and tests performed at the 9-month time point.

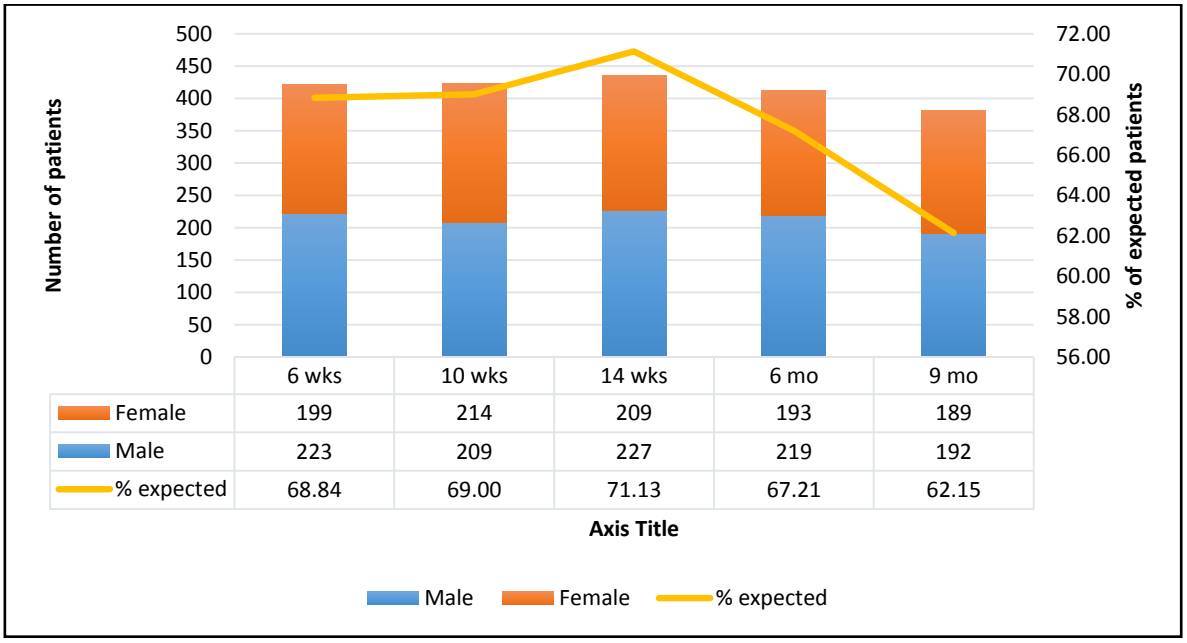


Figure 1: Patients, male and female, seen at each scheduled time point and the percentage of expected patient-visits per time point.

