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IDENTIFYING INTERVENTIONS TO IMPROVE OUTCOMES OF THE SOUTH AFRICAN PREVENTION OF MOTHER-TO-CHILD TRANSMISSION PROGRAMME

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A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science in Medicine

Johannesburg, 2013

DECLARATION

I, Rivka R. Lilian, declare that this dissertation is my own work. It is being submitted for the degree of Master of Science in Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

…….

10th day of September, 2013

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

Publications

1) Lilian RR, Kalk E, Bhowan K, Berrie L, Carmona S, Technau K, et al. Early diagnosis of in utero and intrapartum HIV infection in infants prior to 6 weeks of age. J Clin Microbiol. 2012 Jul;50(7):2373-7 (Appendices A and B).

2) Lilian RR, Kalk E, Technau K, Sherman GG. Birth diagnosis of HIV infection in infants to reduce infant mortality and monitor for elimination of mother-to-child transmission. Pediatr Infect Dis J. 2013: In press (Appendices C and D).

Oral conference presentations (presented by E Kalk and G Sherman)

3) Kalk E, Hampton G, Bhowan K, Lilian RR, Technau K, Selepe N, et al. HIV testing at 6 weeks – too little too late? [Abstract 435]. The 26th International Pediatric Association Congress of Pediatrics; 4-9 August 2010; Johannesburg, South Africa.

4) Sherman GG. Tackling paediatric HIV: Time for an 'earlier' diagnosis [Abstract 1546]. The 26th International Pediatric Association Congress of Pediatrics; 4-9 August 2010; Johannesburg, South Africa.

5) Sherman GG. Diagnosing HIV infection in infants: Are we there yet? [Abstract 112]. 19th Conference on Retroviruses and Opportunistic Infections; 5-8 March 2012; Seattle, USA.

ABSTRACT

South Africa's Prevention of Mother-to-Child Transmission (PMTCT) programme is critical for eliminating vertical HIV transmission and reducing infant mortality. Early treatment of HIV-infection to curb infant deaths requires earlier diagnostic testing than the currently recommended six-week test. This study describes the continuum of PMTCT care at a Johannesburg hospital to identify interventions for improvement and investigates birth HIV testing for infants. Data from a cohort study at the hospital evaluating diagnostic assays in HIV-exposed infants were collated with routine clinical data, validated and analysed. Among 838 mother-infant pairs, 38% of mothers attended antenatal clinics early enough to receive optimal antenatal prophylaxis. Only 72% of infants accessed six-week testing at the hospital; a further 10% underwent testing elsewhere. Of 38 HIV-infected infants, 29 were infected *in-utero* and could have been identified at birth (sensitivity of 76.3% for birth testing), compared to only 26 (68%) diagnosed by six-week testing at the hospital. Majority (88%) of these 26 infants accessed antiretroviral therapy, but treatment was only initiated at a median age of 16.0 weeks and 43% of HIV-infected infants who initiated treatment had defaulted or died before the end of the study. Mathematical modelling demonstrated that birth testing would be superior to a six-week test to maximise infants diagnosed and life years saved, with the ideal algorithm being a birth and ten-week test. The PMTCT programme can be enhanced by earlier antenatal care for women and earlier infant diagnosis. Birth testing would diagnose HIV-infection before infants die or default from the PMTCT programme, thereby enabling effective monitoring of MTCT, and would allow earlier treatment initiation to reduce early infant mortality.

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NOMENCLATURE

3TC	Lamivudine
AFASS	Referring to infant feeding practice as: Affordable, Feasible, Accessible, Safe and Sustainable
AIDS	Acquired Immune Deficiency Syndrome
ANC	Antenatal Clinic
ANOVA	Analysis of Variance
AZT	Zidovudine
CAP/CTM	COBAS AmpliPrep / COBAS TaqMan
CD4	Cluster of Differentiation 4
CI	Confidence Interval
DBS	Dried Blood Spot
DNA	Deoxyribonucleic Acid
EID	Early Infant Diagnosis
EID Study	Earlier Infant Diagnosis Study
eMTCT	Elimination of Mother-to-Child Transmission
ePP	Early Postpartum
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
IP	Intrapartum
IU	*In Utero*

LIS	Laboratory Information System
LTFU	Lost to Follow-Up
MTCT	Mother-to-Child Transmission
NHLS	National Health Laboratory Service
NPV	Negative Predictive Value
NVP	Nevirapine
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PEPFAR	President's Emergency Plan For AIDS Relief
PMTCT	Prevention of Mother-to-Child Transmission
PP	Postpartum
PPV	Positive Predictive Value
QALY	Quality-Adjusted Life Year
RMMCH	Rahima Moosa Mother and Child Hospital
r_s	Spearman's correlation coefficient
sdNVP	Single Dose Nevirapine
UNAIDS	Joint United Nations Programme on HIV/AIDS
UNICEF	United Nations Children's Fund
VL	Viral Load
WHO	World Health Organisation

1. INTRODUCTION

Paediatric human immunodeficiency virus (HIV) infection is one of the major health challenges facing South Africa and is responsible for significant infant morbidity and mortality (1-3). In children under five years of age, the most common route of HIV-infection is via mother-to-child transmission (MTCT) which can occur antenatally (*in utero* (IU) transmission), during labour and delivery (intrapartum (IP) transmission) or via breast feeding (postpartum (PP) transmission) (4). Thirty percent of pregnant women in South Africa are infected with HIV (5) and their infants are therefore at risk of being infected through MTCT. An effective Prevention of MTCT (PMTCT) programme to curb vertical transmission of HIV-infection is thus essential to improve infant outcomes in South Africa. The PMTCT programme has been given high priority by the National Department of Health in order to achieve the two-thirds reduction in childhood mortality prescribed in Millennium Development Goal four (6) and the elimination of MTCT (eMTCT) by 2015 (7).

1.1 PREVENTION OF MOTHER-TO-CHILD TRANSMISSION

1.1.1 The need for an effective PMTCT programme: mortality and morbidity in HIV-infected infants

Infants with HIV-infections are at significantly increased risk of mortality (8, 9), with an early peak of HIV-related infant deaths at two to three months of age (3). Among untreated infants who are infected by perinatal (IU or IP) transmission, 20% die by three months of life and 48% die by one year (2). Infants with perinatal HIV-infections have a significantly

higher risk of mortality compared to infants who acquire HIV later via breastfeeding, even accounting for increased neonatal mortality (2). Younger infants are at increased risk of dying, as are children with severe immunodeficiency and increased morbidity (including tuberculosis, gastroenteritis and pneumonia) (10, 11). HIV-infected children with poor growth, including those who are underweight, wasted or microcephalic, also have increased odds of dying (10-12). Infant mortality can be decreased by initiating treatment immediately after diagnosis of HIV-infection, as opposed to waiting for immunological or clinical deterioration (13).

In addition to increased mortality, HIV-infected infants who do not access treatment are at risk of rapid disease progression (1). Eighty five percent of untreated infants have severe immunosuppression according to World Health Organisation (WHO) guidelines (CD4 levels <25% (14)) by six months of age (1). Furthermore, HIV-infected infants require hospitalisation and repeated outpatient treatment for infections and thrombocytopenia (1). Growth is impaired in infected infants, with reduced weight and length over time compared to infants who are HIV-uninfected (12, 15-19). Such growth impairment may be related to increasing viral load (VL) levels (20).

Whether disease progression differs in infants with IU versus IP-infections, defined as HIV-infection first detectable at the time of birth and within two to four weeks after birth, respectively (8, 21, 22), is not entirely clear. Newell *et al* 2004 reported comparable mortality in infants with IU and IP-infections (8), but others have described increased risk of mortality and disease progression in IU-infected infants (9, 23). In one setting, the progression of infants with IU-infection to Acquired Immune Deficiency Syndrome

(AIDS) or death was more than double that in IP-infected infants (relative risk of 2.5) (24). If IU-infected infants are at risk of rapid disease progression, their need for early treatment is all the more essential. This can only be achieved with an optimally functioning PMTCT programme.

1.1.2 The PMTCT programme in South Africa

The national PMTCT programme in South Africa, outlined in Figure 1.1, aims to implement a comprehensive strategy for reducing vertical transmission of HIV and providing treatment, care and support for HIV-infected women and children (25). Pregnant women are encouraged to book at an antenatal clinic (ANC) as early in pregnancy as possible (25). All pregnant women attending ANCs are routinely offered HIV counselling and testing to establish their HIV status (25). Where a woman is HIV-infected, a CD4 count is performed on the same day as the HIV test and counselling regarding PMTCT interventions, safe feeding options, family planning and partner testing is provided. According to the 2008 national PMTCT guidelines, women with CD4 counts below 200 x 10^6/l were eligible to receive life-long Highly Active Antiretroviral Therapy (HAART), while women with higher CD4 counts received zidovudine (AZT) prophylaxis from 28 weeks gestation (4). The 2010 guidelines stipulated that women were eligible for HAART with a CD4 count below 350 x 10^6/l and AZT was initiated for women with higher CD4 counts from 14 weeks gestation (25). In both guidelines, AZT was also administered to the mother during labour and delivery, together with single dose nevirapine (sdNVP) (4, 25). New 2013 guidelines stipulate that all pregnant women should receive HAART, irrespective of CD4 count, for the duration of pregnancy (26). In addition to maternal PMTCT interventions, rapid administration of post-exposure prophylaxis to the neonate is critical. Prior to 2008, only sdNVP was administered to the new-born (27). In 2008 a dual

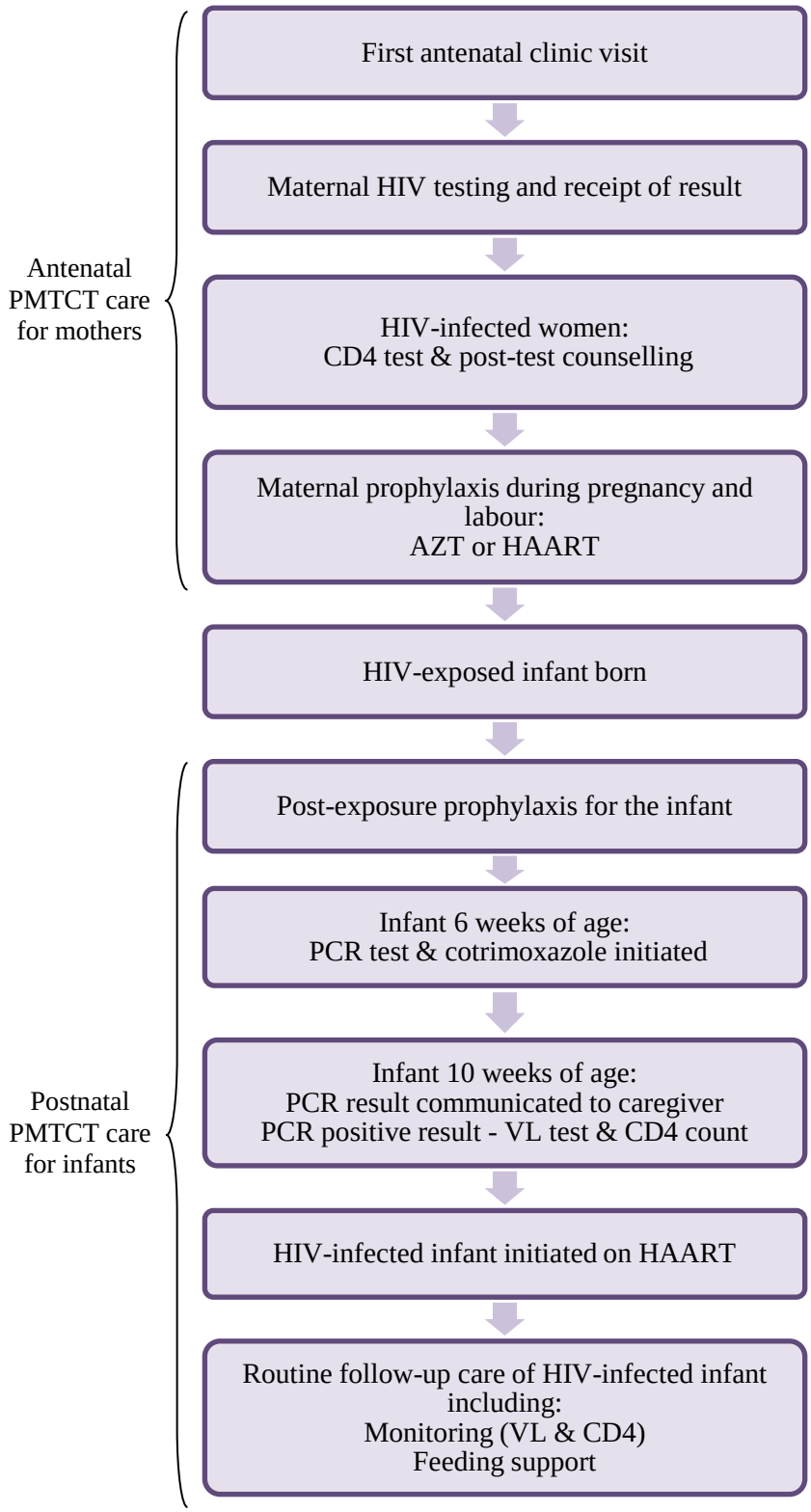


Figure 1.1 – The cascade of PMTCT care for HIV-infected mothers and their infants in South Africa (4, 25, 26, 28).

(AZT, Zidovudine; CD4, Cluster of Differentiation 4; HIV, Humman Immunodeficiency Virus; HAART, Highly Active Antiretroviral Therapy; PCR, Polymerase Chain Reaction; PMTCT, Prevention of Mother-to-Child Transmission; VL, Viral Load)

therapy regimen was introduced where infants received seven days of AZT in addition to sdNVP; 28 days of infant AZT was administered where mothers received less than four weeks of antenatal AZT cover (4). The 2010 guidelines stipulated that infants receive NVP daily for six weeks and continue for the duration of breastfeeding unless the mother was receiving HAART (25). Daily-dose infant NVP for at least six weeks is also recommended in the 2013 guidelines (26). The infection status of HIV-exposed infants is then determined by HIV deoxyribonucleic acid (DNA) polymerase chain reaction (PCR) testing at six weeks of age, as all infants are scheduled to attend an immunisation visit at this age (25, 26). A viral detection assay, as opposed to an HIV antibody detection assay is required, as infant antibodies cannot be differentiated from transplacentally derived maternal HIV antibodies. PCR testing is generally conducted using dried blood spot (DBS) samples that have largely replaced whole blood in routine settings. Results of the six-week PCR test are made available to the infants' caregivers at the ten-week immunisation visit. If the PCR test is positive, a confirmatory baseline VL test is performed together with a baseline CD4 count (28). As of 2010, all HIV-infected infants under one year of age were eligible to receive HAART irrespective of baseline results (28). Subsequently, all children under five years of age also became eligible for immediate treatment initiation irrespective of CD4 count (26). Infants receiving HAART must undergo regular follow-up to monitor treatment adherence, clinical stage, development and CD4/VL results (28). On-going feeding support is also essential – although exclusive formula feeding was supported prior to 2010, HIV-infected mothers are now recommended to exclusively breastfeed their infants for at least six months (29). These mothers receive HAART for the duration of breastfeeding (26).

The efficacy of South Africa's PMTCT programme can only be assessed with on-going monitoring and evaluation, particularly important when implementing new guidelines as

occurred in 2010 and is currently taking place in 2013. Evaluation of the PMTCT programme in South Africa has shown that aspects of antenatal services are well administered – the vast majority (≥90%) of women reportedly access antenatal HIV testing (30-35) and among HIV-infected women, over 78% reportedly access CD4 testing (30, 32, 33, 36) and over 80% receive some form of antiretroviral prophylaxis (30-33, 35). These successes are reflected in the marked reduction in MTCT rates in infants successfully accessing the PMTCT programme (30, 35, 37), with an estimated early vertical transmission rate in South Africa of 2.8% in 2011 compared to 8.2% in 2008 (37). Furthermore, majority of infants who access and remain in care achieve viral suppression (70% to 80% after one year of treatment) (10, 38). The burden of HIV-related morbidity in young infants therefore appears to be declining, with a decrease in HIV prevalence among paediatric hospital admissions (39).

However, optimal implementation of the national PMTCT programme with a view to eliminating MTCT is an on-going challenge. Women present late for antenatal care (33, 36, 40) for a variety of reasons, including fear of HIV testing, facility limitations and a lack of understanding of the importance of antenatal care (41, 42). Late presentation at ANC is a major concern, as MTCT is significantly increased in women who book for antenatal care later in pregnancy and have shorter durations of antenatal PMTCT cover (43). Late presentation for antenatal care and missing CD4 results (32, 33, 44) contribute to inadequate PMTCT and HAART initiation in accordance with national guidelines, as has previously been described (32, 36, 41, 45). In addition, follow-up of HIV-exposed infants through the PMTCT care cascade (Figure 1.1) is poor. Only ±70% of HIV-exposed infants receive sdNVP or adequate dual therapy PMTCT according to national guidelines (41, 46). Six-week PCR testing for early infant diagnosis (EID) has only been reported in 37% to

62% of exposed infants (33, 47, 48), with national coverage of PCR testing in 2010/2011 of 52% to 72% (34, 35). This will likely be an on-going problem, as only 35% of HIV-infected women interviewed in a recent national survey indicated that they intended to access six-week EID services (30). Factors which may hinder EID include the lack of integration of healthcare services (44) and maternal factors, including poverty, lack of social support and a lack of understanding about EID (47, 49). Only 57% of mothers reportedly return to receive PCR test results (46). Once HIV-infection is diagnosed, linkage to care is also problematic, with relatively few children initiating HAART compared to women (50) – in 2010, almost a third of children with positive PCR results were not linked to antiretroviral therapy services (51). Among infants who do initiate treatment, retention in care is also a challenge (10), with losses to follow-up (LTFU) being reported as early as three months after HAART initiation (52). Additionally, infant feeding is an area of confusion for mothers (41), with 18% of HIV-infected mothers in South Africa mixed-feeding during 2010 (30), significantly increasing their risk of PP transmission (53, 54).

Many reasons have been cited for the failures within the PMTCT programme. A critical concern is missed opportunities for the identification and care of HIV-infected mothers and their infants at all points of the PMTCT cascade. Missed opportunities for antenatal HIV testing, administration of PMTCT prophylaxis and HAART initiation among pregnant women have been described (32, 36, 44, 45, 48). Failure of antenatal HIV testing may account for 46% of missed opportunities for reducing MTCT, as HIV-infected women who are not identified do not access antiretroviral prophylaxis and neither do their infants (45). Similarly, missed opportunities for PCR testing of HIV-exposed infants (48) prevent the identification of infected infants for care. In addition, lack of integration of healthcare

services is an often cited problem, with diagnostic and treatment services for HIV-exposed and infected infants operating in a vertical manner to routine antenatal and postnatal services, thus hindering the continuum of care (33, 44, 55). Furthermore, shortages of resources, insufficient staff and a lack of clinical knowledge among healthcare workers hinder HIV service delivery, while inadequate data and information systems prevent meaningful monitoring and evaluation (44, 55-57).

As we aspire to eMTCT by 2015 (7), it is essential to prevent missed opportunities through the PMTCT programme in order to improve coverage of PMTCT care for all HIV-infected mothers and their infants. Additionally, infants who become HIV-infected, with or without PMTCT interventions, need to access appropriate care for childhood mortality rates to be reduced and Millennium Development Goal four (6) to be achieved. One way of improving these outcomes is to evaluate implementation of the PMTCT programme to identify gaps and barriers for targeted interventions. Since national data collection systems like the District Health Information System are dysfunctional (56), reporting on local experience currently offers the best insights.

1.2 DIAGNOSIS OF PERINATAL HIV-INFECTION IN INFANTS

1.2.1 Timing of early infant diagnostic testing

The recommendation to perform diagnostic testing at six weeks of age was based on the finding that a single PCR test at six weeks detects virtually all IU and IP transmissions of HIV (58). Six-week testing was also programmatically sound, as a routine immunisation visit is scheduled at six weeks of age. At the time the recommendation for six-week testing

was made, sdNVP was the only available prophylaxis and HAART was only initiated in HIV-infected infants following immunological or clinical deterioration.

Diagnosing HIV-infection earlier than six weeks of age is an intervention for reducing infant mortality that became obvious when the CHER study demonstrated that early initiation of HAART at a median age of seven weeks significantly decreases infant mortality and HIV progression (13). In response, paediatric treatment guidelines were updated to recommend that all infants under one year of age initiate HAART as soon after diagnosis as possible (28), but no evidence was available to inform programmes on how to make an earlier diagnosis. In South Africa, results of the six-week PCR test are generally only available at the ten-week immunisation visit and additional time is then required to prepare the primary caregiver to administer HAART (28). In 2010, there was a five-week delay from the time of the positive PCR to the first VL test performed as part of the HAART work-up (51); therefore, the earliest time HAART could be initiated following a six-week PCR test would be after 11 weeks of age. However, this is too late to influence the peak of HIV-related deaths centred at eight to 12 weeks of age (3) or the 20% of perinatally infected infants who die by 13 weeks (three months) of age (2). Initiating treatment after 11 weeks of age is also more than four weeks later than the median age of HAART initiation in the CHER Study (13). Earlier initiation of HAART to reduce infant mortality can only be achieved by an earlier diagnosis and the recommendation for a six-week PCR, made at a time when HAART was only commenced after clinical and immunological deterioration, therefore requires reconsideration.

The timing of diagnostic testing in infants depends on how early in life vertically

transmitted HIV-infection can be detected. Prior to PMTCT interventions, most vertical transmissions occurred IP, during late gestation or labour and delivery (59), and IU transmission detectable at birth only accounted for 38% of perinatal HIV-infections (21). However, with the provision of PMTCT interventions, the relative proportion of IU to IP transmission has changed. PMTCT prophylaxis administered in the late antenatal and intrapartum periods, when IP transmission occurs, reduces IP-infections. Even in the absence of antenatal cover, post-exposure infant prophylaxis for up to six weeks also effectively reduces IP transmission (60). Therefore, with intensified cover during the mid-late antenatal, intrapartum and postpartum periods to reduce IP transmission, proportionately more perinatally infected infants are likely to be infected IU. This has been reported in the context of sdNVP and post-exposure infant prophylaxis, where IU-infections comprise 69% to 74% of perinatal HIV transmissions detectable by four to eight weeks of age (1, 27, 60). Similarly, in the United States, the proportion of IU relative to IP transmissions has increased over time from 27% in 1990-1992 to 80% in 1999-2001 (61). In the context of prolonged and intensified PMTCT regimens, as are currently recommended, a higher proportion of perinatal HIV-infections are IU transmissions detectable at birth. Performing PCR testing at birth would therefore provide the opportunity to detect the majority of perinatal HIV-infections. Birth testing would also assist in monitoring the PMTCT programme, as eMTCT can only be assessed if an HIV-infection status is determined for all exposed infants. Since 28% to 48% of HIV-exposed infants in South Africa do not undergo six-week testing (34, 35), likely due to LTFU or death, the true vertical transmission rate cannot be determined. Performing EID at birth would allow testing of HIV-exposed infants before they die or are LTFU and would therefore assist in more successful monitoring for eMTCT.