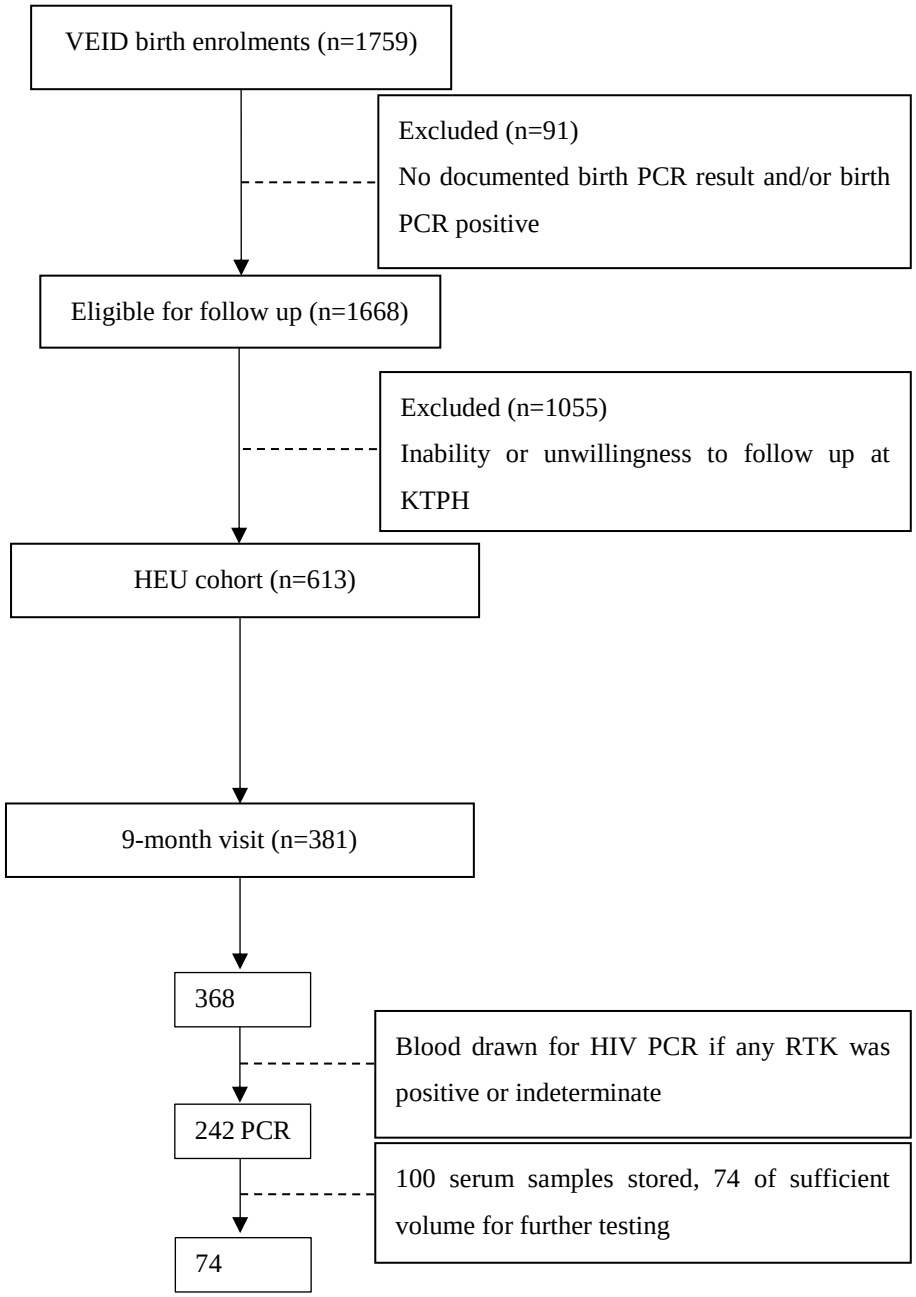


Figure 2: Cohort diagram of VEID birth and HEU follow up cohorts and 9-month visit Rapid Test assays performed

PCR: polymerase chain reaction, VEID: Very Early Infant Diagnosis of HIV study, KPTH: Kalafong Provincial Tertiary Hospital, HEU: HIV exposed uninfected, RTK: HIV rapid test kits, ELISA: Enzyme linked immunosorbent assay

Three hundred and eighty one study participants attended the 9-month follow up visit. The mean age-days was 265 (SD ± 20 days). Most infants were born at term (283/334, 84.73%)

with 14 infants (4.19%) born prematurely at less than 34 weeks' gestation. Of 319 (83.73%) mothers that started cART antepartum, median cART duration at delivery was 160 days (IQR 121 – 671 days). Treatment interruption during the 9-month period was documented in 5/366 (1.4%) of mothers. Most mothers (331/355, 93.25%) elected to breastfeed exclusively from birth, 24 (6.8%) formula feeding. At 9 months an additional 16 mothers (40/355, 11.3%) changed to formula feeds; therefore, most mothers (88.7%) continued to breastfeeding during the weaning period. The maternal viral load, cART duration and feeding choice in infants with seropositive and negative RTK on all 3 assays respectively, are illustrated in Table 2. Infants with 3 seropositive RTK results were all breastfed and born to mothers on cART for a median duration of 20 months (573 days) at delivery and viral loads lower than detectable (at birth).

Table 2: Maternal viral load, cART duration and feeding option at birth

Variable*	**All 3 RTK positive (9)**	**All 3 RTK negative (131)**
Maternal VL group	(N=6)	(N=64)
LDL	5 (83.33%)	42 (65.62%)
<1000	0	11 (17.19%)
>1000	1 (16.67%)	11 (17.19%)
Median maternal cART duration before delivery (days)	573 days (IQR 264 – 1529)	191 days (IQR 125 – 899)
Still on cART	9/9 (100%)	127/130 (97.69%)
Milk-feeding options	(N=9)	(N=126)
BF	9 (100%)	117 (92.86%)
FF	0 (0%)	9 (7.14%)

**Data were missing for some of the variables*

RTK results were documented in 368 infants. Nine patients had seropositive results from all 3 rapid tests, while 131 infants tested seronegative on all assays (Table 3). The ADVANCE RTK had more frequent seronegative results (95.27%), followed by the ABON (71.47%). The Determine was seropositive in almost half the patients (49.86%).

Table 3: Seropositive, seronegative and indeterminate percentages of three rapid test assays at 9-month-old HIV exposed infants of the VEID study cohort

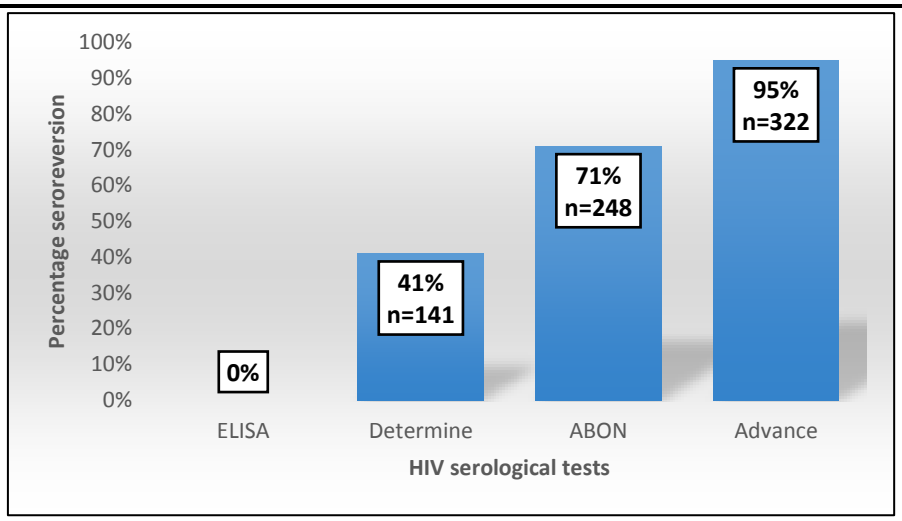
Results (N=total)	**Positive**	**Negative**	**Indeterminate**
ABON (N=347)	85 (24.50%)	248 (71.47%)	14 (4.03%)
ADVANCE (N=338)	10 (2.96%)	322 (95.27%)	6 (1.78%)
Determine (N=345)	172 (49.86%)	141 (40.87%)	32 (9.28%)
All 3 RTK same results (N=338)	9 (2.66%)	131 (38.76%)	0 (0%)

ABON: ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test, ADVANCE: OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test, Determine: Alere Determine™ HIV-1/2 Ag/Ab Combo, RTK: Rapid test kit

All participants tested PCR negative; therefore, no sensitivity or positive predictive values were calculated. The ADVANCE RTK showed the highest specificity (96.99%) to detect HIV exposed uninfected infants followed by the ABON (74.47%), while the Determine was least specific (45.05%).

Further serological testing was done on 74/100 samples. Seroreversion, with loss of maternal antibodies as detected by a negative HIV ELISA, was not demonstrated in any patient-sample. Supplementary digital content 1 illustrates the RTK, HIV PCR result with laboratory turnaround times, and ELISA result.

The ability of each serological test to predict seroreversion at 9-months differed widely and ranged from 0% (ELISA) to 97% (ADVANCE) as illustrated in Figure 3.



ELISA: Enzyme-linked Immunosorbent Assay; Determine: Alere Determine™ HIV-1/2 Ag/Ab Combo; ABON: ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test, ADVANCE: OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test

Figure 3: Percentage seronegative tests per serological test in an HIV exposed uninfected 9-month cohort.

4.5 DISCUSSION

We describe seroreversion in a breastfed, maternal-cART-exposed HIV exposed PCR-negative 9-month cohort of the VEID study. We have observed that different serology assays vary in performance regarding identifying seroreversion at 9 months of age among HIV exposed infants. Rapid test kit-assays differed in specificity (45 – 97%). No seroreversion was documented in our cohort with the use of an HIV ELISA test.