In addition to increasing the proportion of IU-infections, multi-drug or extended PMCT regimens to mothers and/or infants may delay the detection of IP-infections beyond four to six weeks of age (60, 62-64). In the context of six weeks of postpartum infant AZT, with or without other drugs, 32% of IP-infected infants detectable by three months of age were PCR negative at four to six weeks (60). This concurs with findings of reduced VL levels in the first week of life among IU-infected infants receiving perinatal NVP, with 38% of these infants having VL levels below the limit of detection at five days of age (1). The reduced sensitivity of diagnostic assays following PMTCT prophylaxis further emphasises the need to re-evaluate the timing of the six-week PCR test, particularly in view of the 2013 national guideline recommendation for extensive maternal and infant PMTCT prophylaxis (26).

1.2.2 Assays for early infant diagnostic testing

The earliest time at which HIV can be diagnosed in infants also depends on the assay being used, as sensitivity for detecting HIV-infection varies between different assays at various ages. The Amplicor HIV-1 DNA PCR assay (Roche Molecular Systems Inc., Branchburg, NJ) has been widely used for EID. Version 1.0 of this assay on DBS yielded sensitivities of 27.3% and 88.9% for detecting perinatal infections at less than four days and ten to 15 days of age, respectively, with a specificity of 100% at both ages (65). This study was included in a review of DNA PCR testing, together with other studies which used in-house PCR assays on whole blood, prior to PMTCT therapy being available; the review yielded sensitivities of 38% at birth, 93% at 14 days and 96% at 28 days (21). Later studies which exclusively assessed the Roche HIV DNA PCR assay on whole blood demonstrated birth sensitivities of 38% to 62% (66-68) and sensitivities by one week, two weeks and one month of age of 29%, 67% and 67-92%, respectively (66, 67, 69). The assay was 100%

specific at birth (excluding cord blood), one week, two weeks and one month of age (66, 68, 69). In some of these studies, selected women received antenatal AZT (66, 68). The current HIV DNA PCR assay, Amplicor version 1.5, was 100% sensitive and 99.6% specific using DBS samples at six weeks of age (70), but has not been evaluated prior to six weeks.

Newer assays that are more sensitive than HIV DNA PCR may allow improved diagnosis of HIV-infection prior to six weeks of age. Two such assays, which offer high sample throughput and improved automation, are the COBAS AmpliPrep / COBAS TaqMan (CAP/CTM) HIV-1 assay (Roche Molecular Systems, Inc., Branchburg, NJ) and the APTIMA HIV-1 Screening Assay (Gen-Probe Inc., San Diego, CA) (71, 72). These assays use different technologies to the HIV DNA PCR assay to detect both HIV DNA and RNA. The CAP/CTM and APTIMA assays yielded sensitivities of 99.7% and 100%, respectively and specificities of 100% and 97%, respectively using DBS samples from infants over six weeks of age (71, 72). The manual version of the APTIMA assay was also sensitive and specific on DBS in HIV-exposed infants and children (99.2% and 100%, respectively) (73). Using serum samples, APTIMA was 100% specific in HIV-exposed, uninfected children aged less than 18 months (74). Although the CAP/CTM and APTIMA assays have demonstrated remarkable sensitivity, their performance for the diagnosis of HIV-infection in infants prior to six weeks of age, including testing at birth, has not been assessed.

1.3 DETERMINANTS OF MOTHER-TO-CHILD TRANSMISSION

One of the key determinants of MTCT risk is the administration of antiretroviral

prophylaxis to the mother-infant pair. Many studies have demonstrated the potent efficacy of antenatal, intrapartum and/or postpartum prophylaxis in reducing MTCT of HIV. AZT monotherapy administered during pregnancy, delivery and postpartum to the infant reduces MTCT risk by 68% compared to placebo (75). Intrapartum interventions alone, with sdNVP administered to mothers and infants at the time of delivery, also effectively reduce HIV transmission by 50% (27). Short-course prophylaxis from 36 weeks gestation successfully reduces perinatal infections versus placebo (76-78). However, short maternal and infant regimens are inferior to long regimens in reducing perinatal HIV transmission (79), with transmitting mothers having significantly shorter durations of antenatal PMTCT cover (43). Combination therapy including AZT, lamivudine (3TC) and/or sdNVP effectively decreases MTCT (80-83), though concerns regarding serious adverse events and resistance to lamivudine have been raised (81). In the absence of antenatal cover, six weeks of postpartum infant AZT combined with either NVP or nelfinavir and lamivudine decreases IP transmission more effectively than AZT alone (60). HAART administered antenatally and through the breastfeeding period is highly effective in reducing overall MTCT in the perinatal and postnatal periods (84, 85).

Maternal PMTCT and HAART regimens reduce VL levels. Maternal VL is therefore also a key determinant of MTCT, with higher antenatal and/or perinatal VL levels significantly increasing the risk of perinatal HIV transmission (86-92), even after adjusting for other predictors of MTCT (87, 88, 90-92). High VL levels are associated with low CD4 counts (89) and MTCT has also been linked to low maternal CD4 levels. Increased transmission has been recorded from mothers with CD4 counts below 200 x 10^6/l (83, 92-94) and mothers with CD4 levels under 350 x 10^6/l account for 76.6% of perinatal transmissions (91). Some studies have demonstrated that this association is independent of other

predictors of perinatal transmission (83, 91). However, in other cohorts, no significant association between maternal CD4 count and MTCT could be demonstrated (87, 89, 90) or the association was only significant if the analysis did not adjust for VL or other predictors of MTCT (43, 88, 92). This suggests that CD4 count may not be the strongest determinant of vertical transmission.

Although no significant association between maternal age and risk of MTCT has been found (1, 87, 89, 92, 95), failed coverage of antiretroviral prophylaxis is more likely in younger than older mothers (96, 97) which may in turn increase their transmission risk. The odds of failed prophylaxis coverage are also increased where mothers have fewer antenatal care visits (45, 96) and these mothers may be more likely to transmit HIV to their infants (98). Later presentation for antenatal care also significantly increases the risk of perinatal HIV transmission (43).

Intrapartum factors have been associated with transmission risk, with caesarean section preventing MTCT (81, 99) and premature rupture of membranes significantly increasing the risk of transmission (95, 100). A higher proportion of preterm deliveries occur from HIV-infected women compared to uninfected women (32) and preterm deliveries have also been associated with perinatal HIV transmission (43, 87, 101).

1.3.1 Predictors of IU transmission

Although birth testing would detect the majority of perinatal HIV-infections, allow earlier initiation of HAART and assist in monitoring for eMTCT, testing of all HIV-exposed

infants at birth may not be appropriate in all settings. Predictors of IU transmission, namely a positive PCR test at birth, would assist in identifying HIV-exposed infants at increased risk of IU-infection who would benefit from an HIV test at birth. Both IU- and IP-infections have been associated with less optimal maternal antiretroviral cover (61), increasing maternal VL levels (53, 61, 101-103), advanced clinical stage of maternal HIV disease (102) and birth weight <2 500g (61). Some studies have also found that low maternal CD4 count predicts for IU (102) and IP (102-104) transmission. Unique predictors of IU versus IP-infection have also been described – IP transmission can be predicted by duration of membrane rupture (24, 53, 61, 104) and pre-term birth (24, 53, 61), whereas IU-infections have been associated with delayed initiation of maternal AZT prophylaxis (101) and maternal weight loss during pregnancy (105). Understanding the unique predictors of IU and IP transmission may direct targeted interventions to reduce these infections and guide targeted testing aimed at identifying IU-infected infants who are possibly at risk of rapid progression (9, 23, 24).

1.4 MATHEMATICAL MODELLING OF EARLY INFANT DIAGNOSIS

Clinical studies are limited by the number of subjects that can be recruited, funding, time constraints and implementation concerns. Mathematical models can overcome these limitations and have therefore been widely used to investigate the HIV epidemic and the PMTCT programme (50, 106-113). None of these models have specifically investigated the optimal time for EID prior to six weeks of age in terms of maximising the identification of HIV-infected infants and reducing infant mortality. Investigating these questions in a mathematical model would allow the impact of earlier testing at a national level to be assessed before recommendations for earlier PCR testing are made.

1.5 AIMS AND OBJECTIVES

Given the critical role of South Africa's PMTCT programme in achieving eMTCT (7) and reducing childhood mortality (6), the poor quality of national PMTCT monitoring and evaluation data (56), the paucity of information regarding diagnosis of HIV-infection prior to six weeks, the availability of new HIV test technologies (71, 72) and the evolution of PMTCT programmes since the recommendation for six-week testing was made, this study aims to:

- Describe the continuum of PMTCT care at an urban tertiary setting to identify interventions to improve outcomes of the PMTCT programme.
- Investigate the optimal time for EID of HIV prior to six weeks of age, with a focus on HIV diagnostic testing at birth.

Specifically, the study objectives are:

1. Describe the routine PMTCT programme at Rahima Moosa Mother and Child Hospital (RMMCH) to identify interventions for improvement.
    - Construct coverage cascades depicting antenatal PMTCT care to HIV-infected mothers and postnatal PMTCT care to HIV-exposed infants.
    - Describe the demographic and clinical profile of HIV-infected women delivering at RMMCH, and their antenatal and peripartum PMTCT care, in relation to MTCT.
    - Describe the characteristics and outcomes of HIV-exposed infants born at RMMCH, and PMTCT care received, in relation to HIV-infection status.

2. Evaluate and compare the performance of three HIV detection assays (namely, HIV DNA PCR, CAP/CTM and APTIMA) on DBS for the diagnosis of perinatal (IU and IP)

HIV-infection at time points prior to six weeks of age.

3. Determine whether antenatal or peripartum factors can predict IU-infection to identify a subgroup of infants most likely to benefit from an HIV test at birth.

4. Using a mathematical model, determine the optimal time to perform early PCR testing to detect perinatal (IU and IP) HIV-infection and minimise the HIV disease burden.

2. METHODS

For the purpose of this dissertation, a secondary data analysis was performed. A master dataset was compiled using multiple data sources, including laboratory data from the Earlier Infant Diagnosis Study (EID Study) and clinical data from operational databases maintained by RMMCH's routine services. These data were collated and thoroughly validated. This dissertation is an analysis of the newly compiled master dataset.

2.1 EARLIER INFANT DIAGNOSIS STUDY

A prospective, observational cohort study was conducted at RMMCH, Johannesburg, South Africa to determine whether IU and IP HIV-infections could be identified prior to six weeks of age. HIV-infected women over 18 years of age who were aware of their HIV status antenatally and planned to attend RMMCH for postnatal care were eligible to participate in the study. Recruitment took place in the postnatal ward three mornings per week, at which time written informed consent was obtained and a study number assigned to the mother-infant pair. Demographic and clinical data, including information concerning antenatal PMTCT care, were collected from women at the time of enrolment. Recruitment took place from August 2008 to July 2010 and follow-up continued until December 2010.

All mother-infant pairs participating in the operational study were offered the routine PMTCT care available at RMMCH as per national guidelines at the time. The 2008 guidelines, described in section 1.1.2, were applicable for most of the study period (4),

with the 2010 guidelines only being implemented toward the end of the study (25, 28). Routine peripheral blood HIV DNA PCR testing at six weeks of age determined the HIV status of enrolled infants throughout the study (Amplicor HIV-1 DNA PCR, version 1.5; Roche Molecular Systems Inc., Branchburg, NJ). Peripheral blood samples were taken as opposed to DBS samples, as skilled phlebotomists were available at RMMCH. Test results were provided to the infants' caregivers at the ten-week immunisation visit in accordance with national guidelines. HIV-uninfected infants were discharged from the programme; HIV-infected infants were referred for HAART initiation at RMMCH and followed until December 2010.

Study visits occurred at birth, two, four, six and ten weeks of age, at which time a medical practitioner examined each infant. For study purposes, DBS samples were made at each visit using Schleicher & Schuell 903 (S&S 903 W-041) filter paper cards, which were then stored at RMMCH at ambient room temperature with desiccant sachets. Where the routine six-week HIV DNA PCR test identified an infant as HIV-infected, stored DBS samples from birth, two, four and six weeks were retrieved and retrospectively tested together with samples from two age-matched, HIV-exposed, uninfected controls. DBS samples were tested on three assays, namely: HIV DNA PCR, the quantitative CAP/CTM HIV-1 assay (version 1.0; Roche Molecular Systems, Inc., Branchburg, NJ) and APTIMA HIV-1 screening assay with automated Tigris analyser (Gen-Probe Inc., San Diego, CA). Repeat testing was conducted where invalid, equivocal or discrepant results were obtained at a single time point and sufficient sample was available.

If an infant was LTFU before accessing routine six-week testing at RMMCH, PCR data

from the National Health Laboratory Service's (NHLS) Laboratory Information System (LIS) were checked to determine if the infant had accessed routine diagnostic testing at any other healthcare facility. Where the infant's surname and date of birth matched, the PCR result recorded on the LIS was used to assign an HIV status. At the end of the study, birth DBS samples from all infants with an unknown HIV status were tested for the presence of HIV using the APTIMA assay; positive results were confirmed with CAP/CTM and HIV DNA PCR. This APTIMA screen identified all remaining IU-infected infants in the cohort. In addition, all available stored DBS samples from the four-week visit that could be used to assign a definitive HIV-uninfected status (114), as well as samples from infants known to have died, were tested. All laboratory testing was conducted by accredited hospital-based laboratories.

2.2 DATA COLLATION AND VALIDATION

2.2.1 Laboratory Database from the EID Study

HIV DNA PCR, CAP/CTM and APTIMA results had been recorded in a Microsoft Excel workbook during the EID Study. All qualitative results were checked against the raw laboratory data (viz. the quantitative output readings) to identify data capturing errors or missing data. These were resolved using the original laboratory reports. Erroneous results due to sample swops were also identified and the correct results from repeat testing recorded. Negative results from the APTIMA birth screen had not originally been captured in the Laboratory Database; these were added so that a complete set of birth data would be available for analysis.

2.2.2 Creating the Master EID Database

For the purpose of this dissertation, a Master EID Database containing all laboratory, demographic and clinical data for mother-infant pairs enrolled in the EID Study was created. The Master EID Database was compiled by collating the EID Laboratory Database with data from multiple sources, namely:

- **Clinical E-database**: This operational Microsoft Access database was used in RMMCH's routine clinic to record maternal and infant demographic and clinical data, including findings at each study visit. Anonymous clinic identification numbers were linked to EID Study numbers using a master list provided by study clinicians; patient identifying details were not accessed at any time. Of 271 variables in the Clinical E-database, approximately 201 were validated and analysed, including 45 new variables that were added into the database (Appendix E). These new variables were re-coded from the original variables, calculated from dates or figures in the database or imported from other data sources (for example, the province and district where women lived and where ANCs were located were imported from a Department of Health mapping document).
- **EID Study Records**: These paper-based study records were the primary source documents in which study clinicians noted demographic and clinical findings from study visits. Reports from routine laboratory testing were also filed with these records.
- **Patient Tracking Tool**: This Microsoft Excel visit log was used during the EID Study to record expected and actual visit dates, as well as follow-up of infants.
- **Miscellaneous workbooks**: The EID Study clinician provided a number of Microsoft Excel workbooks with additional infant data, including details of hospital admissions, antibiotic treatment and summaries of infant outcomes and testing.

2.2.2.1 Data validation and coding

The Clinical E-database, which formed the majority of the Master EID Database, was extracted to Microsoft Excel and thoroughly validated prior to being merged with the Laboratory Database and other data sources. Outlying, missing or nonsensical values were identified using histograms and Excel filters. Discrepancies were detected by comparing variables that had been duplicated in the various data sources, for example, attendance at study visits recorded in the Clinical E-database was compared to visit attendance in the Patient Tracking Tool. Queries were discussed with the EID Study clinician and resolved using the Study Records. Variables in the Clinical E-database that were particularly problematic in terms of validity or coding included:

Maternal variables:

- **'Gestational age at antenatal clinic booking'**: In the original Clinical E-database, gestational age was calculated using the reported date of last menstrual period. However, study clinicians advised that this variable was highly inaccurate. Gestational age at ANC booking was therefore calculated as follows:
  - The antenatal booking date was subtracted from the infant's date of birth to calculate the number of weeks before delivery that the booking took place.
  - Gestation was assumed to be 39, 36 or 41 weeks for term, preterm or postterm deliveries, respectively, based on working definitions from the study clinician. The number of weeks before delivery that the booking occurred was subtracted from 39, 36 or 41, as applicable, to calculate the gestational age at booking.

  This calculation led to a number of negative results, uncovering incorrectly captured booking dates. These were corrected using the EID Study Records. Where the correct

date could not be ascertained, 'Gestational age at booking' was not calculated.

- **'Maternal PMTCT'**: Maternal PMTCT was highly variable, comprising 59 codes for the various combinations of sdNVP, AZT and HAART (Appendix F). In some instances, the PMTCT code indicated that HAART had been administered even though a HAART initiation date had not been recorded. These PMTCT regimens were checked in the EID Study Records and appropriately re-coded. Three additional variables relating to maternal PMTCT were added to the Master EID Database:

1. *PMTCT rank:* The EID Study clinician had ranked the 59 PMTCT regimens from least to most optimal; these rankings were incorporated using Excel's VLOOKUP function.
2. *Ordinal categorisation:* PMTCT regimens were categorised as follows:
    - Suboptimal: No antenatal prophylaxis.
    - Intermediate: Less than ten weeks of antenatal AZT or HAART.
    - Optimal: Ten or more weeks of antenatal AZT or HAART.

    A ten week cut-off was used as the 2008 PMTCT guidelines recommend that women initiate AZT from 28 weeks gestation (4), equating to ten to 12 weeks of treatment.
3. *Length of antenatal treatment:* Each PMTCT regimen detailed in Appendix F was translated into a binary variable indicating whether or not sdNVP had been received and continuous variables indicating how many weeks of antenatal AZT and/or HAART had been administered. Where a woman had received pre-conception HAART, the number of weeks of antenatal treatment was based on the duration of gestation (39, 36 or 41 weeks for term, preterm or postterm deliveries, respectively).

- **'Antenatal CD4 count'**: CD4 counts were measured using single platform pan-leukogate technology. Two sets of antenatal CD4 counts were recorded in the Clinical E-database, namely 'Last recorded count' (the count closest to delivery) and 'Second last recorded count' which was performed earlier in pregnancy. These were combined

into a single variable indicating the *earliest* antenatal CD4 count. Where antenatal CD4 counts had not been captured, every effort was made to find the missing data. The study numbers of mothers without CD4 counts were sent to a clinician at RMMCH who populated each mother's first name, surname, date of birth and delivery date. The NHLS data warehouse unit then identified women with matching details who had had CD4 tests within 40 weeks of the delivery date. These antenatal counts, which were relinked to anonymous study numbers by the NHLS data warehouse unit, were added to the Master EID Database. 'Gestational age at CD4 test' was calculated as described above for 'Gestational age at booking'.

- **'Maternal VL'**: Some mothers had plasma samples stored at delivery and VL testing was performed on these samples using the CAP/CTM HIV-1 assay (Roche Molecular Systems, Inc., Branchburg, NJ). VL testing was performed retrospectively; it was therefore not possible to check results that were not credible, for example, where VL levels were undetectable in women who had only received sdNVP and three to four weeks of antenatal AZT. Undetectable VL results that were not plausible in the context of the PMTCT regimen were excluded from all analyses. Where undetectable VL results were included in the analyses, they were assigned a value of zero.

Infant variables:

- **'Infant PMTCT'**: This variable originally comprised 13 codes (Appendix F) which were categorised in the Master EID Database as follows:
  - Suboptimal: no PMTCT, sdNVP only, sdNVP and less than one week of AZT
  - sdNVP and one to two weeks of AZT
  - sdNVP and four weeks of AZT
  - Daily-dose NVP

- **'Date of 1st PCR'**: Where an infant was more than seven weeks of age or less than 5.5 weeks of age at the time of PCR testing, the test date was checked in the EID Study Records. The records were also consulted where the date was obviously incorrect, for example, where a test was recorded before a date of birth. Age at first PCR test was calculated so that the spread of ages at which infants were first tested could be described.
- **'Date of 2nd PCR'**: Initially, a number of second PCR test dates indicated projected booking dates. Projected dates were deleted so that dates were only recorded where PCR testing actually occurred.
- **'1st/2nd/3rd PCR results'**: Some infants had incorrect 'intermediate' PCR results or had no PCR results despite test dates being recorded. In one case, a PCR result had been entered without a date and it was ascertained that this result had been captured against the incorrect infant. In all instances, the EID Study Records were consulted and the PCR results correctly captured.
- **Growth measurements**: Weight, length and head circumference at birth were recorded twice in the Clinical E-database (under birth details and the first study visit). These were compared and discrepant measurements corrected. Across all study visits, missing weight, length and head circumference measurements were captured from the Study Records and outlying z-scores used to identify measurements that had been incorrectly captured. Where a growth measurement was corrected, the corresponding z-score was re-calculated using the WHO Anthropometric Calculator (version 3.2.2; WHO, Geneva, Switzerland) available at http://www.who.int/childgrowth/software/en/. Z-scores were also re-calculated where a study visit date was corrected, as these scores are based on infant age at the time of the measurement.
- **'Age at study visits'**: This variable was calculated by subtracting infant date of birth

from the visit date. A number of negative ages were recorded, uncovering incorrect birth and/or study visit dates that were corrected using the EID Study Records.

2.2.2.2 Classification of study visits

A number of visit dates in the Clinical E-database were not actual visits where infants attended RMMCH, but rather dated notes (for example, a date that a PCR result had been found on the NHLS LIS). An additional 'Information only' section was therefore added to the Master EID Database so that these dates would not be confused with visit dates. The LTFU status of each infant was then compared to the visits recorded in the database. For example, all five scheduled study visits should have been captured for infants who were not classified as LTFU. Where necessary, the EID Study Records were consulted and missing study visits manually entered into the Master EID Database.

The timing of all visits was checked to ensure that they had been captured in chronological order. Particular attention was paid to visits where infants were more than one week older or less than one week younger than the expected visit age. The six- and ten-week visits were not classified by age, but rather as 'PCR' and 'results' visits (viz. the visits at which routine PCR tests were performed and test results given, respectively). The spread of ages at which infants presented for the six-week visit and returned for results could then be analysed.

2.2.2.3 Assigning infant HIV status

Infant HIV status was classified as follows in the Master EID Database:

- **HIV-infected** – Infants who had a positive routine HIV DNA PCR test or at least two positive test results on different assays using study DBS samples.
- **HIV-uninfected** – Infants who had a negative routine HIV DNA PCR test or at least two negative results on study DBS samples at four weeks of age or older, in accordance with WHO guidelines (114).
- **Unknown but no IU-infection** – Infants who had a negative HIV DNA PCR test or negative study DBS results under four weeks of age, thereby excluding IU-infection, but no later test results to confirm a diagnosis.

HIV-infected infants were further classified according to the timing of transmission:

- **IU-infected** infants had at least two positive test results on different assays at birth.
- **IP-infected** infants had negative test results at birth, but tested positive on two or more assays by six weeks of age.
- **PP-infected** infants tested negative at birth and six weeks of age, but had a positive HIV DNA PCR test thereafter in the context of breastfeeding. It is possible that infants with an early PP transmission are included in the IP-infected group.

2.2.2.4 Determining the method of infant diagnosis

The HIV status of infants enrolled in the EID Study was established in one of three ways:

- Diagnostic PCR testing was conducted through RMMCH's routine PMTCT programme as part of the EID Study. In this scenario, mothers were fully compliant with the study requirements.
- Some infants were LTFU from RMMCH but had accessed routine PCR testing at

another healthcare facility and were identified by searching the NHLS's LIS. In this scenario, mothers were partially compliant with the study's PMTCT care requirements.

- Some infants did not access diagnostic PCR testing at any facility (non-compliant) and DBS study samples were therefore tested, where possible, to assign an HIV status.

It was initially difficult to determine the method of infant diagnosis, as the Clinical E-database did not differentiate PCR tests that were performed as part of the EID Study from those that were found by trawling the NHLS LIS. Various documents, including the Patient Tracking Tool, were therefore consulted. Where an infant had attended a study visit on the date the PCR sample was drawn, the PCR test was recorded as having been performed at RMMCH as part of the study.

2.2.2.5 Classification of infants' final outcomes – LTFU and death

The Clinical E-database originally classified infants' final outcomes as in care, discharged, LTFU, transferred out (n = 1) or died. However, these outcomes were not mutually exclusive; for example, an infant could have died after being LTFU from the study. LTFU status and death were thus re-coded as two separate variables so that both could be recorded for each infant. The 'discharged' outcome was not required, as HIV-uninfected infants who were not LTFU were known to be discharged from the study. Whether HIV-infected infants accessed care was recorded separately in the database of HIV-infected infants (see section 2.2.3 for details).

LTFU status was based on visit attendance once all missing study visits had been captured from the EID Study Records. A date of LTFU was recorded where required. The

'Alive/Dead' variable detailed whether each infant was alive at the end of the study (December 2010). This was ascertained using multiple source documents, including the Clinical E-database, Patient Tracking Tool, EID Study Records and additional summaries compiled by the study clinician. A second variable was created in the Master EID Database indicating whether infants were alive or dead at ten to 14 weeks of age (viz. the end of the study for each infant). A date of death was recorded for infants who died.

2.2.3 Creating the Database of HIV-Infected Infants

In addition to the Master EID Database, a second database was compiled to record the follow-up of HIV-infected infants. Data were collated from the following sources:

- **Empilweni database**: In addition to the Clinical E-database, a second operational database was obtained from RMMCH. This database recorded the routine follow-up care of HIV-infected infants attending Empilweni Clinic, the treatment clinic at RMMCH, including details of HAART treatment and CD4/VL results to 31 December 2010. Anonymous Empilweni Clinic identification numbers were linked to EID Study numbers using a master list provided by Empilweni clinicians.
- Two additional databases detailing the follow-up of HIV-infected infants, including HAART treatment and laboratory (CD4 and VL) results, were also available. These databases had been compiled from patient records, one by the Empilweni Medical Officer and the other by the EID Study clinician.

Whether infants had initiated HAART and the date of treatment initiation was ascertained from the three databases of HIV-infected infants. Data were considered confirmed where at least two of the three data sets concurred; Empilweni clinicians were contacted to resolve

any discrepancies. Infants were classified as having remained in care if they attended Empilweni Clinic in the last nine months of the study. This time period was chosen as virologically suppressed children required VL testing every six months (28) and an additional three-month leeway was provided. Viral suppression was achieved when VL levels dropped below 400 copies/mL as per South African Guidelines at the time (28).

CD4 and VL measurements over time were collated and validated using the three databases of HIV-infected infants. CD4 counts were measured using a single platform pan-leukogate technology. Viral load testing was initially performed with the NucliSENS EasyQ HIV-1 assay (version 1.2 (bioMerieux, Boxtel, Netherlands) until November 2009 and version 2.0 (bioMerieux, Marcy L'Etoile, France) from November 2009 to September 2010), followed by CAP/CTM HIV-1 testing during the last three months of the study (Roche Molecular Systems, Inc., Branchburg, NJ). Where the CD4/VL test was performed prior to the date of HAART initiation or where the infant did not initiate HAART, the test was considered to be a baseline measure. For CD4/VL tests performed after HAART initiation, the time (in months) from treatment initiation to the test date was calculated. Tests were then grouped into six-monthly intervals (viz. tests performed zero to six months after HAART initiation, six to 12 months after HAART initiation etcetera). Where more than one test was performed in a single six-month period, only the first test was included.

2.3 DATA ANALYSIS

All statistical analyses were performed using Microsoft Excel 2010, Statistica 10 (StatSoft Inc., Tulsa, OK) and STATA 12.0 (StataCorp LP, College Station, TX) using data from the

Master EID Database and Database of HIV-Infected Infants. α was set at 0.05, viz. two-tailed p-values < 0.05 were considered significant.

Categorical variables were described using frequencies and proportions. Mean ± standard deviation and median (range and interquartile range) were used to describe normal and non-normal continuous variables, respectively. Normality was assessed using Shapiro-Wilk's Test, where p>0.20 indicates a normal distribution.

Categorical variables were compared with Chi^2 or Fisher's exact tests, as appropriate. Where multiple comparisons between groups were performed, the level of significance was adjusted using a Bonferroni correction, viz. α/the number of comparisons.

Mann-Whitney U test was used to compare two groups of independent, continuous variables that were not normally distributed. Non-parametric comparisons of three or more independent groups were performed with Kruskal-Wallis Analysis of Variance (ANOVA). Where the ANOVA was significant, two-by-two comparisons with the Mann-Whitney U test identified which of the groups were significantly different; the significance level was adjusted using a Bonferroni correction. A Friedman ANOVA was used for non-parametric comparisons of three or more groups of repeated measures, namely outcomes that were not independent, to determine whether the outcome changed significantly over time (a time-effect).

Differences between three or more groups of continuous measures that were not independent but were normally distributed were assessed using an ANOVA for repeated measures. Mauchley's Sphericity Test was performed to check that the assumption of sphericity, namely equal variances of the differences between all possible combinations of the groups, was not violated. Where this assumption had been violated ($p<0.05$ with Mauchley's Test), a Greenhouse-Geisser correction was applied. Greenhouse-Geisser adjusted p-values were calculated for the time-effect and for between-group comparisons to detect differences between two or more groups over time.

Box-plots were used to present median and interquartile range of non-normal, continuous variables that were grouped by categorical parameters. Scatterplots were used to visually compare two continuous variables. Spearman's correlation coefficient (r_s) was calculated to assess the correlation between non-normally distributed continuous variables.

Time-to-event analyses were performed using Kaplan Meier survival curves and log rank tests to compare overall survival between groups. Hazard ratios with 95% confidence intervals (CI) were calculated using cox regression analysis where the assumption of proportional hazards had not been violated.

2.3.1 Objective 1 – RMMCH's PMTCT programme and the study cohort

2.3.1.1 The antenatal and postnatal PMTCT coverage cascades

Antenatal care provided to HIV-infected women enrolled in the EID Study was depicted in a coverage cascade. This presented the proportion of women who accessed care at key

points in the PMTCT programme, namely attendance at an ANC, antenatal CD4 testing and receipt of PMTCT prophylaxis. Data were not available for other antenatal cascade indicators, including HIV testing. Since ANC attendance is the entry point into the antenatal cascade, the correlation between how soon women booked for antenatal care and their PMTCT cover and VL levels was investigated. The antenatal cascade was then stratified according to the three methods of infant diagnosis which indicate compliance with the PMTCT programme (section 2.2.2.4), allowing the link between antenatal health seeking behaviours and compliance with infant PMTCT care to be investigated.

The postnatal follow-up of HIV-exposed infants was also depicted in a coverage cascade, indicating how many infants accessed care at each step of the PMTCT programme (including NVP prophylaxis, PCR testing, receipt of results and for HIV-infected infants, HAART initiation, retention in care and virological suppression). Data for cotrimoxazole administration were not available. The postnatal PMTCT cascade was stratified by method of infant diagnosis and the performance of RMMCH's routine PMTCT programme in providing diagnostic services for HIV-exposed infants and treatment for HIV-infected infants described. Time to initiation of treatment was calculated from the ten-week result visit to the date of HAART initiation. For infants who received HAART, trends in VL levels and CD4 counts at six-monthly intervals following HAART initiation were evaluated using a Friedman ANOVA. Infants could only be included in this analysis where CD4 and VL results were recorded consistently in every six-month period.

2.3.1.2 Maternal characteristics by MTCT status

Maternal demographic and clinical characteristics, antenatal PMTCT care, peripartum and

delivery characteristics and infant feeding practices were compared in 1) mothers of IU- and IP-infected infants versus mothers of HIV-uninfected infants and 2) mothers of IU-infected infants versus mothers of IP-infected infants, using the statistical methods described above.

2.3.1.3 Infant characteristics by HIV-infection status

Infant gender, birth anthropometry, PMTCT prophylaxis, morbidity (as measured by hospital admissions and treatment with antibiotics) and mortality were compared in: 1) IU- and IP-infected versus uninfected infants and 2) IU-infected versus IP-infected infants.

Deaths were investigated using Kaplan Meier survival analysis. For infants who died, the event time was imputed as the age at death. For infants who did not die, follow-up time in the study was calculated and data were right censored. Calculation of follow-up time yielded a result of zero for infants who were only seen at birth; a follow-up time of 0.01 days was assigned in these instances, as values of zero are excluded from STATA survival analyses. Only deaths to 14 weeks of age were compared in infected and uninfected infants, as all infants, irrespective of HIV status, were followed to this age and deaths would therefore have been equally detected. When comparing deaths in IU-infected and IP-infected infants, all deaths to December 2010 were included as all HIV-infected infants were followed to the end of the study.

Weight, length and head circumference z-scores over time (at the birth, two-, four-, six- and ten-week visits) were compared in IU- and IP-infected versus uninfected infants using

an ANOVA for repeated measures. Only infants with growth measurements at every visit could be included in the analysis. The calculation of z-scores takes infant age into account and thus made allowance for infants who were slightly younger or older than expected at a study visit. Preterm infants were excluded from the analysis, as many had very low z-scores and this parametric analysis was sensitive to outliers. Growth could not be compared in IU-infected versus IP-infected infants due to the small number of IP-infected infants with repeated measures over time.

2.3.1.4 Implementation of the national PMTCT guidelines over time

The 2008 national PMTCT guidelines (4) were released six months before the first mother-infant pair was enrolled in the EID Study. In order to assess the implementation of these guidelines over time, the proportions of women presenting early for antenatal care and accessing optimal PMTCT from ±28 weeks gestation were compared in women enrolled in the first and second halves of the study. The proportion of infants receiving one month compared to one week of AZT prophylaxis, as an indicator of the length of maternal AZT cover, was also compared. Additionally, IU and IP transmission and time to infant HAART initiation in the first and second halves of the study were evaluated. An improvement in these indicators in the second half of the study would suggest that the guidelines were being implemented over time. The implementation of the 2010 recommendation for exclusive breastfeeding was considered in terms of the proportion of women who elected to exclusively breastfeed. The two cases of mixed feeding were excluded for this analysis.

2.3.2 Objective 2 – Diagnosis of IU and IP HIV-infection under six weeks of age

The performance of the HIV DNA PCR, CAP/CTM and APTIMA assays in identifying

IU- and IP-infected and HIV-uninfected infants prior to six weeks of age was assessed. Results from DBS study samples tested with each assay at birth, two, four and six weeks were compared to the final HIV status of each infant (the reference standard). Sensitivity (true-positive results/(true-positive + false-negative results)) and specificity (true-negative results/(true-negative + false-positive results)) were calculated with 95% CI using the Wilson score method. This method was chosen as the normal Gaussian approximation is ill suited for calculating CIs for sensitivity and specificity of diagnostic tests (115). Positive predictive values (PPV) (true-positive results/(true-positive + false-positive results)) and negative predictive values (NPV) (true-negative results/(true-negative + false-negative results)) were also calculated to determine the proportion of infants with positive and negative results, respectively, with a correct diagnosis. Predictive values were computed as opposed to likelihood ratios, as the prevalence of HIV did not vary in each age group.

Equivocal results obtained with the HIV DNA PCR assay, defined as optical density readings between 0.2 and 1.5 as per the NHLS Standard Operating Procedure (116), were excluded from the sensitivity and specificity analyses. Repeat tests performed for false negative or false positive results were also excluded. Results from repeat testing performed following a sample swop or an initial failed or invalid result were included in the analyses, as routine laboratory practice stipulated that such samples be retested.

2.3.3 Objective 3 – Predictors of IU transmission

Characteristics of mothers of IU-infected infants were compared to those of mothers whose infants were not infected IU. Mann Whitney U test was used to compare continuous variables and Chi^2 or Fisher's exact tests were used for categorical variables. Logistic

regression was then performed to determine whether antenatal or peripartum factors could predict for IU-infection. Variables that were investigated included maternal age, antenatal CD4 count, maternal PMTCT prophylaxis, peripartum maternal VL, preterm delivery, mode of delivery and premature rupture of membranes. The latter two variables were included to investigate whether the decreased risk of IP transmission with caesarean section (99) and increased risk of IP transmission with premature rupture of membranes (24, 61, 100, 104) would proportionately alter the risk of IU transmission. Infant characteristics were not investigated in this analysis.

Univariate logistic regression was performed with each predictor variable and unadjusted odds ratios (OR) were calculated. These indicated whether predictor variables were protective (OR < one) or risk factors (OR > one) for the outcome, namely IU-infection. Predictor variables with $p<0.20$ in the univariate analysis were included in the multivariate model where adjusted ORs were computed. All ORs were calculated with 95% CI.

Interactions between predictor variables in the multivariate model were investigated to ensure independence of these variables. Where predictor variables were correlated, the multivariate model was run with and without an interaction term, the product of the two correlated variables. The -2 log likelihood values, which are overall test statistics, in the two models were then compared. Where there was no difference between the -2 log likelihood statistics in the models with and without the interaction term, the correlation did not impact on the model and the interaction term was excluded from the final analysis.

2.3.4 Objective 4 – Mathematical model of early infant diagnosis

A mathematical model of paediatric HIV incidence and survival in South Africa was developed and published by Dr Leigh Johnson (University of Cape Town) (109-111). In collaboration with Dr Johnson, the model was used to determine the optimal time to perform PCR testing to detect perinatal (IU and IP) infections and minimise the HIV disease burden. To adapt the model for this purpose, data were collated from the EID Study and published literature to estimate: 1) the proportion of perinatal transmissions detectable at birth and 2) the impact of antiretroviral prophylaxis on the diagnosis of perinatal HIV-infections. Dr Johnson used these estimates to modify and run the model. Details of the methodology used in the original and adapted models are available in Dr Johnson's working paper (109) and Appendix G, respectively (Appendix H).

2.3.4.1 Assumptions regarding perinatal transmissions detectable at birth

The proportion of perinatal transmissions detectable at birth in the presence of various PMTCT regimens was estimated from findings of the EID Study and published literature (8, 9, 21, 27, 53, 60, 75-85, 88, 93, 117-123). These studies were highly variable in terms of the duration of PMTCT prophylaxis, rate of caesarean section, timing of diagnostic testing, assays used, study location and whether the population was predominantly breast or formula fed. It was therefore difficult to merge the findings from these studies for a definitive conclusion regarding the rate of IU relative to IP transmission. An approach was thus taken to focus on landmark studies and those with larger cohorts that used PMTCT regimens similar to those recommended in South Africa. Studies that used commercial or standardised testing technologies and performed testing at comparable time intervals to the EID Study (viz. birth and four- to six-week testing) were also considered. Table 2.1 details the key studies used to determine the proportion of IU transmission in the context of various PMTCT regimens for inclusion in the model.

Table 2.1 Proportion of perinatal transmissions detectable at birth by level of PMTCT cover.

Study	Reason for inclusion	Feeding	Country	PMTCT regimen	Assay	Definition of IU (IP) infection	IU infection as a proportion of perinatal infections
1. No PMTCT cover							
Dunn et al 1995 (21)	Landmark study	Non-breast feeding	Multiple (review of 12 cohorts)		In-house DNA PCR, except Roche Amplicor in 1 instance	**IU**: PCR positive on the day of birth or day after birth	38% (This is ± in the middle compared to: Bertolli 1996 (117) - 16/61 = 26.2%; Shaffer 1999 (78) placebo group - 13/37 = 35.1%; ZVITAMBO 2004 (9) - 89/193 = 46.1%)
							Proportion used in model: 38%
2. IP PMTCT cover							
Guay et al 1999 (HIVNET 012) (27)	Landmark study	Breast feeding	Uganda	**sdNVP group**: NVP to mothers at onset of labour & to infants within 72 hrs of birth	RNA PCR - Roche Amplicor Monitor v1.0 + added primers and then v1.5 primers	**IU**: HIV-infection at day 1-3	**sdNVP group** IU/(all infected by 6-8 wks) = 25/35 = 71.4%
Moodley et al 2003 (SAINT trial) (82)	South African study	Mix of feeding practices	South Africa	**1) sdNVP group:** *a. Mothers:* sdNVP in labour & additional dose 24-48 hrs post-partum *b. Infants:* sdNVP 24-48 hrs after birth **2) Short course AZT/3TC group:** *a. Mothers:* IP AZT+3TC through delivery and post-partum for 1 wk *b. Infants:* AZT+3TC for 1 wk	Roche Amplicor DNA and RNA PCR v 1.0 initially & v 1.5 thereafter	**IU**: PCR positive within 72 hrs of birth **IP/early PP (ePP)**: PCR negative within 72 hrs of birth, then positive 4-wk test **ePP**: PCR negative at 72 hrs and 4 wks, then positive 6-8 wk test	62-68% **1) sdNVP group:** IU/(IU + IP + ePP) = 45/73 = 61.6% **2) Short course AZT/3TC group:** IU/(IU + IP + ePP) = 38/56 = 67.9%
Chasela et al 2008 (118)	Large number of infants; testing at same intervals	Breast feeding	Malawi, Tanzania, Zambia	sdNVP to mothers and infants (HIVNET 012 regimen (27))	RNA: bioMerieux NucliSens NASBA in Malawi and Zambia; Roche Amplicor Monitor v 1.5 in Tanzania	**IU**: Positive test at birth (within 48 hrs) **IP/ePP**: First positive test at the 4-6 week visit	IU/(IU + IP/ePP) = 153/281 = 54.4%
							Proportion used in model: 65%

Study	Reason	Feeding	Country	PMTCT regimen	Assay	Definition of IU (IP) infection	IU infection as a proportion of perinatal infections
3. IP and antenatal PMTCT cover							
Lallemant et al 2004 (Perinatal HIV Prevention Trial) (80)	Similar PMTCT regimens	Non-breast feeding	Thailand	*All mothers:* AZT initiated at median of 28.7 wks gestation through delivery *All infants:* AZT for 1 wk; PLUS **1) NVP-NVP group** *a. Mothers:* sdNVP at labour onset *b. Infants:* sdNVP 48-72 hrs after birth **2) NVP-placebo group** *a. Mothers:* sdNVP at labour onset *b. Infants:* placebo **3) Placebo-placebo group** *a. Mothers:* placebo *b. Infants:* placebo	Roche Amplicor DNA PCR v 1.5	**IU**: Test positive for HIV within 3 days after birth	50-58%; combined cases 29/55=53% **1) NVP-NVP group** IU/(all infected infants) = 7/14 = 50.0% **2) NVP-placebo group** IU/(all infected infants) = 11/19 = 57.9% **3) Placebo-placebo group** IU/(all infected infants) = 11/22 = 50.0%
Shapiro et al 2006 (88)	Similar PMTCT regimens	Mix of feeding practices	Botswana	**1) AZT + maternal sdNVP** *a. Mothers:* AZT from 34 wks gestation, through delivery & sdNVP during labour *b. Infants:* sdNVP at birth & 1 mo AZT **2) AZT + maternal placebo** *a. Mothers:* AZT from 34 wks gestation, through delivery *b. Infants:* sdNVP at birth & 1 mo AZT	Roche Amplicor DNA PCR	**Birth positive**: PCR positive within 15 days, but median time to birth testing in both groups = 1 day; 99% infants in sdNVP group & 98% in placebo group had birth testing ≤4 days	62-87%; combined cases 21/28=75% **1) AZT + maternal sdNVP** Birth positive/(1 mo positive) = 13/15 = 86.7% **2) AZT + maternal placebo** Birth positive/(1 mo positive) = 8/13 = 61.5%
Dabis et al 2005 (ANRS 1201/1202 DITRAME PLUS Study Group) (83)	Testing at same time intervals; similar PMTCT regimen in group 1	Mix of feeding practices	Cote d'Ivoire	**1) AZT + sdNVP group** *a. Mothers:* AZT from 36 wks gestation AZT + sdNVP at labour onset *b. Infants:* sdNVP (day 2) & 1 wk AZT **2) AZT + sdNVP + 3TC group** *a. Mothers:* AZT + 3TC from 32 wks gestation; AZT + sdNVP + 3TC at labour onset; AZT + 3TC for 3 days PP *b. Infants:* sdNVP (day 2) & 1 wk AZT	Real-time PCR (quantitative Taqman technology)	**IU**: Day 1-4 specimen positive **IP/ePP**: Day 1-4 specimen negative but week 4-6 specimen was positive	64-77%; combined cases 24/35=69% **1) AZT + sdNVP group** All IU/(All IU + IP/ePP) = 14/22 = 63.6% **2) AZT + sdNVP + 3TC group** All IU/(All IU + IP/ePP) = 10/13 = 76.9%
							Proportion used in model: 76%*
4. HAART							
							IU/IP split not known
							Proportion used in model: 80%†

* The EID Study, which also provided IP and antenatal cover, found that 76% of perinatal infections were detectable at birth.

† Conservative estimate – only slightly higher than that used in the context of IP and antenatal cover.

2.3.4.2 Assumptions regarding detection of perinatal HIV-infections in the context of antiretroviral prophylaxis

The model was designed to be consistent with the 2010 South African PMTCT guidelines which recommend prolonged, daily-dose infant NVP for at least six weeks (25). Although initial studies of AZT monotherapy did not find significantly delayed diagnosis of infant HIV-infection or reduced VL levels (68, 75, 124), more recent studies have found that prolonged and/or multi-drug prophylactic regimens do delay diagnosis of HIV-infection beyond four to six weeks and dampen VL levels in HIV-infected infants (60, 62-64, 125). In the context of six weeks of postpartum infant AZT, with or without other drugs, 32% of IP-infected infants detectable by three months of age were PCR negative at four to six weeks (60). In the case of NVP, even a single perinatal dose reduced VL levels below the limit of detection in 38% of IU-infected infants at five days of age (1). Consistent with this finding, 17% of IU-infected infants detectable at birth in the EID Study had false negative results at two weeks of age – a two-week sensitivity of only 83% – most likely due to sdNVP at birth. Prolonged daily-dose NVP may similarly adversely impact detection of HIV-infected infants at six weeks of age. Based on the two-week sensitivity of 83% among IU-infected infants receiving sdNVP in this cohort, sensitivity of PCR testing for detecting IU-infections at six weeks of age was assumed to be 85% in the context of daily-dose NVP. A lower six-week sensitivity of 70% was assumed for detecting IP-infections, as these infections are being established at the time of daily-dose NVP and would therefore be more susceptible to NVP's dampening effect. The 70% sensitivity for detecting IP-infections also concurs with findings of the HPTN 040 study (60). PCR sensitivity was assumed to recover over time as NVP resistant strains accumulate.