Our study cohort had a seroreversion rate of 41% using the Determine rapid test-assay. Previous South African data from 2005 suggested that 82% of HIV exposed infants had seroreverted around the age of 9 months using the Determine rapid test kit-assay.[10] Our findings are in keeping with delayed seroreversion within the context of increasing access to maternal cART. [6] Although the ABON and ADVANCE assays performed slightly better in detecting seronegativity at 9-months of age (71% and 95% respectively), a large proportion of infants (61%) remained seropositive on one or more rapid assays. As all infants with a positive rapid assay or indeterminate result at 9 months require HIV PCR testing, these finding support the WHO recommendations to move away from rapid test kits and to perform routine PCR testing for infant-HIV diagnosis in HIV exposed infants at 9 months of age.

None of the 74 infants tested using a 4th generation HIV ELISA had seroreverted by 9 months of age. Nine study patients with no evidence of seroreversion on the assays were born to mothers that were on cART for close to 20 months (573 days) and most had undetectable viral loads. This supports a previous report by Gutierrez *et al.* that suggested that maternal cART with subsequent suppression in viral load can delay seroreversion in the exposed infant.[6] Seroreversion rates have changed since the first report by Moodley et al in 1995 that described that all HEU infants were seronegative by 15 months of age.[11] This finding highlights the need for further research to determine the age at seroreversion within the context of the PMTCT option B+ / universal maternal access to lifelong cART.

4.6 FUNDING

Two dedicated research nurses and hosting of the REDCAP database were funded by the South African Medical Research Council. Additional HIV PCR testing was performed by the National Health Laboratory Services (NHLS) and supported by National Department of Health. Rapid test assays were funded by the Paediatric Infectious Diseases Division of the University of Pretoria.

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Supplementary digital content 1: Test results of three rapid test assays, HIV ELISA and HIV PCR tests done at the 9-month follow up visits of the VEID study cohort

ABON	**ADVANCE**	**Determine**	**HIV PCR**	**PCR TAT (hrs)**	ELISA (S/CO)	Result Interpretation
neg	neg	pos	neg	76.03333	0.99	Equivocal
neg	indeterminate	pos	neg	96.76667	1.13	Reactive
neg	neg	neg	neg	38.68333	1.44	Reactive
neg	neg	neg	neg	61.05	2.32	Reactive
neg	neg	neg	neg	63.4	3.31	Reactive
neg	neg	neg			3.6	Reactive
neg	neg	neg	neg		3.73	Reactive
neg	neg	pos	neg	44.58333	3.77	Reactive
neg	neg	neg	neg	56.5	5.2	Reactive
neg	neg	pos	neg		6.67	Reactive
neg	neg	pos	neg	44.36667	7.29	Reactive
neg	neg	pos	neg	45.83333	8.12	Reactive
pos	pos	pos	neg		12.43	Reactive
pos	neg	pos	neg	55.35	13.27	Reactive
neg	neg	neg	neg	17691.55	13.85	Reactive
indeterminate	neg	indeterminate	neg	42.45	14.75	Reactive
neg	neg	pos	neg	55.15	15.11	Reactive
neg	neg	pos	neg	37.53333	15.79	Reactive
neg	neg	indeterminate	neg	56.55	16.42	Reactive
pos	neg	pos	neg	50.98333	16.81	Reactive
neg	neg	pos	neg	45.15	19.98	Reactive
neg	neg	pos	neg	45.16667	21.56	Reactive
neg	neg	pos	neg	51	23.93	Reactive
pos	neg	pos	neg	54.18333	24.28	Reactive
pos	pos	pos	neg	169.7	25.27	Reactive
neg	neg	pos	neg	45.05	25.31	Reactive
indeterminate	neg	indeterminate	neg	62.78333	26.39	Reactive
neg	neg	pos	neg	660	28.98	Reactive
neg	neg	pos			29.65	Reactive
neg	indeterminate	pos	neg	95.78333	30.25	Reactive
neg	neg	pos	neg	47.01667	34.46	Reactive
neg	neg	pos	neg	57.23333	35.28	Reactive
neg	neg	pos	neg	121.5	37.12	Reactive