2.3.4.3 Model of PCR testing and infant mortality

The adapted model estimated infant outcomes in a cohort of 240 000 HIV-exposed infants; this is the estimated number of exposed infants born in South Africa in 2010 as per the ASSA2008 AIDS and Demographic model (126). The number of IU- and IP-infected infants diagnosed by a single PCR test at birth, six weeks and ten weeks of age was compared to the number diagnosed using two PCR tests, with the first test at birth and the second at six, ten or 14 weeks of age. These ages were chosen because they correspond to routine healthcare visits according to the immunisation schedule in South Africa. Testing at ten and 14 weeks also allowed for delayed diagnosis of perinatal HIV-infection beyond the currently recommended six-week test. The number of HIV-infected infants diagnosed at each time point was indicative of those who received PCR test results (Appendix G, Table G2). When calculating the number of PCR tests required at each age, testing of symptomatic children and routine screening post-cessation of breastfeeding was taken into account. The number of life years and quality-adjusted life years (QALYs) saved by testing at each time point was also estimated (calculation of QALYs is described in Appendix G). For the purpose of this analysis, postnatal transmission rates were set to zero.

A number of sensitivity analyses were performed to compare outcomes in the baseline scenario to alternative scenarios with different assumptions:

- The *baseline scenario* was consistent with the 2010 South African PMTCT guidelines which prescribed antenatal AZT for pregnant women not eligible for HAART and daily-dose NVP for HIV-exposed infants (25). The latter was assumed to reduce sensitivity of PCR testing (60, 62-64). The baseline scenario assumed equal mortality in IU- and IP-infected infants (8).
- *Scenario one* assumed that NVP prophylaxis did not reduce PCR sensitivity.

- *Scenario two* investigated the impact of changing maternal prophylaxis to antenatal HAART for all HIV-infected women (WHO option B (127)), as was implemented in South Africa from April 2013 (26).
- *Scenario three* assumed that IP-infected infants progressed less rapidly than infants infected IU. Based on reported cumulative mortality rates of 0.29 and 0.42 in IP- and IU-infected infants, respectively (9), the rate of progression in IP-infected infants was assumed to be 0.70 times that of IU-infected infants.

2.4 ETHICAL CONSIDERATIONS

The original EID Study was approved by The University of the Witwatersrand's Ethics Committee in 2008 (M080626; Appendix I) and written informed consent was obtained from all study participants. An application to conduct a secondary data analysis for degree purposes was subsequently approved by the Ethics Committee (Medical) of the University of the Witwatersrand (M10366; Appendix J). Missing antenatal CD4 counts were identified using the NHLS LIS under the ethics approval for the PMTCT programme at RMMCH (Appendix K). Patient identifying details were not accessed at any point during the analysis and reporting process.

2.5 FUNDING

The EID Study and secondary data analysis were funded by the NHLS, the President's Emergency Plan For AIDS Relief (PEPFAR) and United Nations Children's Fund (UNICEF).

3. RESULTS

3.1 OBJECTIVE 1 – RMMCH'S PMTCT PROGRAMME AND THE STUDY COHORT

3.1.1 The antenatal and postnatal PMTCT coverage cascades

3.1.1.1 Cascade of antenatal PMTCT care

A convenience sample of 848 mother-infant pairs was enrolled in the EID Study. Ten mother-infant pairs withdrew during the course of the study and have been excluded from all analyses. The remaining 838 infants included nine sets of twins; a total of 829 mothers were therefore enrolled.

Majority (97.5%) of the 829 enrolled women lived in City of Johannesburg, the Gauteng district where RMMCH is located, and 92.9% attended ANCs in the district. Two percent of enrolled women had not booked at any ANC. Only 21.8% of the 829 mothers attended an ANC before 20 weeks gestation and 4.8% attended before 14 weeks (Figure 3.1). After presenting for antenatal care, majority of mothers underwent antenatal CD4 testing (86.5%) and received some form of antenatal PMTCT prophylaxis (89.4%). However, only 38.0% accessed optimal PMTCT from 28 weeks gestation as stipulated in the 2008 guidelines (Figure 3.1).

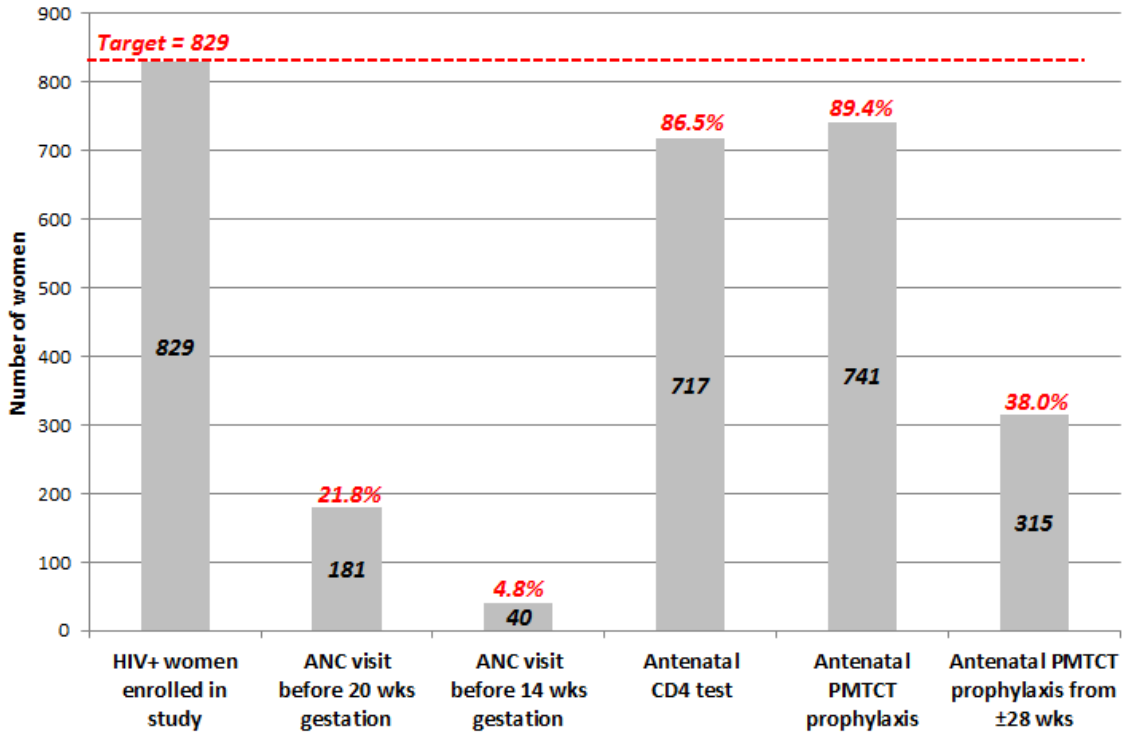


Figure 3.1 – Coverage cascade of antenatal PMTCT care provided to study participants.

Women who booked at antenatal clinics later in pregnancy (median of 29 weeks gestation) were less likely to access CD4 testing than women who booked earlier at a median of 25 weeks ($p<0.001$). Women who booked later in gestation also accessed less optimal PMTCT prophylaxis ($r_s = 0.46$, $p<0.001$, Figure 3.2). These women tended to have higher peripartum VL levels ($r_s = 0.17$, $p = 0.003$, Figure 3.3), as VL levels increase with deteriorating PMTCT rank ($r_s = 0.43$, $p<0.001$, Figure 3.4). Thus, women who booked at antenatal clinics late in pregnancy were less likely to undergo CD4 testing, accessed less optimal PMTCT, tended to have higher VL levels, and most early transmissions, indicated by red dots in Figures 3.2 to 3.4, occurred from these women.

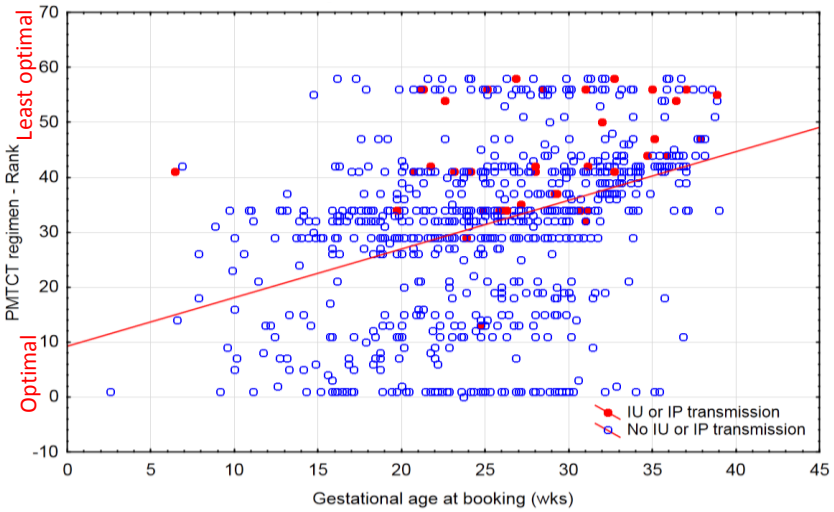


Figure 3.2 – Relationship between timing of antenatal clinic booking and maternal PMTCT (r_s = 0.46, p<0.001).

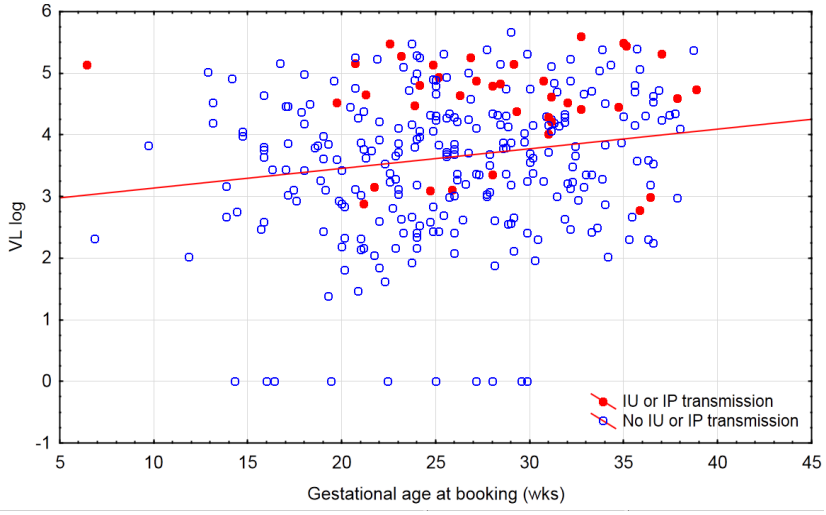


Figure 3.3 – Relationship between timing of antenatal clinic booking and peripartum maternal VL (r_s = 0.17, p = 0.003). Mothers with undetectable VL levels (VL log = 0.00; n = 10) had all initiated HAART pre-conception.

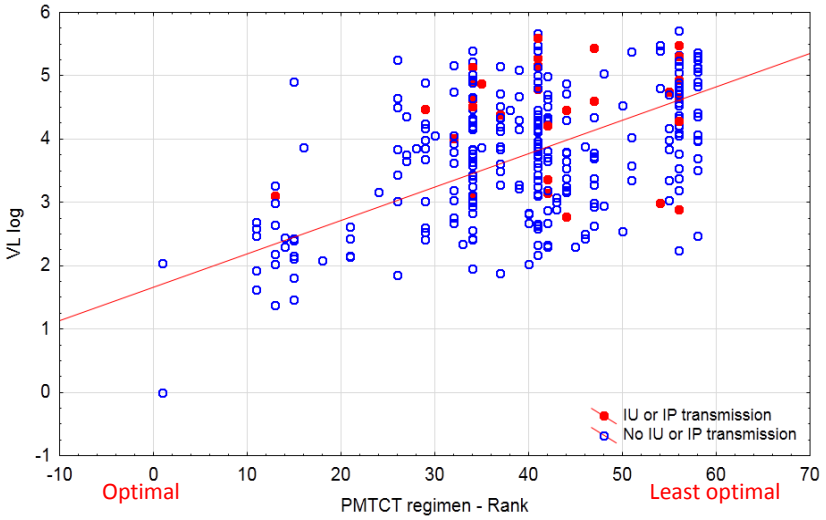


Figure 3.4 – Relationship between antenatal PMTCT and peripartum maternal VL (r_s = 0.43, p<0.001).

The antenatal PMTCT cascade and related maternal characteristics were stratified according to the method of infant diagnosis as an indicator of compliance with infant PMTCT care (see section 2.2.2.4) (Table 3.1). Mothers who were fully compliant in bringing their infants back to RMMCH for diagnostic testing were also the most compliant with their own antenatal care. Compared to mothers who were less compliant with their infants' PMTCT care, a greater proportion of fully compliant mothers attended an ANC ≤20 weeks gestation ($p = 0.005$), underwent CD4 testing ($p<0.001$) and accessed antenatal PMTCT prophylaxis ($p<0.001$) (Table 3.1). This difference was particularly significant when comparing mothers who were fully compliant to those who were not compliant at all. Furthermore, a significantly greater proportion of compliant mothers accessed optimal antenatal PMTCT from 28 weeks gestation ($p = 0.006$); consequently, their VL levels around the time of birth were significantly lower ($p<0.001$) (Table 3.1). Mothers who were not compliant with their infants' PMTCT care were younger ($p = 0.002$) and fewer were South African ($p<0.001$) (Table 3.1). Thus, infants born to mothers with poor antenatal health seeking behaviours, who were younger and of a foreign nationality, were at greatest risk of being lost to follow-up from the PMTCT programme and of not accessing an HIV diagnosis.

3.1.1.2 Cascade of postnatal PMTCT care for the diagnosis and treatment of infants with early (IU and IP) HIV-infections

The cascade of postnatal PMTCT care for infants enrolled in the EID Study is depicted in Figure 3.5. All but one infant (99.9%) received NVP at birth. All 838 enrolled infants should have undergone diagnostic PCR testing at RMMCH and received their test results at ten weeks. Instead, only 72.3% (n = 606) accessed routine diagnostic testing at RMMCH and 67.4% (n = 565) returned for the ten-week result visit, though the latter represents the

Table 3.1 – The antenatal PMTCT cascade and maternal characteristics by method of infant diagnosis as an indicator of compliance with infant PMTCT care.[a]

	1 – Fully compliant: mothers of infants diagnosed at RMMCH	**2 – Partially compliant: mothers of infants diagnosed elsewhere**	**3 – Non-compliant: mothers of infants not tested routinely**	**Overall comparison (1 vs 2 vs 3): p-value[b]**	**2-by-2 comparisons: p-value[c]**
n Mothers Infants	 602 606	 82 85	 145 147		
Antenatal clinic visit ≤20 weeks gestation	148/588 (25.2)	15/79 (19.0)	18/140 (12.9)	**0.005**	1 vs 2: 0.230 **1 vs 3: 0.002** 2 vs 3: 0.223
Antenatal clinic visit ≤14 weeks gestation	34/588 (5.8)	3/79 (3.8)	3/140 (2.1)	0.180	1 vs 2: 0.607 1 vs 3: 0.088 2 vs 3: 0.670
Accessed antenatal CD4 testing	551/602 (91.5)	68/82 (82.9)	98/145 (67.6)	**<0.001**	**1 vs 2: 0.013** **1 vs 3: <0.001** **2 vs 3: 0.012**
Accessed any antenatal PMTCT prophylaxis	555/602 (92.2)	71/82 (86.6)	115/144 (79.9)	**<0.001**	1 vs 2: 0.087 **1 vs 3: <0.001** 2 vs 3: 0.203
Accessed antenatal PMTCT from ±28 weeks gestation	243/602 (40.4)	34/82 (41.5)	38/144 (26.4)	**0.006**	1 vs 2: 0.849 **1 vs 3: 0.002** 2 vs 3: 0.019
CD4 ≤350 & on HAART / total with CD4 ≤350	142/268 (53.0)	15/37 (40.5)	20/54 (37.0)	0.054	1 vs 2: 0.156 1 vs 3: 0.032 2 vs 3: 0.736
CD4 ≤200 & on HAART / total with CD4 ≤200	75/99 (75.8)	8/12 (66.7)	11/17 (64.7)	0.495	1 vs 2: 0.494 1 vs 3: 0.373 2 vs 3: 1.000
Peripartum maternal VL (log)[d] - median (range; n)	3.73 (0.00 - 5.72; 259)	4.16 (0.00 - 5.48; 43)	4.84 (4.38 - 5.59; 8)	**<0.001**	**1 vs 2: 0.005** **1 vs 3: <0.001** 2 vs 3: 0.023
Maternal age (years) - median (range; n)	28.3 (18.2 - 44.8; 602)	27.4 (18.6 - 42.1; 82)	26.5 (16.2 - 41.1; 145)	**0.002**	1 vs 2: 0.248 **1 vs 3: <0.001** 2 vs 3: 0.186
Maternal nationality: South African	354/584 (60.6)	47/82 (57.3)	58/138 (42.0)	**<0.001**	1 vs 2: 0.568 **1 vs 3: 0.<001** 2 vs 3: 0.028

[a] Data are n/total (%) except continuous variables where data are median (range; n) as indicated. The total number of mothers varies due to missing data.

[b] Chi^2 or Fisher's exact test for binary and categorical variables; Kruskal Wallis ANOVA for continuous variables. Significant p-values are indicated in bold ($p<0.05$).

[c] Chi^2 or Fisher's exact test for binary and categorical variables; Mann Whitney U test for continuous variables. Significant p-values are indicated in bold ($p<0.017$, Bonferroni correction).

[d] 310 VL results were included in this analysis. An additional 20 undetectable VL results (6.1% of the original 330 VL tests) were not plausible in the context of the antenatal PMTCT regimen and were therefore excluded.

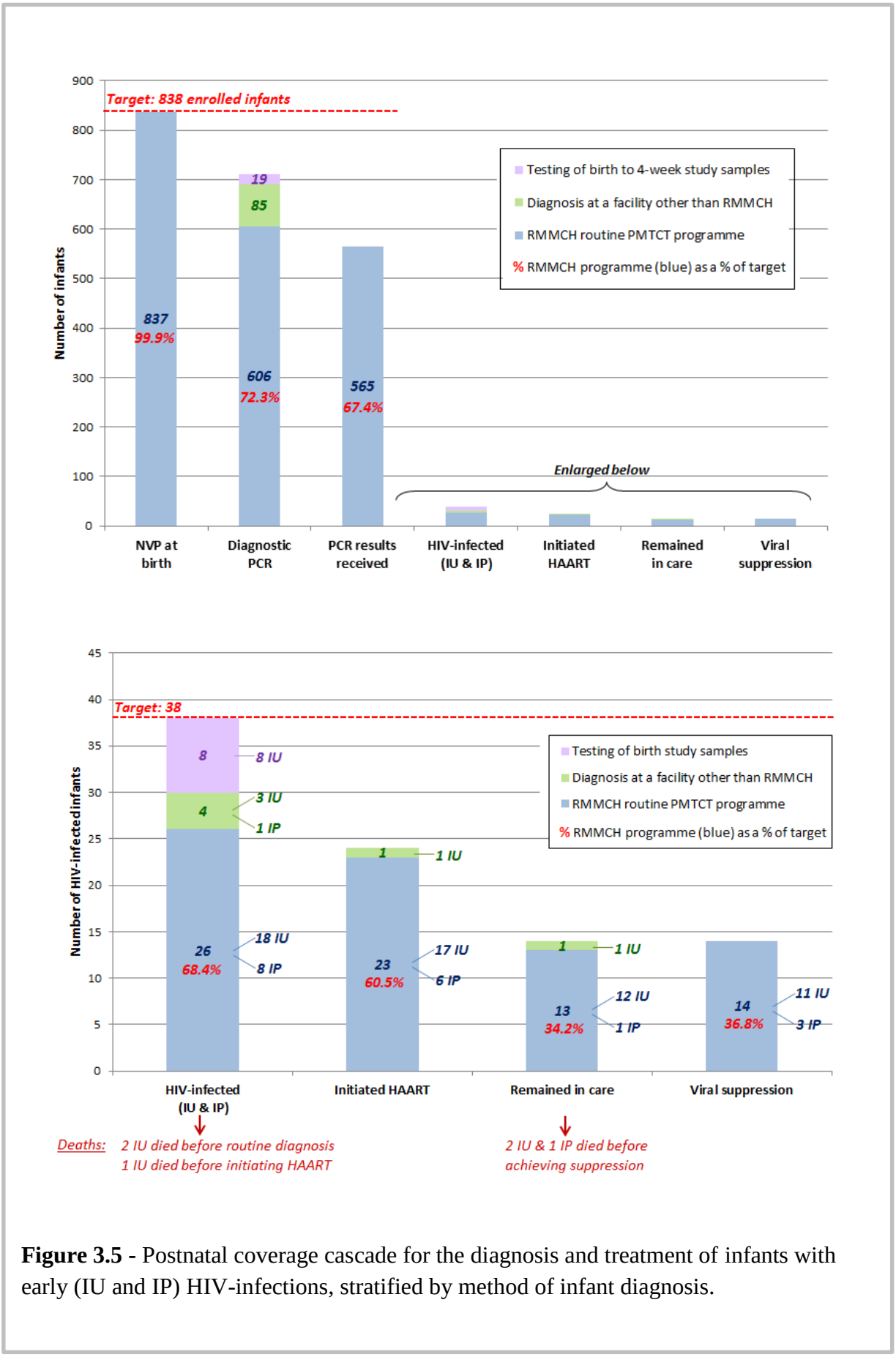


Figure 3.5 - Postnatal coverage cascade for the diagnosis and treatment of infants with early (IU and IP) HIV-infections, stratified by method of infant diagnosis.

majority of infants who were tested (565/606 = 93.2%) (Figure 3.5). Infants ranged from 1.7 to 11 weeks of age at their first PCR test, with a median age of 6.5 weeks (Figure 3.6 a). HIV-uninfected and infected infants underwent testing at comparable ages (median of 6.4 and 6.6 weeks, respectively). In total, 60 (9.9%) of the 606 infants tested at RMMCH had two PCR tests, most often as a result of clinical concern. Majority of the 565 infants who returned to RMMCH for their results did so timeously, at a median age of 10.6 weeks (range 6.6 – 20.9 weeks) (Figure 3.6 b). HIV-infected and uninfected infants received their PCR results at the same median age of 10.6 weeks.

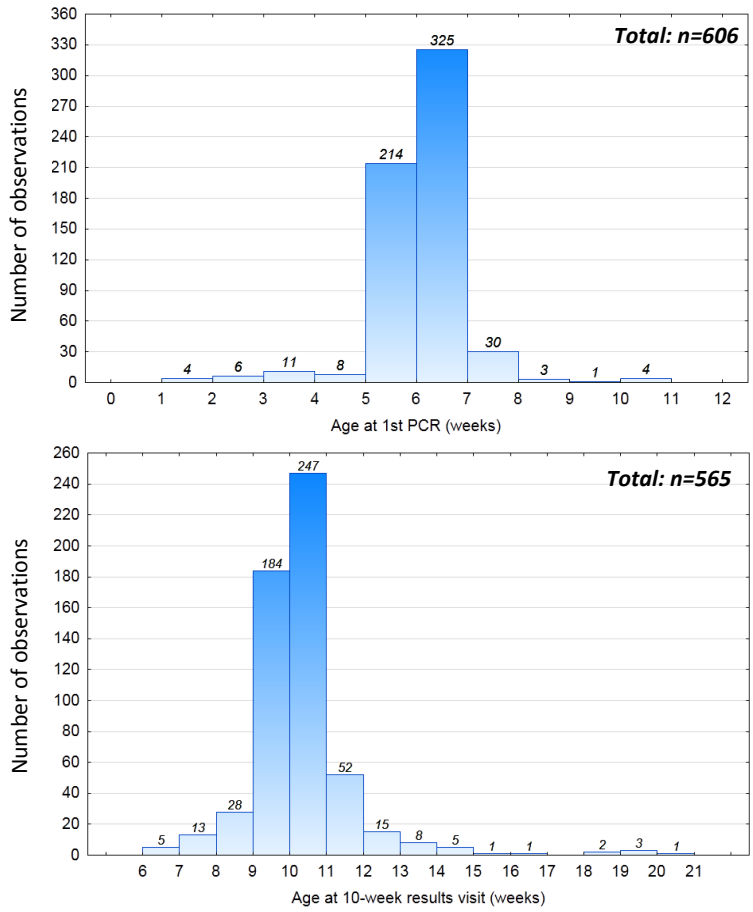


Figure 3.6 – Timing of a) the first PCR test and b) the result visit for HIV-exposed infants accessing diagnostic testing at RMMCH.

In addition to the 606 infants who underwent routine PCR testing at RMMCH, the LIS search identified an additional 85 infants who had accessed diagnostic testing at other healthcare facilities (Figure 3.5). It is not known whether these infants received their PCR results, but it is likely that some did. The routine PMTCT programme therefore provided a definitive diagnosis for 82.5% (n = 691) of the 838 infants in the cohort through testing at RMMCH and other facilities. In addition to routine PCR testing, a further 19 infants were diagnosed by testing DBS study samples (Figure 3.5). A definitive diagnosis was thus available for a total of 710 (84.7%) infants in the cohort.

Of the 606 infants who were tested at RMMCH, 26 were diagnosed as HIV-infected, comprising 18 IU and eight IP-infections (Figure 3.5). The LIS search identified an additional four HIV-infected infants (three IU and one IP) and testing of birth samples identified a further eight IU-infected infants, bringing the total number of IU- and IP-infected infants in the cohort to 38 (Figure 3.5). Thus, a total of 29 IU-infected infants could have been identified through birth testing, in comparison to the 26 infected infants diagnosed by RMMCH's routine six-week testing programme. Compared to the total number of early HIV-infections (n = 38), RMMCH's PMTCT programme identified 68.4% of infected infants (Figure 3.5) i.e. a third of all early HIV-infections in this cohort were missed by six-week testing at RMMCH.

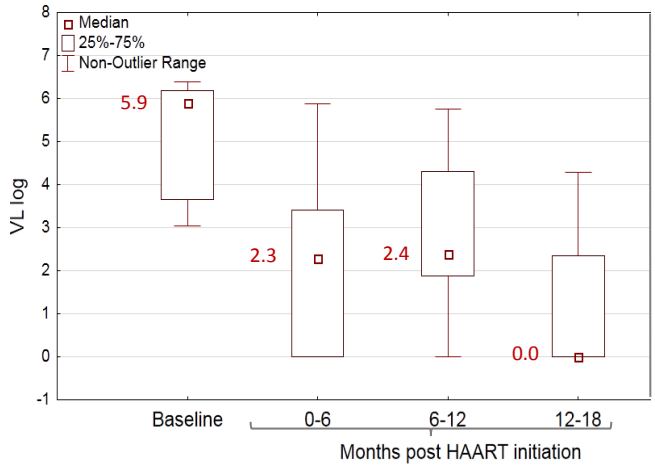
Of the 11 IU-infected infants who did not present for routine six-week testing at RMMCH (Figure 3.5), six were LTFU before accessing a diagnosis, three died, one returned for care and one reportedly moved to Malawi. Thus, of these 11 infants who could have been diagnosed at birth, nine (81.8%) were completely lost from the PMTCT programme

(assuming that the infant who moved to Malawi did access treatment). Although birth testing would not have identified the nine IP-infected infants in the cohort, four of these infants presented with clinical symptoms. Two were symptomatic at six weeks, one with significant adenopathy and the other with suspicion of tuberculosis and poor weight gain. An additional two were symptomatic between seven and ten weeks, with wasting and significant adenopathy being the most common symptoms. Thus, clinical stigmata should have prompted HIV testing in four (44.4%) of the nine IP-infected infants, even if six-week testing was not routine. In this cohort, a birth diagnosis together with clinical follow-up of symptomatic infants at the routine six- and ten-week visits would have identified the highest number of HIV-infected infants – testing at birth would have detected the 29 IU-infections and clinical stigmata should have prompted testing of four IP-infected infants. Thirty three (86.8%) of the 38 HIV-infected infants could therefore have been identified, compared to 26 (68.4%) diagnosed by RMMCH's routine six-week testing programme.

HAART was initiated in 24 IU- and IP-infected infants in the cohort (Figure 3.5). One infant who had accessed testing at a facility other than RMMCH later returned to the hospital for HAART initiation at 20.1 months of age. The remaining 23 infants who initiated treatment had been diagnosed through RMMCH's routine programme. These infants represent 88.5% of the 26 infants diagnosed at RMMCH, but only 60.5% of the total early HIV-infections in the cohort. Infants initiated HAART at a median age of 16.0 weeks (range 6.3 – 30.3 weeks) due to a delay of 6.1 weeks from the ten-week result visit to HAART initiation (range -3.3 – 20.3 weeks) (Lilian *et al* 2013 (128) Figure 3; Appendix C). The 23 infants who initiated HAART were followed up for a median of 51.6 weeks (range 0.0 – 112.1 weeks) and by the end of the study, ten (43.5%) were not in care –seven were LTFU and three had died shortly after initiating treatment (Figure 3.5).

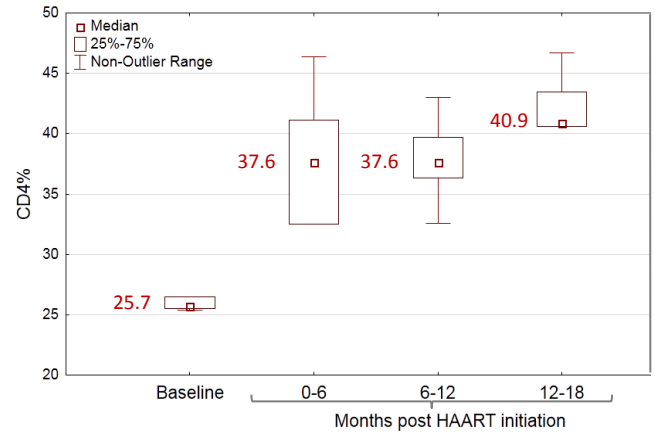
For those infants who remained in care at RMMCH, treatment with HAART was successful in suppressing viral replication and reversing immunosuppression. Prior to treatment, median baseline VL was 5.4 log (range 3.0 – 7.0 log) and baseline CD4 percentage was 25.7% (range 9.2 – 38.2%). Among infants with repeated VL and CD4 measures, VL decreased significantly over time (p = 0.002), with a corresponding increase in CD4 percentage to levels over 40% (p = 0.033) (Figure 3.7). In total, viral suppression was achieved in 14 (60.9%) of the 23 infants who initiated HAART through RMMCH's routine programme; this equates to only 36.8% of the 38 infected infants in the cohort (Figure 3.5).

a)



***n*=7, *p*=0.002 (Friedman ANOVA)**

b)

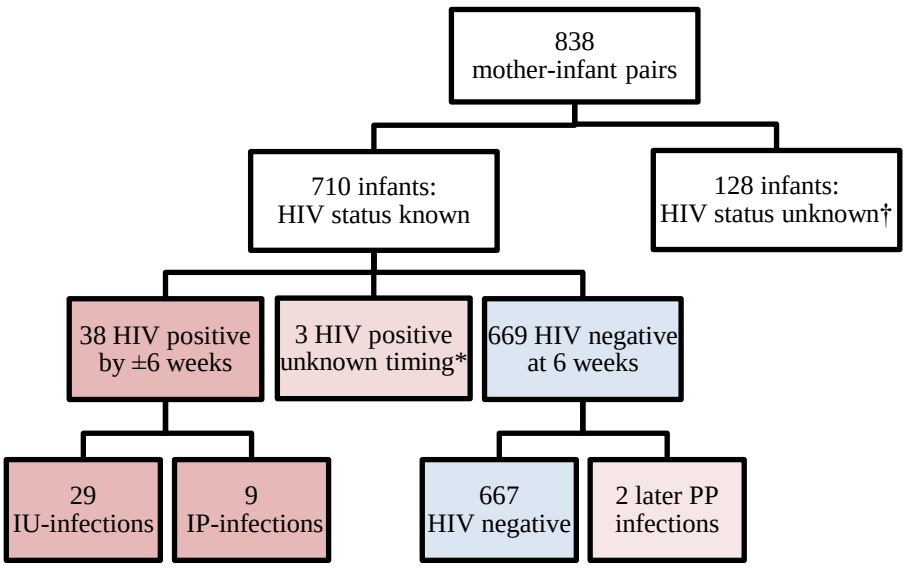


***n*=5, *p*=0.033 (Friedman ANOVA)**

Figure 3.7 – a) VL and b) CD4 measurements over time in HIV-infected infants who initiated HAART at RMMCH and had repeated measures at six-monthly intervals.

3.1.2 Mother-to-child transmission of HIV in the EID cohort

In addition to the 38 confirmed cases of early (IU or IP) transmission, five more instances of MTCT occurred in the cohort (Figure 3.8). Three infants who defaulted from the study before the two-week visit were HIV-uninfected at birth and were first diagnosed as HIV-infected at six months or older. Since these infants did not undergo routine six-week testing, it is unclear whether the infections were vertical IP transmissions or occurred later as a result of breastfeeding (PP transmission). These three infants have therefore been excluded from the cohort of IP-infected infants. An additional two reportedly breastfed infants acquired HIV through PP transmission. These are two of the 669 infants who had negative six-week PCR results, but repeat tests at 9.4 and 15.6 months of age were positive. No definitive diagnosis was available for 128 infants, though IU-infection was excluded by virtue of negative tests on birth study samples (Figure 3.8).



† Six-week HIV status unknown, but negative test results on birth samples excluded IU-infection in these infants.
* These infants tested negative at birth, defaulted from the study at two weeks and first tested positive after six months of age. It is therefore unclear whether they were infected IP or later as a result of breastfeeding (PP transmission).

Figure 3.8 – Mother-to-child transmission in the 838 mother-infant pairs enrolled in the EID Study.

3.1.3 Maternal characteristics by MTCT status

Table 3.2 compares the characteristics of mothers of IU- and IP-infected infants to mothers of infants who were uninfected at six weeks of age. Mothers of infected infants were significantly younger ($p = 0.007$) and had had fewer pregnancies compared to non-transmitting mothers ($p = 0.031$). Few mothers declared drinking alcohol or smoking and few had clinical illnesses, irrespective of transmission. The proportion of preterm deliveries and caesarean sections did not differ in mothers of infected and uninfected infants ($p = 0.523$ and 1.000, respectively). A significantly higher proportion of transmitting mothers intended to breastfeed and ultimately did breastfeed their infants ($p = 0.007$ and 0.002, respectively).

Mothers of perinatally infected infants presented for antenatal care significantly later in pregnancy than non-transmitting mothers ($p = 0.002$) (Table 3.2). It thus follows that transmitting mothers underwent CD4 testing later in pregnancy ($p = 0.023$), fewer accessed optimal PMTCT or HAART ($p<0.001$) and for a shorter duration ($p<0.001$); consequently, their peripartum VL levels were higher ($p<0.001$) (Table 3.2). Transmission from mothers who received intermediate antenatal PMTCT of less than ten weeks was not significantly different to transmission from mothers who received no antenatal cover ($p>0.017$), indicating that more than ten weeks of prophylaxis was required to protect against transmission. However, if a ten-week cut-off was not applied to the data and duration of PMTCT was instead analysed continuously, it was evident that a median of eight weeks of antenatal prophylaxis was also protective against transmission ($p<0.001$) (Table 3.2). The apparently higher rate of transmission from mothers who received sdNVP ($p = 0.018$) is indicative of increased risk in the absence of HAART, as sdNVP was administered to mothers who had not received HAART.

Table 3.2 – Maternal characteristics of IU- and IP-infected versus uninfected infants.

Maternal characteristics	**Mother-infant pairs with IU or IP transmission (n=38)**	**Mother-infant pairs with no transmission at 6 weeks (n=669)**	**p-value[a]**
Age (years) - median (range; n)	26.1 (18.9 - 37.6; 38)	28.3 (16.2 - 44.8; 669)	**0.007**
Nationality: South African - n/total (%)	19/37 (51.4)	391/652 (60.0)	0.299
Parity (viable births) - median (range; n)	2.0 (1.0 - 4.0; 38)	2.0 (1.0 - 10.0; 669)	0.142
Gravidity (pregnancies) - median (range; n)	2.0 (1.0 - 4.0; 38)	2.0 (1.0 - 10.0; 669)	**0.031**
NVP in a previous pregnancy - n/total (%)	2/38 (5.3)	61/646 (9.4)	0.566
Clinical history - n/total (%)			
Alcohol	1/38 (2.6)	15/669 (2.2)	0.591
Smoking	0/37 (0.0)	6/669 (0.9)	1.000
Syphilis	0/34 (0.0)	9/643 (1.4)	1.000
Diabetes	0/38 (0.0)	1/669 (0.1)	1.000
Hypertension	1/38 (2.6)	43/669 (6.4)	0.504
Tuberculosis	2/37 (5.4)	42/665 (6.3)	1.000
Antenatal factors			
Gestational age at antenatal booking (weeks) - median (range; n)	28.2 (6.4 - 38.9; 38)	25.0 (2.6 - 39.0; 652)	**0.002**
Antenatal CD4 testing			
Accessed a CD4 test - n/total (%)	33/38 (86.8)	602/669 (90.0)	0.577
Gestational age at CD4 test (weeks) - median (range; n)	28.1 (4.0 - 38.9; 33)	26.0 (4.0 - 39.0; 601)	**0.023**
CD4 count (x 10^6/l) - median (range; n)	291.0 (58.0 - 760.0; 33)	356.5 (24.0 - 1221.0; 602)	0.089
PMTCT: ordinal categorisation - n/total (%)			**<0.001[b]**
Suboptimal (no antenatal prophylaxis)	10/38 (26.3)	49/669 (7.3)	
Intermediate (<10 wks AZT/HAART)	25/38 (65.8)	342/669 (51.1)	
Optimal (≥10 wks AZT/HAART)	3/38 (7.9)	278/669 (41.6)	
PMTCT: length of treatment			
Antenatal AZT or HAART (weeks) - median (range; n)	3.0 (0.0 - 12.0; 38)	8.0 (0.0 - 41.0; 666)	**<0.001**
Mothers receiving HAART			
Number on HAART - n/total (%)	2/38 (5.3)	193/669 (28.8)	**0.001**
Most common WHO stage at initiation	Stage 1	Stage 1	
Facilities most commonly providing HAART antenatally and postnatally	Witkoppen Clinic, Helen Joseph Hospital	Witkoppen Clinic, RMMCH, Helen Joseph Hospital	
Delivery			
PMTCT: sdNVP - n/total (%)	33/38 (86.8)	454/666 (68.2)	**0.018**
Peripartum viral load (log) - median (range; n)	4.65 (2.78 - 5.60; 38)	3.73 (0.00 - 5.70; 269)	**<0.001**
Gestation: preterm delivery - n/total (%)	4/38 (10.5)	50/669 (7.5)	0.523
Mode of delivery: caesarean - n/total (%)	5/38 (13.2)	102/669 (15.2)	1.000
Premature rupture of membranes - n/total (%)	1/29 (3.4)	24/614 (3.9)	1.000
Feeding - n/total (%)			
Feeding intention: breastfeeding	10/38 (26.3)	70/668 (10.5)	**0.007**
Final feeding practice: breastfeeding	10/38 (26.3)	57/669 (8.5)	**0.002**

[a] Mann-Whitney U test for continuous and discrete variables; Fisher's exact or Chi^2 test for binary and categorical variables. The total number of mothers varies due to missing data.

[b] Significant at $p<0.017$ for suboptimal vs optimal and intermediate vs optimal (Bonferroni correction).

Antenatal CD4 count was not significantly lower in mothers of IU- and IP-infected infants compared to mothers of uninfected infants (p = 0.089) (Table 3.2). Almost half the enrolled mothers had CD4 counts below 350 x 10^6/l (44.4%) and 20 IU/IP transmissions occurred from these women (Figure 3.9). A comparable proportion of mothers had high CD4 counts (45.4%) and there were 13 instances of IU/IP transmission (Figure 3.9). Thus, of the 33 perinatal infections from mothers who had accessed CD4 testing, 60.6% occurred from mothers with CD4 counts below 350 x 10^6/l, giving a comparable rate of transmission from mothers with high and low CD4 counts (p = 0.213). This may be due to the comparable peripartum VL levels in both groups of women (median of 4.47 and 4.73 log in mothers with high and low CD4 counts, respectively; p = 0.726). Maternal VL testing was performed in 43.3% (n = 139) of women with high CD4 counts and 44.9% (n = 141) of women with low CD4 counts and thus appears representative in both groups.

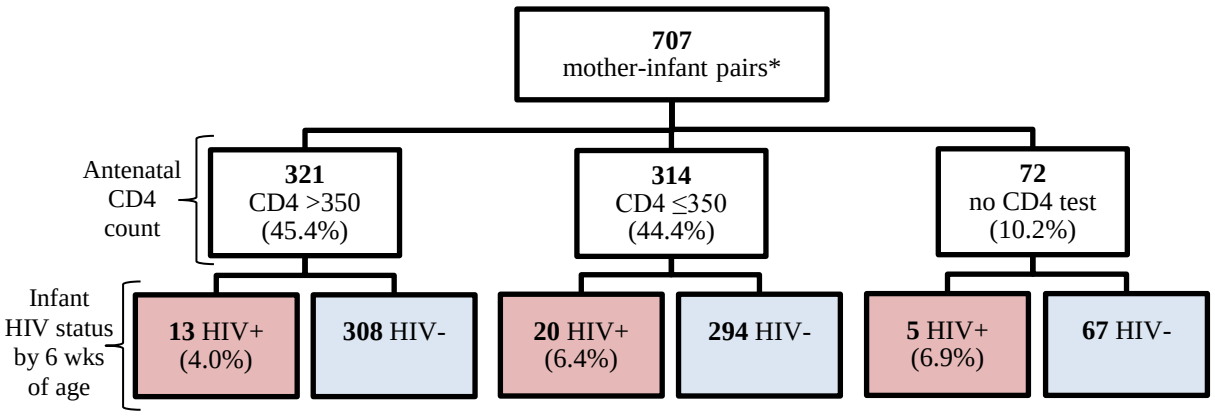


Figure 3.9 – Early (IU and IP) mother-to-child transmission stratified by antenatal CD4 count.

Demographic characteristics, clinical and obstetric history, antenatal and peripartum

factors and feeding practices, as detailed in Table 3.2, were also compared in mothers of IU-infected infants versus mothers of IP-infected infants. No significant differences could be demonstrated in any of these characteristics (data not shown).

3.1.4 Infant characteristics by HIV-infection status

Characteristics of the 38 IU- and IP-infected infants compared to the 669 infants uninfected at six weeks of age are detailed in Table 3.3.

Table 3.3 – Characteristics of IU- and IP-infected versus uninfected infants.

Infant characteristics	**IU- and IP-infected infants (n=38)**	**Infants not infected at 6 weeks (n=669)**	**p-value[a]**
Gender: male - n/total (%)	18/38 (47.4)	345/669 (51.6)	0.614
PMTCT - n/total (%)			**0.019[b]**
Suboptimal	0/38 (0.0)	11/669 (1.6)	
sdNVP and 1-2 week AZT	20/38 (52.6)	474/669 (70.9)	
sdNVP and 4 weeks AZT	16/38 (42.1)	130/669 (19.4)	
Daily-dose NVP	2/38 (5.3)	54/669 (8.1)	
Birth weight			
Weight (kg) - median (range; n)	2.8 (1.1 - 3.7; 38)	3.0 (0.9 - 4.4; 669)	0.076
Weight-for-age z-score - median (range; n)	-1.0 (-5.7 - 1.1; 38)	-0.6 (-6.1 - 1.8; 669)	0.072
Birth length			
Length (cm) - median (range; n)	48.5 (38.0 - 57.0; 38)	50.0 (33.0 - 59.0; 667)	**0.039**
Length-for-age z-score - median (range; n)	-0.6 (-6.0 - 3.7; 38)	-0.0 (-8.8 - 4.8; 667)	**0.038**
Birth head circumference			
Head circumference (cm) - median (range; n)	34.0 (27.0 - 38.0; 38)	34.0 (24.0 - 39.0; 667)	0.770
Head circumference-for-age z-score - median (range; n)	-0.2 (-5.8 - 3.4; 38)	0.0 (-8.4 - 4.3; 667)	0.631
Hospital admissions - n/total (%)	7/38 (18.4)	67/669 (10.0)	0.104
Antibiotic treatment - n/total (%)	5/38 (13.2)	42/669 (6.3)	0.100
Deaths (to 14 weeks of age) - n/total (%)	5/32 (15.6)	5/573 (0.9)	**<0.001**

[a] Mann-Whitney U test for continuous variables; Chi^2 or Fisher's exact test for binary and categorical variables. The total number of infants varies due to missing data.

[b] Significant at $p<0.008$ for sdNVP and 1-2 weeks AZT versus sdNVP and 4 weeks AZT (Bonferroni correction).

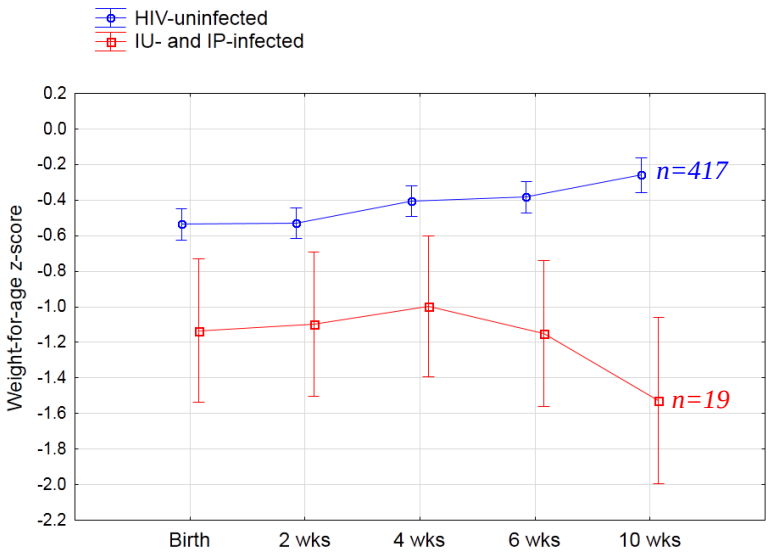
PMTCT prophylaxis of IU- and IP-infected versus uninfected infants

Majority of infants (90.5%) received sdNVP and one to four weeks of AZT (Table 3.3). Very few (7.9%) received daily-dose NVP, as this regimen was only implemented toward the end of the study. A significantly higher proportion of HIV-infected infants received four weeks of AZT compared to HIV-uninfected infants who mostly received one to two weeks of AZT ($p<0.008$) (Table 3.3). A month of infant AZT was only administered where mothers had received less than four weeks of antenatal AZT cover, indicating that mothers of HIV-infected infants were less compliant with their own PMTCT care. This confirms maternal findings that shorter courses of maternal antenatal PMTCT place infants at increased risk of transmission.