pos	neg	pos	neg	65.18333	37.76	Reactive
pos	neg	pos	neg	161.5	38.14	Reactive
pos	indeterminate	pos	neg	91.35	38.4	Reactive
neg	neg	neg	neg	93.11667	41.36	Reactive
neg	neg	indeterminate	neg	56.05	42.86	Reactive
neg	neg	indeterminate	neg		43.08	Reactive
pos	neg	pos	neg	651.7167	44.01	Reactive
pos	neg	pos	neg	76.83333	45.92	Reactive
neg	neg	neg	neg	62.45	56.23	Reactive
pos	neg	pos	neg	43.75	62.4	Reactive
indeterminate	neg	pos	neg	38.1	63.1	Reactive
pos	neg	pos	neg	46.65	63.66	Reactive
pos	neg	pos	neg	47.75	66.6	Reactive
neg	neg	neg	neg	46.33333	68.5	Reactive
neg	neg	neg			73.11	Reactive
neg	neg	neg	neg	43.9	76.17	Reactive
neg	neg	pos	neg	56.41667	76.66	Reactive
pos	neg	pos	neg	37.33333	83.94	Reactive
neg		pos	neg	45.65	93.21	Reactive
pos	neg	neg	neg	92.58333	93.84	Reactive
pos	neg	pos	neg	46.05	100.89	Reactive
neg	neg	pos	neg	240.3333	106.93	Reactive
pos	neg	pos	neg	50	115.32	Reactive
neg	neg	pos	neg	61.88333	117.73	Reactive
pos	neg	pos	neg	45.15	126.24	Reactive
indeterminate	neg	indeterminate	neg	74.81667	126.66	Reactive
pos	neg	pos	neg	45.25	134.72	Reactive
neg	neg	pos	neg	0.383333	144.08	Reactive
pos	indeterminate	pos	neg	45.91667	147.32	Reactive
neg	neg	pos	neg	65.66667	149.53	Reactive
neg	neg	pos	neg	76.31667	154.45	Reactive
pos	neg	neg	neg	48.1	172.16	Reactive
pos	neg	pos	neg	141.1833	250.79	Reactive
neg	neg	indeterminate	neg	76.56667	271.71	Reactive
neg	neg	pos	neg	145.1	274.77	Reactive

neg: seronegative; pos: seropositive; TAT: turnaround time; ELISA: Enzyme-linked immunosorbent assay; S/CO: Signal-to-cutoff ratio; ABON: ABON™ HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test, ADVANCE: OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test, Determine: Alere Determine™ HIV-1/2 Ag/Ab Combo, RTK: Rapid test kit

CHAPTER 5

General discussion and conclusions

This PhD dissertation reports data from the very early infant diagnosis (VEID) study that was conceptualized when universal birth HIV PCR testing was mandated at Kalafong Provincial Tertiary Hospital. Three research questions were evaluated: (1) What factors are associated with early infant HIV acquisition?; (2) What are the growth outcomes of HIV exposed but uninfected infants?; and (3) What is the 9-month infant HIV seroreversion rate in the context of PMTCT Option B+ with the use of different serological tests?

The first research chapter aimed to enumerate the number of additional early infant HIV infections identified using a universal HIV testing approach for all HIV exposed infants, compared with a targeted HIV testing approach for mother-infant pairs who meet specified criteria. Additionally, we investigated the predictive values of individual and combined clinical and laboratory characteristics in identifying very early infant HIV infection. From the results, the maternal HIV prevalence during the study period was 22.12% and overall birth PCR positivity rate was 2.2%. Targeted birth HIV testing had a sensitivity of 80% (0.02 probability level in 3- and 4-risk models); therefore, 20% (1-in-5) of infected infants would additionally be identified with universal HIV testing. When considering predictive values of maternal and infant characteristics, a detectable maternal HIV viral load, maternal cART duration of <1 month, and an infant that was symptomatic at birth were the most significant risk factors predicting early infant HIV acquisition. Small-for-gestational-age was included with the above three characteristics in multivariate analyses and a two-, three-, and four-risk model for newborn HIV acquisition was developed with a predictive probability score of a newborn PCR positive test at 0.28, 0.498, and 0.57 respectively. These modelling scores can guide a targeted

approach algorithm. Additionally, risk models could be used to identify exposed uninfected infants that could benefit from a multiple drug (2- or 3-drug) post-exposure prophylaxis regimen compared to a single drug regimen. However, because close to 1 in 5 infected infants will not receive targeted testing, we strongly support universal birth PCR testing within the South African PMTCT context.