Growth of IU- and IP-infected versus uninfected infants

HIV-infection did not appear to markedly affect anthropometric measurements at birth. Median birth weight and head circumference were comparable in infected and uninfected infants ($p = 0.076$ and 0.770, respectively) (Table 3.3). Although birth length and length-for-age z-scores were significantly lower in infants with IU- or IP-infections ($p = 0.039$ and 0.038, respectively), this was reportedly a measuring artefact (personal communication, K. Technau). In order to determine whether HIV status had an effect on growth over time, an ANOVA for repeated measures was performed. Weight-for-age z-scores in infected infants were consistently lower than in uninfected infants ($p<0.001$) (Figure 3.10). They dropped from -1.00 at the four-week visit to -1.53 at the ten-week visit, unlike weight-for-age z-scores in uninfected infants which improved over time. In contrast, neither length nor head circumference z-scores to ten weeks differed significantly between HIV-infected and uninfected infants ($p = 0.521$ and 0.292, respectively), nor did they

change significantly over time (p = 0.197 and 0.207, respectively) (data not shown).



Repeated measures ANOVA:

- *Time effect*: p=0.144
- *Between-group effect* (difference in HIV-infected versus uninfected infants): **p<0.001**

Figure 3.10 – Weight-for-age z-scores in HIV-infected (red) and uninfected (blue) infants over time. Vertical bars denote 95% confidence intervals.

Outcomes of IU- and IP-infected versus uninfected infants

Increased illness, as measured by hospital admissions and treatment with antibiotics, did not show a sicker population in HIV-infected compared to uninfected infants (p = 0.104 and 0.100, respectively) (Table 3.3). However, significantly more IU- and IP-infected infants died under 14 weeks of age compared to those who were uninfected (p<0.001) (Table 3.3). This is depicted in the Kaplan Meier curve of infant deaths (Figure 3.11) which illustrates the significantly higher mortality rate in infected infants (log rank test, p<0.001). Uninfected infants were 92% less likely to die than infants with an IU- or IP-infection (unadjusted hazard ratio (cox regression) = 0.08, 95% CI 0.02-0.30, p<0.001). Only a single infected infant was known to have died after 14 weeks of age; this occurred in an IU-infected infant aged 39.0 weeks.

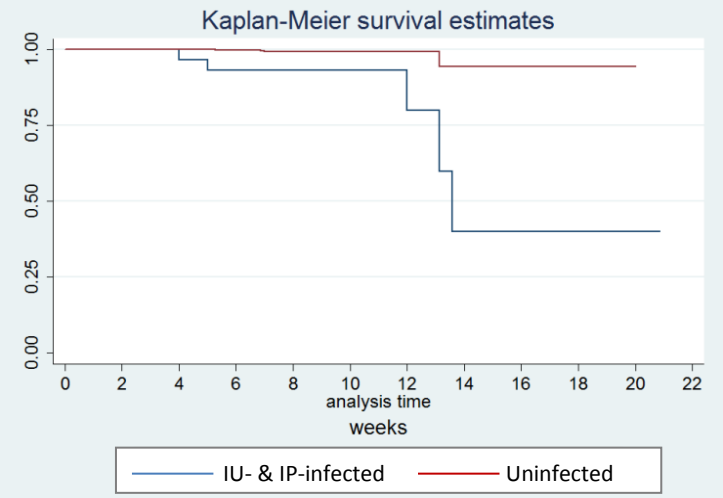


Figure 3.11 – Kaplan Meier survival curve of deaths to 14 weeks in IU- and IP-infected versus uninfected infants (log rank test, p<0.001). The five deaths in infected infants occurred at 4.0, 5.0, 12.0, 13.1 and 13.6 weeks of age and those in uninfected infants at 5.3, 5.6, 6.9, 7.0 and 13.1 weeks of age.

Characteristics of IU- versus IP-infected infants

Gender, PMTCT prophylaxis and birth anthropometry, as detailed in Table 3.3, were also compared in IU-infected versus IP-infected infants. No significant differences were found (data not shown). There was also no evidence to suggest that infants with IU-infections progressed more rapidly than those with IP-infections – hospital admissions, antibiotic treatment, deaths, WHO stage at diagnosis and baseline VL were all comparable in IU- and IP-infected infants (p = 1.000, 1.000, 0.647, 0.468 and 0.254, respectively).

3.1.5 Implementation of the national PMTCT guidelines over time

Compared to women enrolled in the first half of the study, significantly more women enrolled in the second half presented for antenatal care before 20 weeks gestation (p = 0.016) and accessed PMTCT from 28 weeks as per the 2008 guidelines (p<0.001) (Figure 3.12 a). Similarly, significantly fewer infants enrolled in the second half of the study

received AZT for four weeks (p = 0.001), indicating that their mothers were more compliant with their antenatal PMTCT care (Figure 3.12 b). MTCT decreased from 6.1% to 4.4% in the first and second halves of the study, respectively, but this decrease was not significant (p = 0.325) (Figure 3.12 b). The improvements in antenatal PMTCT care and the trend to decreasing transmission indicate that the 2008 guidelines were slowly being implemented over time. However, in the second half of the study, over 18 months after the 2008 guidelines had been released, only 47.8% of women received PMTCT prophylaxis from 28 weeks gestation (Figure 3.12 a). Furthermore, there was no improvement in the time it took infants to initiate HAART from the ten-week result visit (median delay of 5.8 and 6.2 weeks in infants enrolled in the first and second halves of the study, respectively; p = 0.843).

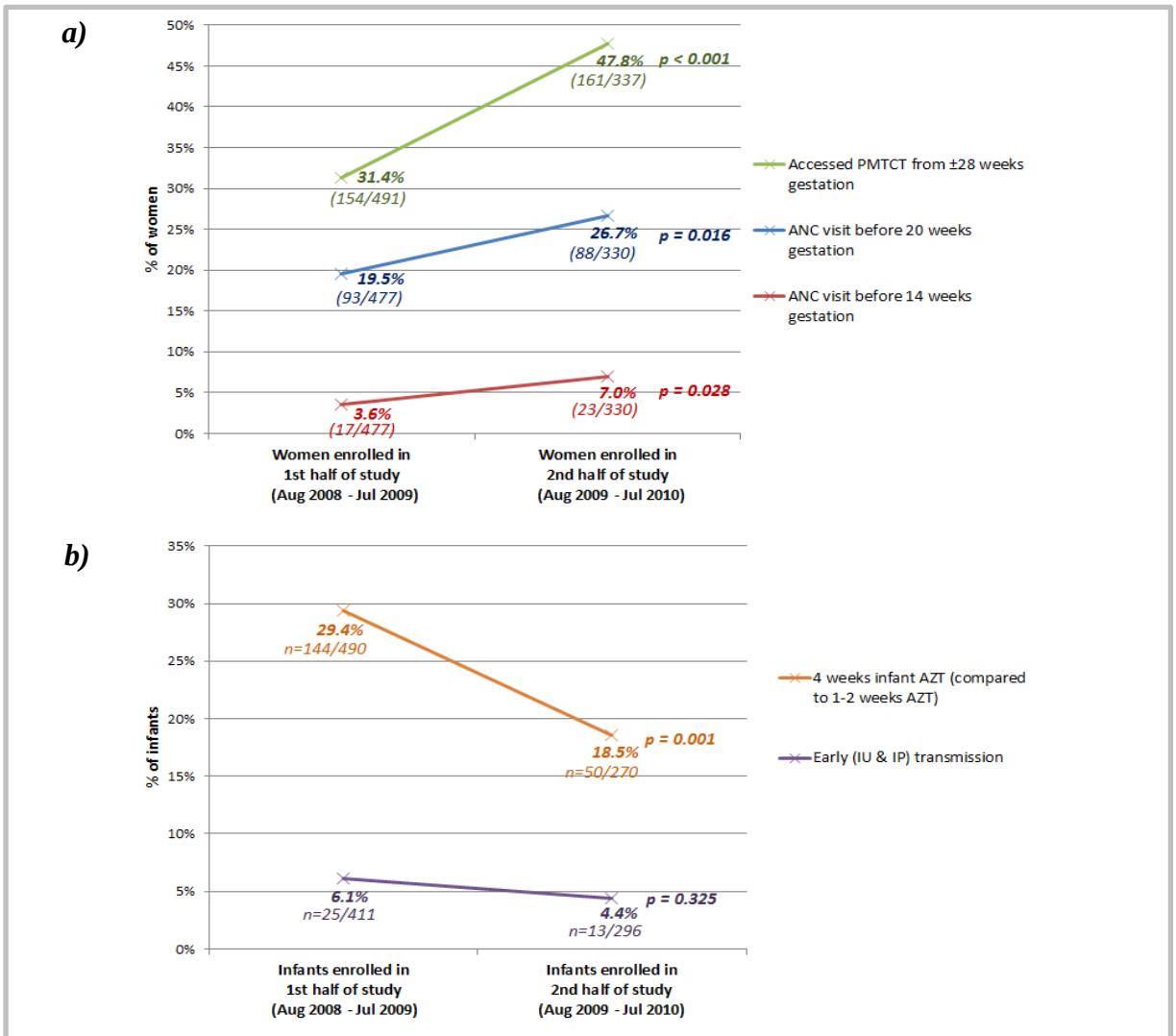


Figure 3.12 – a) Antenatal and b) postnatal PMTCT indicators by timing of enrolment (p-values calculated with Chi^2 test).

The proportion of women at RMMCH who elected to exclusively breastfeed was low, but increased over time to 21.2% in women enrolled in the final quarter of the study when exclusive breastfeeding was supported (Figure 3.13).

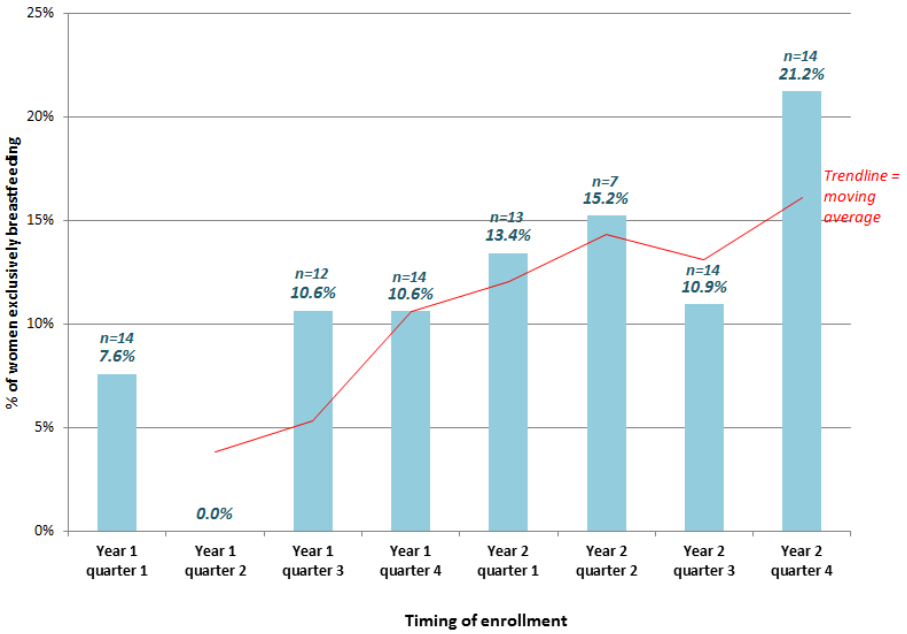


Figure 3.13 – Proportion of women choosing to exclusively breastfeed versus exclusively formula feed by timing of enrolment.

3.2 OBJECTIVE 2 – DIAGNOSIS OF IU AND IP HIV-INFECTION UNDER SIX WEEKS OF AGE

3.2.1 EID Study visits and sample testing

Study visits attended by the 710 infants with a known HIV status are detailed in Table 3.4. Of the 606 infants who underwent diagnostic PCR testing at RMMCH during the EID Study (Figure 3.5), 595 attended the six-week visit. The remaining 11 infants had earlier PCR tests that provided a definitive diagnosis and therefore did not attend the visit at six weeks. Between the birth and six-week visits, 115 (16.2%) infants were LTFU, 12 of whom were IU- or IP-infected (Table 3.4). The prevalence of infected infants at each visit

was consistent at 4.4% to 5.4%. A total of 366, 365 and 403 samples from IU- and IP-infected and HIV-uninfected infants were tested with the HIV DNA PCR, CAP/CTM and APTIMA assays, respectively (Table 3.4).

Table 3.4 – Description of study cohort and total number of samples from HIV-uninfected and *in utero*- and intrapartum-infected infants tested with each diagnostic assay (Lilian *et al* 2012 (129) Table 1; Permission to reproduce these data given in Appendix B).

	Study cohort			No. of samples tested with each diagnostic assay[b]		
Time of visit	**Median age (wk)**	**Total no. of infants**	**No. (%) HIV infected[a]**	**HIV DNA PCR**	**CAP/CTM**	**APTIMA**
Birth	0.1	710	38 (5.4)	107	107	144[c]
2 wk	2.1	566	25 (4.4)	86	86	86
4 wk	4.6	556	25 (4.5)	88	88	88
6 wk	6.6	595	26 (4.4)[d]	85	84[e]	85
Total		**710**	**38 (5.4)**	**366**	**365**	**403**

[a] *In utero* and intrapartum infections. HIV infection status was determined retrospectively where the routine 6-week PCR test identified an infant as HIV-infected.
[b] Samples were obtained from infants later determined to be HIV-infected, together with two age-matched uninfected controls, as well as from infants who died; birth samples were also obtained from infants of an unknown status.
[c] The screen of birth samples from infants with an unknown HIV status using the APTIMA assay accounts for the additional APTIMA tests.
[d] Samples were not prepared for two HIV-infected infants who attended the 6-week visit.
[e] One 6-week sample produced an invalid CAP/CTM result and could not be retested.

3.2.2 Assay performance to six weeks of age

HIV DNA PCR was the only assay that yielded equivocal results, three from HIV-infected infants (one at four weeks and two at six weeks) and four from HIV-uninfected infants (two at birth, one at two weeks and one at four weeks). Equivocal results were omitted from the sensitivity and specificity analyses.

3.2.2.1 Detection of IU- and IP-infected infants

At birth, CAP/CTM and APTIMA were the most sensitive assays for identifying HIV-infected infants, detecting 76.3% of all early HIV-infections detectable by six weeks of age (Table 3.5). HIV DNA PCR was less sensitive (68.4%), missing three IU-infected infants. The sensitivities of all three assays declined at two weeks of age, with four infants having positive tests at birth but negative two-week results. This occurred on a single assay for two infants (one each on the CAP/CTM and APTIMA assays) and on two assays for the other two infants (CAP/CTM and APTIMA for one and CAP/CTM and HIV DNA PCR for the other). The four-week CAP/CTM and APTIMA results were positive in all four cases, though HIV DNA PCR still yielded a negative four-weeks result for one IU-infected infant; no six-week HIV DNA PCR sample was available for this infant. All four IU-infected infants with negative two-week results had received sdNVP at birth. By four weeks of age, CAP/CTM and APTIMA were 96.0% sensitive, missing a single IP-infected infant (Table 3.5). This infant, who was reportedly exclusively formula fed, was only detectable by any assay from six weeks of age. At six weeks, all three assays were 100% sensitive and therefore had NPVs of 100% (Table 3.5). However, two IP-infected infants had equivocal HIV DNA PCR results which would have necessitated repeat testing before a definitive diagnosis could be made.

Only one equivocal HIV DNA PCR result at six weeks was retested. The repeat test yielded a false negative result; however, the six-week CAP/CTM and APTIMA tests for this infant were both positive. Repeat testing following false-negative results in IU-infected infants was conducted for three samples (one each from CAP/CTM and APTIMA at two weeks and one from HIV DNA PCR at four weeks). All three remained negative upon repeat testing, with no obvious reasons for the false negative results.

Table 3.5 – Performance of the HIV DNA PCR, CAP/CTM and APTIMA assays in identifying *in utero*- and intrapartum-infected and HIV-uninfected infants in the first 6 weeks of life (Lilian *et al* 2012 (129) Table 2; Permission to reproduce these data given in Appendix B).[a]

Infection status and time of visit	**HIV DNA PCR**				**CAP/CTM**				**APTIMA**			
Infected	Total no. of infants tested[b]	No. with true-positive result	% Sensitivity (95% CI)	PPV (%)	Total no. of infants tested	No. with true-positive result	% Sensitivity (95% CI)	PPV (%)	Total no. of infants tested	No. with true-positive result	% Sensitivity (95% CI)	PPV (%)
Birth	38	26	68.4 (52.5-80.9)	100	38	29	76.3 (60.8-87.0)	100	38	29	76.3 (60.8-87.0)	93.5
2 wk	25[1]	16	64.0 (44.5-79.8)	84.2	25[3]	15	60.0 (40.7-76.6)	100	25[2]	16	64.0 (44.5-79.8)	100
4 wk	24	21	87.5 (69.0-95.7)	91.3	25	24[c]	96.0 (80.5-99.3)	100	25	24[c]	96.0 (80.5-99.3)	100
6 wk	22	22	100 (85.1-100)	91.7	24	24	100 (86.2-100)	100	24	24	100 (86.2-100)	96.0
Uninfected	Total no. of infants tested[d]	No. with true-negative result	% Specificity (95% CI)	NPV (%)	Total no. of infants tested	No. with true-negative result	% Specificity (95% CI)	NPV (%)	Total no. of infants tested	No. with true-negative result	% Specificity (95% CI)	NPV (%)
Birth	67	67	100 (94.6-100)	84.8	69	69	100 (94.7-100)	88.5	106	104	98.1 (93.4-99.5)	92.0
2 wk	60	57	95.0 (86.3-98.3)	86.4	61	61	100 (94.1-100)	85.9	61	61	100 (94.1-100)	87.1
4 wk	62	60	96.8 (89.0-99.1)	95.2	63	63	100 (94.3-100)	98.4	63	63	100 (94.3-100)	98.4
6 wk	61	59	96.7 (88.8-99.1)	100	60	60	100 (94.0-100)	100	61	60	98.4 (91.3-99.7)	100

[a] Superscripts denote numbers of HIV-infected infants with positive tests at birth but negative 2-week results. CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.
[b] Excluding 3 equivocal tests (n = 1 at 4 weeks and n = 2 at 6 weeks).
[c] Same IP-infected infant missed by CAP/CTM and APTIMA assays at 4 weeks.
[d] Excluding 4 equivocal tests (n = 2 at birth, n = 1 at 2 weeks, and n = 1 at 4 weeks).

3.2.2.2 Detection of HIV-uninfected infants

CAP/CTM was the best performing assay for detecting HIV-uninfected infants, with a specificity of 100% at all ages (Table 3.5). HIV DNA PCR was the least specific, yielding seven false positive results between two and six weeks of age, five of which were from testing on a single day. Retesting was performed for all false positive and three equivocal HIV DNA PCR tests, yielding true negative results in all cases. The APTIMA assay produced two false positive results at birth and one at six weeks (specificities of 98.1% and 98.4%, respectively) (Table 3.5). All false positive APTIMA results were negative upon repeat testing.

3.3 OBJECTIVE 3 – PREDICTORS OF IU TRANSMISSION

3.3.1 Characteristics of mothers of IU-infected infants

The characteristics of mothers of IU-infected infants were comparable to those described above for mothers of IU- and IP-infected infants (Table 3.2), as IU-infections comprised the majority of early transmissions (29/38 = 76.3%). In comparison to mothers of infants who were not infected IU, IU-transmitting mothers were significantly younger ($p = 0.018$), booked at antenatal clinics later in pregnancy ($p = 0.004$), had less optimal PMTCT ($p = 0.001$), higher peripartum VL levels ($p<0.001$) and breastfed more often ($p = 0.030$) (Table 3.6).

3.3.2 Predictors of IU transmission

3.3.2.1 Univariate analysis

Logistic regression was performed to determine if antenatal or peripartum factors could

Table 3.6 – Maternal characteristics of IU-infected infants versus infants not infected IU.

Maternal characteristics	**Mother-infant pairs with IU transmission (n=29)**	**Mother-infant pairs with no IU transmission (n=809)**	**p-value[a]**
Age (years) - median (range; n)	26.1 (18.9 - 37.6; 29)	28.1 (16.2 - 44.8; 809)	**0.018**
Nationality: South African - n/total (%)	13/28 (46.4)	450/785 (57.3)	0.253
Parity (viable births) - median (range; n)	2.0 (1.0 - 4.0; 29)	2.0 (1.0 - 10.0; 809)	0.262
Gravidity (pregnancies) - median (range; n)	2.0 (1.0 - 4.0; 29)	2.0 (1.0 - 10.0; 809)	0.088
Nevirapine in a previous pregnancy - n/total (%)	2/29 (6.9)	70/784 (8.9)	1.000
Clinical history - n/total (%)			
Alcohol	0/29 (0.0)	16/808 (2.0)	1.000
Smoking	0/29 (0.0)	6/807 (0.7)	1.000
Syphilis	0/26 (0.0)	10/773 (1.3)	1.000
Diabetes	0/29 (0.0)	2/809 (0.2)	1.000
Hypertension	1/29 (3.4)	55/809 (6.8)	0.715
Tuberculosis	0/28 (0.0)	48/804 (6.0)	0.400
Antenatal factors			
Gestational age at antenatal booking (weeks) - median (range; n)	29.3 (19.7 - 38.9; 29)	25.6 (2.6 - 39.0; 787)	**0.004**
Antenatal CD4 testing			
Accessed a CD4 test - n/total (%)	25/29 (86.2)	699/809 (86.4)	1.000
Gestational age at CD4 test (weeks) - median (range; n)	28.1 (4.0 - 38.9; 25)	26.1 (4.0 - 39.0; 698)	0.096
CD4 count (x 10^6/l) - median (range; n)	286.0 (58.0 - 674.0; 25)	354.0 (11.0 - 1221.0; 699)	0.175
PMTCT: ordinal categorisation - n/total (%)			**0.001[b]**
Suboptimal (no antenatal prophylaxis)	7/29 (24.1)	80/808 (9.9)	
Intermediate (<10 wks AZT/HAART)	19/29 (65.5)	414/808 (51.2)	
Optimal (≥10 wks AZT/HAART)	3/29 (10.3)	314/808 (38.9)	
PMTCT: length of treatment			
Antenatal AZT or HAART (weeks) - median (range; n)	3.0 (0.0 - 12.0; 29)	8.0 (0.0 - 41.0; 805)	**<0.001**
Mothers receiving HAART			
Number on HAART - n/total (%)	1/29 (3.4)	217/809 (26.8)	**0.002**
Most common WHO stage at initiation	Stage 1	Stage 1	
Delivery			
PMTCT: sdNVP - n/total (%)	27/29 (93.1)	557/805 (69.2)	**0.004**
Peripartum viral load (log) - median (range; n)	4.60 (2.78 - 5.59; 29)	3.78 (0.00 - 5.72; 281)	**<0.001**
Gestation: preterm delivery - n/total (%)	3/29 (10.3)	63/809 (7.8)	0.493
Mode of delivery: caesarean section - n/total (%)	4/29 (13.8)	117/809 (14.5)	1.000
Premature rupture of membranes - n/total (%)	1/22 (4.5)	28/736 (3.8)	0.581
Feeding			
Feeding intention: breastfeeding - n/total (%)	7/29 (24.1)	96/806 (11.9)	0.076
Final feeding practice: breastfeeding - n/total (%)	7/29 (24.1)	84/806 (10.4)	**0.030**

[a] Mann-Whitney U test for continuous and discrete variables; Fisher's exact or Chi^2 test for binary and categorical variables. The total number of mothers varies due to missing data.

[b] Significant at $p<0.017$ for suboptimal vs optimal and intermediate vs optimal (Bonferroni correction).

identify mothers at greatest risk of IU transmission whose infants would benefit most from a birth diagnosis (Table 3.7). In univariate analysis, maternal sdNVP increased the risk of IU transmission six-fold (OR = 6.01, 95% CI 1.42 – 25.47, p = 0.015), indicative of increased risk in the absence of HAART. Maternal peripartum VL was also a risk factor for IU transmission, with each log VL increase more than doubling the risk of IU transmission (OR = 2.19, 95% CI 1.38 – 3.46, p = 0.001). Each additional week of antenatal AZT or HAART reduced the risk of IU transmission by 15% (OR = 0.85, 95% CI 0.77 – 0.93, p = 0.001). Maternal age was also a protective factor, with the probability of IU transmission decreasing by 9% with each additional year (OR = 0.91, 95% CI 0.84 – 0.99, p = 0.021).

Table 3.7 – Odds ratios for IU transmission according to antenatal and peripartum factors (logistic regression).

	Univariate regression			**Multivariate regression**		
	Unadjusted odds ratio	**95% CI**	**p-value**	**Adjusted odds ratio**	**95% CI**	**p-value**
Maternal age (years)	0.91	0.84 - 0.99	**0.021**	0.93	0.86 - 1.01	0.093
Antenatal CD4 count	0.44	0.11 - 1.73	0.238			
Maternal PMTCT sdNVP AZT or HAART (weeks)	 6.01 0.85	 1.42 - 25.47 0.77 - 0.93	 **0.015** **0.001**	 0.97	 0.88 - 1.07	 0.588
Maternal peripartum viral load (log)	2.19	1.38 - 3.46	**0.001**	2.03	1.25 - 3.28	**0.004**
Preterm delivery	1.37	0.40 - 4.64	0.617			
Caesarean section	0.95	0.32 - 2.77	0.920			
Premature rupture of membranes	1.20	0.16 - 9.27	0.858			

3.3.2.2 Multivariate analysis

The multivariate model included maternal age, duration of AZT or HAART and peripartum VL (Table 3.7). Antenatal HAART was included in place of sdNVP. The interaction between duration of AZT or HAART and VL did not significantly affect the model (-2 log likelihood ratios of -87.2 and -87.6 with and without the interaction term,

respectively). In the final multivariate model, the only independent predictor of IU transmission was VL, with the likelihood of IU transmission doubling with each VL log increase (adjusted OR = 2.03, 95% CI 1.25 – 3.28, p = 0.004) (Table 3.7).

3.4 OBJECTIVE 4 – MATHEMATICAL MODEL OF EARLY INFANT DIAGNOSIS

3.4.1 Baseline scenario

3.4.1.1 Single PCR test for early infant diagnosis

Testing at six weeks of age, as is currently recommended, would identify double the number of perinatal HIV-infections compared to a scenario where no routine testing was performed (Table 3.8). However, a single PCR test at six weeks of age would diagnose the fewest perinatally infected infants compared to routine testing at any other age, reflecting reduced assay sensitivity at six weeks due to NVP prophylaxis, early infant deaths and LTFU. Testing at six weeks would also save the least total number of life years and QALYs, and would therefore be the least effective time to perform a single PCR test for early infant diagnosis. Compared to six-week testing, a single PCR test at birth would diagnose a comparable number of HIV-infected infants (Table 3.8). However, since the number of HIV-exposed infants available for testing would be higher at birth that at six weeks, a higher total number of PCR tests would be performed at birth. Birth testing would therefore be less efficient than six-week testing in terms of new diagnoses, life years saved and QALYs saved *per additional PCR*. Compared to birth and six-week testing, a single PCR test at ten weeks would identify the highest number of infected infants and save the most life years and QALYs in total and per additional PCR (Table 3.8). Nevertheless,

almost 62 000 fewer PCR tests would be performed at ten weeks than at birth, as fewer HIV-exposed infants would be available for diagnostic testing at ten weeks because of early mortality and LTFU. Birth testing would deliver only minimally less than ten-week testing – at birth, only 161 fewer HIV-infected infants would be diagnosed and 5-7% fewer life years and QALYs would be saved. However, birth testing would only diagnose IU-infected infants, compared to testing at ten weeks where both IU- and IP-infected infants could be detected.

Table 3.8 – Impact of PCR testing at different ages on identification of IU- and IP-infected infants and life years / quality-adjusted life years (QALYs) saved in 240 000 HIV-exposed infants (*baseline scenario*).

	No routine testing*	**1 routine PCR test at ages**			**2 routine PCR tests at ages**		
		Birth	**6 wks**	**10 wks**	**Birth + 6 wks**	**Birth + 10 wks**	**Birth+ 14 wks**
Total PCR tests	18 001	256 515†	207 461	194 535	442 797	430 462	418 224
Total HIV-infected infants diagnosed	3 467	7 139	6 962	7 300	8 979	9 257	9 085
New HIV diagnoses per additional PCR		0.0154	0.0184	0.0217	0.0130	0.0140	0.0140
Life years saved		44 107	40 258	46 999	66 121	69 494	66 938
Life years saved per additional PCR		0.1849	0.2125	0.2662	0.1558	0.1686	0.1674
QALYs saved		21 167	19 337	22 360	31 680	33 384	32 098
QALYs saved per additional PCR		0.0887	0.1021	0.1267	0.0746	0.0810	0.0803

Figures in blue are the highest in each row with 1 routine PCR test; figures in red are the highest in each row with 2 routine PCR tests.

* PCR testing only performed post cessation of breastfeeding or where a child was symptomatic. These PCR tests were included as a baseline at all other ages.

† Total PCR tests exceeds 240 000 as screening performed post cessation of breastfeeding and for symptomatic children was also included.

3.4.1.2 Two PCR tests for early infant diagnosis: birth testing in conjunction with testing at older ages

Birth testing, which detects IU-infections, would need to be followed by a second test at an older age to detect IP-infections. Performing the second test at six weeks of age would diagnose the fewest perinatally infected infants and save the least number of life years

(Table 3.8). The ideal time for the second test to diagnose the highest number of perinatal HIV-infections would be at ten weeks of age, when IP-infected infants not detectable at six weeks due to NVP prophylaxis would also be identified. Birth and ten-week testing would therefore also save the maximum number of life years and QALYs. Even though the dampening effects of NVP prophylaxis would be further reduced by 14 weeks of age, a higher number of infected infants would be LTFU or would have died; birth and 14-week testing would therefore be less effective than a birth and ten-week test in detecting perinatal transmission and saving lives (Table 3.8). Testing at birth and ten weeks would diagnose 1 957 (26.8%) more infants than a single test at ten weeks by virtue of detecting IU-infected infants before they are LTFU or die. This would have a considerable impact on infant mortality, with 22 495 (47.9%) additional life years and 11 024 (49.3%) additional QALYs saved by testing at birth and ten weeks compared to testing at ten weeks alone (Table 3.8). The expected rate of perinatal transmission in the model was 5.02%, equating to 12 048 IU- and IP-infected infants. Compared to this expected number of infections, the ideal testing scenario with a birth and ten-week test would only detect 76.8% of perinatal infections; the remaining infants would have died, been LTFU, failed to receive test results or escaped detection due to reduced assay sensitivity in the context of NVP prophylaxis. Nevertheless, this exceeds the 60.6% of expected perinatal HIV-infections that could be diagnosed with a single PCR test at ten weeks of age.

3.4.2 Sensitivity analyses

Scenario one: If it was assumed that NVP prophylaxis had no impact on PCR sensitivity, two PCR tests at birth and six weeks would diagnose the highest number of perinatal HIV-infections and save the most life years and QALYs (Table 3.9). In the baseline scenario, the lowest sensitivity due to NVP prophylaxis occurred at six weeks of age; *scenario one*

Table 3.9 – HIV-infected infants diagnosed, life years saved and quality-adjusted life years (QALYs) saved under different scenarios.

Scenario	1 routine PCR test at ages			2 routine PCR tests at ages		
	Birth	**6 wks**	**10 wks**	**Birth + 6 wks**	**Birth + 10 wks**	**Birth + 14 wks**
Baseline						
HIV-infected infants diagnosed	7 139	6 962	7 300	8 979	9 257	9 085
QALYs saved	21 167	19 337	22 360	31 680	33 384	32 098
Life years saved	44 107	40 258	46 999	66 121	69 494	66 938
1. No loss in PCR sensitivity due to NVP prophylaxis						
HIV-infected infants diagnosed	6 815	7 594	7 219	9 578	9 333	9 112
QALYs saved	19 985	23 989	22 104	35 556	34 108	32 439
Life years saved	41 284	49 848	46 171	72 873	69 887	66 475
2. WHO option B						
HIV-infected infants diagnosed	4 708	4 759	5 025	6 094	6 320	6 185
QALYs saved	14 944	13 581	15 292	21 705	23 213	22 206
Life years saved	31 483	28 068	31 737	44 761	48 007	45 745
3. Lower mortality in IP-infected children						
HIV-infected infants diagnosed	6 696	6 755	7 104	8 804	9 126	8 972
QALYs saved	20 514	18 484	20 886	30 535	32 551	31 396
Life years saved	41 892	37 913	42 887	63 755	68 114	65 762

Figures in blue are the highest in each row with 1 routine PCR test; figures in red are the highest in each row with 2 routine PCR tests.

therefore had the greatest impact at this age, with six-week testing diagnosing more HIV-infections and saving many more life years compared to baseline. In the remaining two scenarios, like in the baseline scenario, six-week testing was the least effective for minimising the HIV-disease burden:

- *Scenario two:* If all HIV-infected women received HAART during pregnancy, the overall transmission rate would be reduced; PCR testing at any age would therefore diagnose fewer infants and save fewer life years compared to baseline (Table 3.9). In this scenario, like in the baseline scenario, ten-week testing alone or in combination with birth testing would be most effective in diagnosing perinatal HIV-infections and saving lives. However, combined birth and ten-week testing would be most effective by virtue of saving the lives of IU-infected infants who would otherwise fall ill, die or be LTFU. Birth and 14-week testing would only diagnose 135 fewer HIV-infected

infants than testing at birth and ten weeks, but would be less effective in saving life years and QALYs.

- *Scenario three:* If IP-infected infants were assumed to have a lower mortality than infants with IU-infections, birth and ten-week testing, and to a lesser extent birth and 14-week testing, would diagnose the most HIV-infected infants (Table 3.9). Testing at birth and ten weeks would save the most life years and QALYS. In this scenario, fewer life years and QALYs would be saved at each time point compared to baseline, as the reduced mortality would result in fewer life years lost due to HIV-infection and therefore fewer available life years to be saved with improved HIV diagnostic testing.

4. DISCUSSION

The EID Study successfully enrolled 838 mother-infant pairs over two years, representing approximately one third of the HIV-infected women expected to deliver at RMMCH over a two-year period (130). The women who were recruited knew their HIV status antenatally, possibly creating a more adherent sample, as these women are more likely to return for follow-up than women identified as HIV-infected at the time of delivery (130). This sample may therefore not be representative of all HIV-infected women delivering at RMMCH. Furthermore, exposed infants born to HIV-infected women identified through perinatal counselling and testing would not have been included in the cohort. Fewer HIV-infected infants may therefore have been identified than were available at RMMCH at the time. However, among the infants who were enrolled, it is likely that the study identified all HIV-infected and uninfected infants who would have accessed diagnostic testing through routine PMTCT services, as 82% of enrolled infants accessed testing at RMMCH or other routine facilities. This is equivalent to the reported 2010 - 2011 coverage for early infant diagnostic testing of 76% - 85% in Gauteng, the province where RMMCH is located (37). The overall early transmission rate in the study (5.4%) is comparable to that of 4.4% reported for South Africa in 2010 (37).

4.1 OBJECTIVE 1 – RMMCH'S PMTCT PROGRAMME AND THE STUDY COHORT

4.1.1 The antenatal and postnatal PMTCT coverage cascades

Consistent with previous findings (33, 36, 40), the antenatal PMTCT care cascade

highlighted the significant problem of women presenting at ANCs late in pregnancy, thereby hampering effective antenatal PMTCT care. Even in the second half of the study, less than a third of women presented for antenatal care before 20 weeks gestation and only half accessed optimal PMTCT cover from 28 weeks in accordance with the 2008 guidelines (4). MTCT and/or infant deaths are increased with later booking at ANC and shorter durations of antiretroviral cover during pregnancy (43, 131). Women who book late for antenatal care thus represent critical missed opportunities for achieving eMTCT (7) and reducing infant mortality. Efforts need to ensure that women access antenatal care earlier in pregnancy, especially in view of the 2013 PMTCT guidelines which call for all HIV-infected women to receive HAART for the duration of pregnancy (26). Earlier presentation for antenatal care has been achieved in other settings by means of quality improvement programmes with enhanced resource allocations and improved processes for linking women to antenatal services (40).

Majority of enrolled women who presented for antenatal care underwent CD4 testing and accessed some form of antenatal PMTCT cover, consistent with findings in other settings of high uptake of CD4 testing (32, 36) and antenatal prophylaxis (30-33, 35). Even though CD4 testing was a prerequisite for PMTCT cover, more women in this cohort received PMTCT prophylaxis than underwent CD4 testing. This may be due to prophylaxis being commenced while waiting for CD4 results, which were then never followed up, or due to data problems, where antenatal CD4 tests and/or results were not documented. Both these scenarios have been reported elsewhere – in one setting, 97% of HIV-infected mothers initiated dual therapy prophylaxis at their first antenatal visit independent of CD4 results (32) and in another, 78% of HIV-infected mothers reported having blood taken for CD4 testing, but a result was only recorded for 34% of these women (33). Improved data

management, including follow-up and recording of test results, is required.

The postnatal cascade also demonstrated good coverage of PMTCT prophylaxis, with NVP being administered to all but one enrolled infant. However, almost a third of HIV-exposed infants were subsequently lost from RMMCH's PMTCT programme prior to accessing routine testing, despite all enrolled mothers expressing intention to return to RMMCH for postnatal care. Comparable testing rates of 62% and 83% have been reported at other PMTCT sites in the country (47, 132) and the Interagency Task Team for monitoring progress towards eMTCT presented an equivalent rate of EID in South Africa of 72% during 2011 (35). However, testing rates as low as 37% in an Eastern Cape local service area (48) and 52% nationally during 2010/2011 (34) have also been described. Lower testing rates in routine settings may be expected, as antenatal care, delivery and EID generally take place at different sites and HIV-exposed infants are therefore not so readily identifiable for testing. In contrast, enrolled infants born at RMMCH were known to be HIV-exposed and were scheduled to return to the same site for diagnosis. It is reassuring that in this cohort some defaulting infants did access diagnostic testing at other healthcare facilities, as identified through the LIS search, and this likely also occurs with defaulting infants in other settings. However, efforts to reduce missed opportunities for testing HIV-exposed infants at all sites are critical. Early infant diagnosis is an essential component in reducing infant morbidity and mortality (13) and improved coverage of PCR testing can facilitate greater survival benefits from early HAART initiation (110).

Interventions to improve coverage of PCR testing have been successfully implemented in other settings, including enhanced referral to EID services (service integration) which

improved the odds of infants returning for EID three-fold (133) and enhanced identification of HIV-exposed infants through a data-based, quality improvement intervention which increased six-week testing from 24% to 68% (134). Data from this study suggest that LTFU prior to diagnostic testing can be reduced by providing increased counselling, support and follow-up to young, foreign mothers, who present late for antenatal care with no CD4 results, as infants born to these women are at increased risk of being LTFU prior to accessing a diagnosis. These findings concur with previous studies which demonstrate that infants born to young mothers (47, 49) who present late for antenatal care (132) with poor PMTCT prophylaxis adherence (47, 135) are less likely to remain in the PMTCT programme and/or access HIV diagnostic testing. Furthermore, foreign mothers are a particularly vulnerable group who may not access EID services due to prejudice, lack of knowledge or financial constraints, and these women also require intensified counselling with active infant follow-up. This in line with the call by the Joint United Nations Programme on HIV/AIDS (UNAIDS) for increased efforts to better health services for vulnerable groups (136).

Losses to follow-up from the PMTCT cascade prior to diagnostic testing can also be addressed by performing testing at birth. Currently, routine EID is only performed if an infant returns for the six-week immunisation visit and is identified as being HIV-exposed (25, 26). This relies on a mother or caregiver presenting patient-held health records or volunteering the infant's HIV-exposure status. In contrast, HIV-exposed infants are readily identified at birth, as maternal HIV status should be determined at the time of delivery so that intrapartum PMTCT prophylaxis can be administered (4, 25, 26). Birth testing would therefore target a captive population, particularly in South Africa where majority of infants are born in healthcare facilities (137). In this cohort, more HIV-infected infants were

identified at birth (n = 29) than at six weeks (n = 26), as birth testing enabled the identification of infants before they were LTFU from the PMTCT programme or died. Even though a birth diagnosis would not have identified IP-infected infants, testing prompted by clinical stigmata at the routine six- or ten-week visits could have identified 44% of IP-infected infants. Offering PCR testing to all HIV-exposed infants at birth and to children with clinical features suggestive of HIV-infection would have identified the highest number of perinatally HIV-infected infants in the cohort without increasing testing costs (assuming only a single early routine PCR test was available per HIV-exposed infant). This would therefore be a highly effective intervention for improving the diagnosis of HIV-infected infants. HIV testing prompted by clinical features or acute illness is the standard of care in South Africa (26, 28) but needs to be reinforced in routine settings.

Almost 90% of HIV-infected infants diagnosed by RMMCH's routine programme initiated HAART. This linkage to care is higher than the 68% to 71% reported for PCR positive infants in the Western Cape between 2008 and 2010 (51). LTFU rates were potentially reduced in the EID cohort, as RMMCH offered both treatment and diagnostic services; such service integration is known to improve paediatric enrolment in care (138). However, the timing of HAART initiation at RMMCH at a median age of 16.0 weeks was concerning and did not improve in the second half of the study. Although a five-week delay between PCR testing and attendance at an ART clinic was reported in the Western Cape (51), suggesting treatment initiation from 11 weeks of age following six-week testing, this does not take into account the delay between presentation for care and HAART initiation. Such delays may be substantial, with a median 2.5-week delay for HIV-infected infants in this cohort presenting for care at RMMCH (Lilian *et al* 2013 (128) Figure 3; Appendix C). HAART initiation may therefore be delayed well beyond 11 weeks of age, as demonstrated

in this study. Commencing treatment around 16 weeks of age is too late to save the infants who die in the early peak of HIV-related mortality at eight to 12 weeks of age (3) or the 20% of perinatally infected infants who die by 13 weeks of age (2). In order to reduce infant mortality, the CHER Study demonstrated that HAART should be initiated at a median age of seven weeks (13). Even if the PMTCT programme were optimised to reduce delays to HAART initiation, it is unlikely that treatment could be commenced by seven weeks of age if diagnostic testing is performed at six weeks. Earlier initiation of treatment can therefore only be achieved with an earlier diagnosis, such as testing at birth.

The need for an earlier diagnosis to ensure earlier initiation of treatment is further emphasised by the median baseline CD4 percentage of 25.7% in infants initiating HAART. This indicates that at the time of treatment initiation, at least half the infants in this cohort were already severely immunosuppressed according to the WHO threshold of 25% (14). There is therefore clearly a need for earlier initiation of HAART to prevent disease progression, as late initiation of treatment in infants who are already symptomatic may hinder the survival benefits of HAART (11).

Over half (61%) of the infants who initiated treatment at RMMCH achieved viral suppression, slightly lower than that reported in other settings of 70% to 80% after one year of treatment (10, 38). However, compared to the total number of HIV-infected infants in the EID cohort, only 37% were known to achieve suppression. The remaining HIV-infected infants were either not diagnosed by the PMTCT programme, thus representing missed opportunities for identifying infected infants for HAART initiation, or if they did

access diagnostic testing, were LTFU or died prior to initiating HAART or achieving suppression. Retention in care following HAART initiation was particularly problematic, with 43% of infants who initiated treatment dying or defaulting before the end of the study. It is essential to enhance retention in care if outcomes of HIV-infected infants are to be improved. Interventions that may reduce attrition of children on treatment include implementing community-based adherence support (10), developing or strengthening home-based services (138) and implementing active tracing of infants who are LTFU (139).

4.1.2 Maternal characteristics by MTCT status

Compared to non-transmitting mothers, mothers of IU- and IP-infected infants were younger and had had fewer pregnancies. Transmitting mothers presented for antenatal care significantly later in gestation and therefore accessed less optimal antenatal HAART and/or PMTCT prophylaxis, concurring with previous studies where shorter durations of antenatal cover were associated with MTCT (43, 95, 97). These findings further emphasise the need for increased counselling and follow-up of younger mothers with poor antenatal health-seeking behaviours – infants born to these women are not only more likely to be lost from the PMTCT programme prior to accessing a diagnosis, but are also at increased risk of acquiring HIV via MTCT. Although HAART initiation should occur prior to 20.4 weeks gestation to achieve undetectable VL levels by delivery (140) and the 2010 guidelines recommend antenatal prophylaxis from 14 weeks of pregnancy (25), this study demonstrates that prophylaxis for a median of eight weeks still protects against transmission. Similarly, in the setting of antenatal HAART, transmission only occurs from mothers receiving less than eight weeks of antenatal cover (97). Thus, for mothers presenting late in pregnancy, initiating PMTCT prophylaxis up to 30 or 32 weeks gestation

may still be protective. Beyond this time, additional infant prophylaxis may be necessary.

The link between low antenatal CD4 count and transmission could not be demonstrated in this cohort. There is evidence to suggest that maternal VL is the strongest predictor of perinatal transmission (43, 91, 92) and it is therefore possible that low maternal CD4 count is a surrogate marker for high VL levels when low CD4 is associated with MTCT. This is supported by studies which demonstrate that maternal CD4 count no longer predicts for transmission in multivariate analyses that adjust for maternal VL (43, 88, 92). If the association between maternal CD4 and MTCT is in fact a proxy for the link between maternal VL and MTCT, then low CD4 counts will only predict for transmission if they are associated with high VL levels. However, this may no longer hold true in the context of increasingly prolonged and/or multidrug PMTCT prophylaxis. Such PMTCT regimens may interfere with the CD4-VL relationship by potently decreasing VL levels to reduce transmission, while having a less potent impact on CD4 counts which recover more slowly over time. Low VL levels may therefore no longer be associated with high CD4 counts and vice versa. This is supported by studies in which majority of women received HAART, a potent VL lowering regimen, and no association between CD4 and perinatal HIV transmission could be demonstrated (90, 97).

Exclusive formula feeding was supported for most of the study period (4) and only one-fifth of women therefore elected to breastfeed in the final quarter of the study when breastfeeding was endorsed. A significantly higher proportion of IU- and IP-transmitting mothers intended to breastfeed and did ultimately breastfeed their infants compared to non-transmitting mothers. This was surprising, as feeding choice is known to impact PP, as

opposed to perinatal, transmission. This finding may suggest that transmitting mothers in the cohort were poorer and may therefore have elected to breastfeed as they did not meet the AFASS criteria, which required that formula feeding be Affordable, Feasible, Accessible, Safe and Sustainable (4). Poverty and low socioeconomic status have been directly associated with MTCT (141, 142), a link which is further supported by findings of reduced access to NVP prophylaxis in women of lower socioeconomic status (86). The possibility that IU- and IP-transmitting mothers were socioeconomically disadvantaged suggests that increased efforts in targeting women of low socioeconomic status may be beneficial in reducing MTCT.