The second research chapter describes growth outcomes in HEU infants during the first year of life in the era of universal maternal cART and in relation to maternal and infant birth characteristics. Mothers were mostly breastfeeding at birth and on universal lifelong cART. HIV infection between birth at 9 months were less than 1% (0.31%) and occurred mostly by 6 weeks of age in infants that were high risk with symptoms of immunosuppression, such as growth faltering, from and after birth. The hospitalization rate amongst the HEU cohort was 41.3/1000 person-years. Longitudinal growth trends over the first year of life showed lower LAZ, especially in male infants, and lower HC in infants with maternal cART exposure more than 1 month prior to delivery. Infant characteristics at birth, namely the presence of symptoms, prematurity and low birth weight had the greatest impact on all the longitudinal growth parameters. Whether these findings translate to poorer outcomes in final height, body mass index and neurodevelopment in HEU children remains uncertain.

Lastly, we describe seroreversion in a breastfed, maternal cART exposed HIV exposed PCR negative 9-month cohort of the VEID study. We observed that different serology assays vary in performance regarding identifying seroreversion at 9 months of age among HIV exposed infants. Rapid test kit-assays differed in specificity (45 – 97%). None of the 74 infants tested using a 4th generation HIV ELISA had seroreverted by 9 months of age. This finding highlights the need for further research to determine the age at seroreversion within the context of the PMTCT option B+ / universal maternal access to lifelong cART. This supports a previous report that suggested that maternal cART with subsequent suppression in viral load can delay seroreversion in the exposed infant. It is possible that uninfected infants will remain seropositive beyond 18 months of age. Hence, future recommendations might include HIV PCR testing in infants up to 24 month of age.

Implications

HIV acquisition at birth is more frequent in infants with certain maternal and infant characteristics. Detectable maternal viral load, especially above 1000 copies/mL, carries a significantly higher risk to the infants than an undetectable viral load. We advocate maternal viral load testing at birth followed by regular repeat testing during the breastfeeding period. Although risk scores can predict high risk infants, we recommend continuing with universal birth PCR testing in the South African context.

Growth monitoring in HEU infants is important. Lower LAZ is seen, especially in male infants. The implications with regard to final height and body mass index are still unclear. Infants that develop symptoms suggestive of immunosuppression warrant regular medical follow up and repeat HIV PCR testing, as suggested in the current SAPMTCT guidelines. Though the HEU hospitalization rates are high, this seems to be decreasing, probably due to better virological control and immunological reconstitution amongst caregivers/adults with access to lifelong cART.

Seroreversion is dependent on the type of serological test, but varies widely between tests. No HIV ELISA tests returned seropositive results in this group of HEU infants at 9 months of age. We recommend HIV PCR testing during infancy to detect true seroreversion.

Future direction and research need

Birth HIV acquisition carries a high morbidity and mortality risk early in infancy. Limited reports on outcomes of infected infants on cART are available. Future research is aimed at describing the VEID study HIV infected cohort health outcomes.

Growth trends in HEU children up to adolescence should be determined. The impact of lower LAZ and HC on neurocognitive outcomes are important research questions that need to be addressed.

Seroreversion beyond the age of 18 months is a concern in the era of universal cART with probable prolonged maternal anti-HIV antibody detection in children. HIV PCR testing up to the age of 24, rather than 18 months, is recommended and should be evaluated.

ETHICS APPROVAL

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 20 Oct 2016.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 22/04/2017.

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Faculty of Health Sciences Research Ethics Committee

31/07/2014

Approval Certificate
New Application

Ethics Reference No.: 285/2014

Title: An Evaluation of Factors Associated with Early Infant HIV Acquisition, Infant Outcomes, and 9-12 Month Infant HIV Seroreversion in the Context of PMTCT Option B+: Prospective Data from an HIV Exposed Birth Cohort.

Dear Dr Nicolette du Plessis

The **New Application** as supported by documents specified in your cover letter for your research received on the 4/07/2014, was approved, by the Faculty of Health Sciences Research Ethics Committee on the 30/07/2014.

Please note the following about your ethics approval:
- Ethics Approval is valid for 3 years.
- Please remember to use your protocol number (**285/2014**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of 6 monthly written Progress Reports, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed.
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

• Tel:012-3541330 • Fax:012-3541367 Fax2Email: 0866515924 • E-Mail: fhsethics@up.ac.za
• Web: //www.healthethics-up.co.za • H W Snyman Bld (South) Level 2-34 • Private Bag x 323, Arcadia, Pta, S.A., 0007