4.1.3 Infant characteristics by HIV-infection status

Many previous studies have failed to demonstrate significant differences in weight, length and head circumference in HIV-infected versus uninfected infants at birth (1, 15-17, 19, 95, 143). Furthermore, there does not appear to be any relationship between antenatal HAART and *in utero* growth retardation (144). In agreement with these findings, birth weight and head circumference did not differ in HIV-infected and uninfected infants in this cohort. The impact of HIV-infection on birth length was difficult to assess due to a reported measurement bias with this subjective measure – the tape measure used by midwives in the labour ward was often not properly aligned along the flexed neonate and birth length was therefore reportedly prone to inaccuracy (personal communication, K. Technau), as has been acknowledged in other settings (143). Since HIV-infection status likely does not affect birth length (15-17, 19) and birth weight (a more objective measure) did not differ in HIV-infected and uninfected infants in this cohort, the lower birth length in infected infants was likely a measuring artefact.

Due to the relatively small number of infected infants in the cohort, this study was not powered to detect differences in growth in HIV-infected versus uninfected infants over time. Nevertheless, lower weight z-scores in HIV-infected infants to ten weeks of age were demonstrated, concurring with previous findings of reduced weight and length in HIV-infected infants over time (12, 15-19). Low weight-for-age z-scores independently increase the risk of mortality in children starting HAART (10) and it is therefore important to identify HIV-infected infants early in order to address nutritional deficits that may impact on mortality.

Mortality was significantly higher in HIV-infected infants in this cohort compared to uninfected infants, as has been well established (8, 9). Since perinatally infected infants are at greater risk of mortality than infants with PP-infections (2), it is essential to focus on reducing early, perinatal transmissions of HIV if infant mortality is to be curbed. This in line with the call by UNAIDS for the elimination of MTCT by 2015 (7). For infants who nevertheless do become infected with HIV, mortality can only be reduced by the immediate initiation of HAART following an early diagnosis (13). In this cohort, no difference in mortality could be demonstrated in IU- versus IP-infected infants, nor were there any indications that IU-infected infants were more prone to rapid progression. However, with the small number of IP-infected infants in the cohort, the study was not powered to detect these differences. If IU-infected infants are indeed at increased risk of mortality (9, 24), efforts need to focus on diagnosing infants with IU-infection at birth. This would allow rapid initiation of treatment before the infants die or progress to symptomatic illness which may reduce the survival benefits of HAART (11).

4.2 OBJECTIVE 2 – DIAGNOSIS OF IU AND IP HIV-INFECTION UNDER SIX WEEKS OF AGE

HIV DNA PCR was the least sensitive assay for EID prior to six weeks of age using DBS. It only achieved a sensitivity greater than 95%, the minimum sensitivity for virological assays recommended by WHO (114), at six weeks of age. In comparison, CAP/CTM and APTIMA achieved this threshold at the four- and six-week visits, in line with WHO guidelines to diagnose HIV-infection in exposed infants from four to six weeks of age (114). Although HIV DNA PCR was the first assay used for EID in the South African PMTCT programme and was in routine use at the time of the EID Study, it has subsequently been replaced by the CAP/CTM assay in routine settings.

At birth, the CAP/CTM and APTIMA assays identified 76.3% of perinatal HIV-infections detectable by six weeks of age. Although version 1.5 of the HIV DNA PCR assay was more sensitive than earlier versions for the detection of perinatal infections at birth (65), it was still less sensitive than the CAP/CTM or APTIMA assays for birth testing. The 76.3% of perinatal infections detectable at birth in this cohort is higher than earlier estimates of 27.3% to 62% (21, 65-68). The earlier studies used older viral detection assays in populations receiving little or no PMTCT prophylaxis, in contrast to the EID Study which used newer assays in the context of improved PMTCT regimens. Enhanced late antenatal, peripartum and postpartum prophylaxis reduce IP transmission, proportionately increasing IU transmission. Together with increased sensitivity of newer assays, this may have resulted in a higher proportion of IU relative to IP-infections in this cohort. This proportion is equivalent to that of 71% and 74% in populations receiving IP and postpartum PMTCT prophylaxis, respectively, which are expected to reduce IP transmissions (27, 60). However, the proportion of IU to IP infections in the context of the new 2013 PMTCT

guidelines, which prescribe HAART for the duration of pregnancy for all HIV-infected women (26), is unclear. Perinatal infections will occur in the context of the new guidelines where PMTCT coverage is not 100%. Eventually, the only infections in mothers will represent very early infections (IU transmission), non-adherence (IU or IP transmission) or resistance (IU or IP transmission).

The EID Study was biased to detect IU-infections, as *all* IU-infected infants in the cohort were identified by testing birth DBS samples which were available for all enrolled infants. In contrast, IP-infected infants who defaulted from the study could only be identified using the LIS search if they accessed diagnostic testing at other facilities; it is therefore possible that some IP-infected infants were missed. Nevertheless, this reflects the reality in routine South African settings, where IU-infected infants are available for testing at birth by virtue of the high delivery rate in health institutions (137) and IP-infected infants are at risk of defaulting prior to six weeks, representing missed opportunities for EID. In this cohort, birth testing using any of the three assays would have detected a higher total number of infected infants compared to six-week testing, as infected infants would have been identified before they defaulted from the study or died. Diagnostic testing at birth would therefore be recommended to improve identification of perinatally infected infants for care.

The lowest sensitivity of all three assays occurred at two weeks of age, with four IU-infected infants having false negative results on one or more assays. All four infants had received sdNVP at birth which likely resulted in the reduced two-week sensitivity, concurring with previous reports of reduced VL levels in IU-infected infants within one

week of receiving sdNVP (1). This raises concerns regarding the impact of daily-dose NVP, as prescribed in the 2010 and 2013 guidelines, on the sensitivity of diagnostic testing at six weeks of age. Evidence supporting this concern is mounting, as delays in the detection of perinatal HIV-infection beyond four to six weeks of age in the context of prolonged or multidrug maternal and/or infant prophylaxis are reported (60, 62-64). The sensitivity of diagnostic assays at six weeks may be further reduced once antenatal HAART for all HIV-infected women is implemented (26). Studies addressing this concern are essential to ensure reliable and accurate diagnosis of HIV-infected infants in the context of changing PMTCT regimens. In the interim, assay sensitivity may be improved by using plasma samples, as HIV detection tends to be higher in plasma than DBS (145), or by using newer assays such as the Roche CAP/CTM version 2.0 which has improved sensitivity and VL quantification compared to version 1.0 (146, 147). However, these options may be difficult to implement in the field where venesection skills and funding for new, expensive assays are seldom available.

CAP/CTM was the only assay which demonstrated 100% specificity for detecting HIV-uninfected infants at all ages and is the confirmatory assay of choice for infants younger than six weeks of age. The APTIMA assay yielded false positive results at birth and six weeks, likely due to low level contamination from the manual sample preparation, but achieved specificities above the 98% threshold recommended by WHO at all ages (114). HIV DNA PCR was the least specific assay, yielding a lower six-week specificity than previously reported for this technology (70). The false positive results in this study may have been caused by contamination due to the manual nature of this assay, as 71% of the false positive results occurred from testing on a single day.

4.3 OBJECTIVE 3 – PREDICTORS OF IU TRANSMISSION

In the univariate analysis, longer duration of antenatal AZT or HAART decreased the risk of IU-infection, as has previously been reported for perinatal (43, 95, 97) and IU (79, 101) transmission. This emphasises the importance of initiating maternal PMTCT prophylaxis early enough in pregnancy to reduce maternal VL to undetectable levels, thereby decreasing the risk of transmission. The univariate analysis also demonstrated reduced IU transmission with increasing maternal age. These findings again emphasise the need for increased counselling and follow-up of younger mothers with poor antenatal care to curb MTCT.

However, the only independent predictor of IU-infection in this cohort was maternal peripartum VL, concurring with previous findings that VL is the strongest and sometimes only independent predictor of perinatal and IU HIV-infection (53, 88, 92). Since maternal VL also predicts for IP-infections (53, 101-103), no prediction could be made regarding which mother-infant pairs were at increased risk of IU transmission. It would therefore have been unclear which infants to target for birth, as opposed to later, testing. However, since most perinatal infections in the cohort occurred IU, targeting all infants born to mothers with high VL at delivery may have been an option to identify IU-infected infants. No comment can be made regarding the suggestion to target low birth weight infants (<2 500g) for birth testing (personal communication, G. Sherman: National PMTCT Guidelines Working Group), as infant characteristics, including low birth weight, were not investigated in the regression analysis. In addition, this study was not powered to analyse the predictors of IP transmission, having too few IP-infected infants in the cohort.

4.4 OBJECTIVE 4 – MATHEMATICAL MODEL OF EARLY INFANT DIAGNOSIS

If perinatal HIV-infections are to be diagnosed with a single PCR test, mathematical modelling demonstrates that the least effective time to perform the test would be at six weeks of age. Birth testing would diagnose a comparable number of perinatal HIV-infections as six-week testing, but would be less efficient *per PCR* due to the higher number of tests that would be required at birth. The relatively fewer tests required at six weeks illustrates the deaths and losses to follow-up that would have accumulated by this age and therefore represents critical missed opportunities for diagnosing perinatal infections in HIV-exposed infants who are lost from the PMTCT programme. Six weeks is also the age which is most sensitive to the dampening effects of daily-dose NVP, as this regimen is administered until at least six weeks of age (25, 26). Although six-week testing would be the most effective if it is assumed that there is no loss in PCR sensitivity due to daily-dose NVP, this scenario is highly unrealistic considering the increasing evidence of delayed diagnosis with multi-drug or extended prophylaxis (60, 62-64). PCR testing at six weeks of age is therefore the least effective time for EID – it is too late to detect the infants who default or die early in life and is too early to detect IP-infections that may be suppressed by daily-dose NVP prophylaxis.

The model suggests that if a single PCR test is to be performed for EID, testing at ten weeks of age would maximise the number of perinatal HIV-infections diagnosed, thereby minimising infant mortality. However, many of the infants missed at ten weeks would likely die, considering the peak of HIV-related infant mortality at eight to 12 weeks of age (3) and the 20% of untreated infants who die by 13 weeks of age (2). In contrast, IP-infected infants who are missed by birth testing may be detected at a later stage if they

present with symptoms suggestive of HIV-infection that prompt PCR testing. For birth testing to be as effective as a ten-week test, an additional 161 IP-infected infants would need to be detected by later testing. Even though the model did allow for limited testing of symptomatic infants, operational data from the EID cohort demonstrate that 44% of IP-infected infants would be symptomatic by ten weeks of age. It is therefore not unrealistic to assume that almost half the IP-infected infants missed by birth testing could be detected by virtue of clinical symptoms at a later stage. Birth testing in conjunction with testing of symptomatic infants, as is routinely recommended (26, 28), may therefore be as, or more, effective than a single ten-week test. Nevertheless, programmatically it would be more challenging to introduce testing at birth than at ten weeks, as the latter, like six weeks, is an established immunisation visit and clinic staff are therefore trained to perform EID. In contrast, birth testing would require EID to take place in postnatal wards where staff are not aware of EID protocols. The programmatic advantage of performing a single test at birth is the centralisation of PCR testing, which would minimise the need for extensive courier services that collect DBS samples from thousands of immunisation clinics throughout the country.

The most effective way to influence neonatal and infant mortality and effectively monitor for eMTCT is to perform at least two PCR tests early in infancy, with the first test at birth and the second at ten weeks of age. Compared to ten-week testing alone, combined birth and ten-week testing would identify 27% more HIV-infected infants and save almost 50% more life years and QALYs by virtue of detecting IU-infected infants who would otherwise die or be LTFU. South African guidelines already recommend two PCR tests for diagnosing HIV-infection, one at six weeks of age and the second after cessation of breastfeeding (4, 25, 26). If rapid HIV tests are introduced to exclude HIV-infection in

breastfed infants (148, 149), the two currently recommended PCR tests could instead be used at birth and ten weeks to diagnose perinatal HIV-infections. Rapid HIV tests exclude HIV-infection in over 80% of HIV-exposed, uninfected infants from eight months of age (149) and these infants would not require a follow-up PCR test. With only a minority of HIV-exposed infants requiring a PCR test post-cessation of breastfeeding, the cost of performing two PCR tests early in infancy to detect perinatal HIV-infections would be roughly comparable to the cost of the currently recommended testing algorithm.

Compared to the expected number of perinatally HIV-infected infants in the model, a ten-week test would only diagnose 61% of infections and the ideal algorithm with two PCR tests at birth and ten weeks would only diagnose 77%. Previous studies demonstrating that a single PCR test at six weeks of age could diagnose 98.8% of IU- and IP-infected infants (58) were performed under study conditions in a defined cohort of infants with known HIV status. In contrast, the proportion of perinatally infected infants diagnosed in the model was calculated out of the total number of *expected* HIV-infections, taking into account infected infants who would not have accessed a diagnosis. This represents the real-life scenario where not all HIV-exposed infants are available for testing due to LTFU and early deaths, and among those who do present for testing, results may not always be received by caregivers and HIV-infection may not always be detectable due to the dampening effects of NVP prophylaxis. Additionally, not all mothers access adequate PMTCT cover. The model assumed a lower proportion of IU- relative to IP-infections from mothers who received less optimal PMTCT prophylaxis; sub-optimal PMTCT coverage would therefore result in fewer infected infants detectable by a birth test. With a higher proportion of IP-infected infants, LTFU and death prior to testing and reduced test sensitivity due to NVP prophylaxis would be more likely. In view of these factors, 23% of infants with perinatal

HIV-infections would be missed even with the ideal scenario of birth and ten-week testing. In order to reduce the number of infections that would be missed, it is critical to improve the performance of the PMTCT programme. Coverage of PMTCT prophylaxis needs to be improved, losses to follow-up need to be reduced by actively following up HIV-exposed infants for diagnostic testing, infants with positive PCR results need to be actively traced to ensure that test results are received and lifesaving HAART immediately commenced, and healthcare workers need to be vigilant for HIV-exposed infants who test PCR negative but later develop clinical features suggestive of HIV-infection.

Conclusions drawn from the mathematical model are sensitive to the assumptions used in the model. The assumption of 85% and 70% sensitivity at six weeks for detecting NVP-exposed IU- and IP-infected infants, respectively, was based on findings in this cohort of 83% sensitivity at two weeks for detecting IU-infections in the presence of sdNVP. However, this does not take into account the possibility of resistance to daily-dose NVP which may reduce the number of IU-infected infants missed at six weeks by virtue of an increasing VL. Conversely, the 70% sensitivity for detecting IP-infections may be overestimated, as a 71% sensitivity for detecting IP-infected infants at four to six weeks of age has been reported in the context of six weeks of infant AZT monotherapy and no antenatal PMTCT prophylaxis (60). Since South African mothers receive antenatal prophylaxis from early in pregnancy and infants receive six weeks of NVP (25, 26), a more potent VL lowering drug than AZT, sensitivity for detecting IP-infections at six weeks may be reduced beyond 70%. As with all the assumptions used in the model, the PCR sensitivity figures will need to be updated as more data become available. However, the model clearly demonstrates that six weeks is no longer the optimal time to diagnose perinatal HIV-infection in infants. Birth testing, with a later test to detect IP-infections,

would be the ideal diagnostic algorithm, but must be coupled with improved performance of the PMTCT programme.

4.5 STUDY LIMITATIONS

Even though all data analysed in the study were collected prospectively, the Master EID Database and Database of HIV-Infected Infants were collated retrospectively. Though every attempt was made to thoroughly validate and cross-check the data, there were some queries that could not be resolved and the data in question therefore had to be excluded. Some data were also not available for all mother-infant pairs, such as peripartum maternal VL. Furthermore, the EID cohort may not be representative of all HIV-infected women delivering at RMMCH or other routine PMTCT facilities, as this was a single centre, hospital-based study that enrolled women who were aware of their HIV status antenatally. These women are more likely to return for follow-up care than women first diagnosed as HIV-infected at the time of delivery (130), possibly creating a more adherent sample. In addition, no record was kept of how many women were eligible to participate in the study but declined to enrol. Delivery and postnatal care in the EID Study all occurred at RMMCH, in contrast to routine settings where delivery, diagnostic and treatment services often take place at different facilities; coverage of EID testing and linkage to care may therefore be lower in routine services, as referral to different facilities may increase the likelihood of LTFU. Additionally, an HIV status could not be established for 15% of infants enrolled in the study. Some instances of IP-infection may therefore have been missed, as IP-infected infants who defaulted from the study could only be detected if they presented for testing at another facility. In contrast, all IU-infected infants in the cohort were detected, therefore biasing the study to detect IU-infections. It is also possible that IP-

infections include early PP transmissions where mothers elected to breastfeed. In addition, the relatively small sample size of HIV-infected infants, particularly infants with IP-infections, limited conclusions regarding the characteristics and outcomes of mother-infant pairs with IU versus IP transmission. Finally, the unknown outcomes of defaulting infants are a limitation in this study. The impact of birth testing would depend on the outcomes of IU-infected infants who could be diagnosed at birth (for example, whether they accessed care or died). Since a proportion of these infants were LTFU, their outcomes were unknown. Similarly, it is unknown whether HIV-infected infants who defaulted from the study accessed treatment elsewhere and HAART coverage for infants diagnosed at RMMCH may therefore be higher than reported.

4.6 CONCLUSIONS AND RECOMMENDATIONS: INTERVENTIONS TO IMPROVE SOUTH AFRICA'S PMTCT PROGRAMME

Although successes have been achieved in implementing the PMTCT guidelines, as demonstrated by improved antenatal care and the trend to reduced MTCT over the course of the study, missed opportunities for identification and care of mother-infant pairs throughout the PMTCT cascade are widespread. Interventions are needed to improve implementation of the programme if eMTCT is to be achieved and infant mortality is to be reduced. Analysis of RMMCH's PMTCT programme has highlighted specific areas that should be targeted to improve outcomes of South Africa's programme, namely:

- ***Target area 1: Timing of diagnostic testing for HIV-exposed infants***

  Six-week testing is no longer the optimal time to diagnose perinatal HIV-infections. Testing at six weeks is too late to detect HIV-infected infants who default or die early in life and is too early to detect IP-infections that may be dampened by extended, daily-

dose NVP prophylaxis. Birth testing with newer, more sensitive assays in the context of enhanced PMTCT regimens would diagnose more HIV-infected infants than a six-week test and would increase EID coverage. Performing diagnostic testing at birth is also essential to allow for earlier initiation of HAART to reduce infant morbidity and mortality. The ideal algorithm for EID to enable detection of all IU- and IP-infections early enough to influence infant mortality and monitor for eMTCT would include two PCR tests early in infancy, with the first test at birth and the second at ten weeks of age. However, identification of HIV-infected infants can only be maximised with improved performance of the PMTCT programme, including rapid follow-up of HIV-exposed infants who develop clinical features suggestive of HIV-infection.

- ***Target area 2: Coverage of PCR testing and diagnosis of HIV-infected infants, with a focus on at-risk mother-infant pairs***

  Coverage of EID needs to be increased in order to improve identification of HIV-infected infants. Targeted interventions to improve EID coverage include:

a. Improved counselling and education of HIV-infected women to emphasise the importance of EID and address concerns surrounding infant testing. Young, foreign women who first present at antenatal clinics late in pregnancy and display poor compliance with their antenatal care need additional counselling, as these women are less likely to bring their infants back for diagnostic testing and are more likely to transmit HIV to their infants. Targeting socioeconomically disadvantaged mothers for additional counselling and follow-up may also improve infant outcomes.
b. Education of healthcare workers to actively seek out HIV-exposed infants who require PCR testing among infants who present for routine healthcare and immunisation visits.

- ***Target area 3: Timely initiation of antenatal PMTCT prophylaxis***

  Women present for antenatal care too late in pregnancy and therefore receive suboptimal durations of antenatal PMTCT cover, increasing the risk of MTCT. Key interventions to ensure that women present for antenatal care earlier in pregnancy include:

a. Improved social mobilisation to educate women of childbearing age about the PMTCT guidelines and the importance of antenatal care for maternal and child health.
b. Training and education of healthcare workers who come into contact with women in all areas of the healthcare system to raise awareness of the PMTCT guidelines and improve linkage of pregnant women to antenatal services.
c. Capacity building to ensure that the necessary resources are available, including increased availability of AZT and/or HAART for antenatal prophylaxis.

As the HIV epidemic evolves, so do the PMTCT guidelines. On-going monitoring and evaluation of the evolving PMTCT programme is critical in order to achieve eMTCT and a two-thirds reduction in childhood mortality by 2015. Particular attention needs to be drawn to the key entry points into the antenatal and postnatal PMTCT cascades, including presentation of pregnant women at antenatal clinics and identification of HIV-exposed infants for diagnostic testing. A pro-active approach toward implementing changes recommended by routine monitoring is essential in order to enhance outcomes of South Africa's PMTCT programme.

First publication arising from this study (129).

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Early Diagnosis of *In Utero* and Intrapartum HIV Infection in Infants Prior to 6 Weeks of Age

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Early initiation of antiretroviral therapy reduces HIV-related infant mortality. The early peak of pediatric HIV-related deaths in South Africa occurs at 3 months of age, coinciding with the earliest age at which treatment is initiated following PCR testing at 6 weeks of age. Earlier diagnosis is necessary to reduce infant mortality. The performances of the Amplicor DNA PCR, COBAS AmpliPrep/COBAS TaqMan (CAP/CTM), and Aptima assays for detecting early HIV infection (acquired *in utero* and intrapartum) up to 6 weeks of age were compared. Dried blood spots (DBS) were collected at birth and at 2, 4, and 6 weeks from HIV-exposed infants enrolled in an observational cohort study in Johannesburg, South Africa. HIV status was determined at 6 weeks by DNA PCR on whole blood. Serial DBS samples from all HIV-infected infants and two HIV-uninfected, age-matched controls were tested with the 3 assays. Of 710 infants of known HIV status, 38 (5.4%) had *in utero* ($n = 29$) or intrapartum ($n = 9$) infections. By 14 weeks, when treatment should have been initiated, 13 (45%) *in utero*-infected and 2 (22%) intrapartum-infected infants had died or were lost to follow-up. The CAP/CTM and Aptima assays identified 76.3% of all infants with early HIV infections at birth and by 4 weeks were 96% sensitive. DNA PCR demonstrated lower sensitivities at birth and 4 weeks of 68.4% and 87.5%, respectively. All assays had the lowest sensitivity at 2 weeks of age. CAP/CTM was the only assay with 100% specificity at all ages. Testing at birth versus 6 weeks of age identifies a higher total number of HIV-infected infants, irrespective of the assay.

Human immunodeficiency virus (HIV) infection is a significant cause of pediatric morbidity and mortality in resource-limited settings, where the majority of HIV infection occurs. The CHER study demonstrated that early initiation of highly active antiretroviral therapy (HAART) at a median age of 7 weeks reduces infant mortality and HIV progression by 76% and 75%, respectively (21). However, early initiation of treatment is dependent on an early diagnosis of HIV infection. The identification of HIV-exposed infants for testing and the diagnosis of pediatric infection remain challenges in resource-limited settings. Strategies to implement accurate and timely diagnostic testing are critical to improve infant outcomes.

Prevention of mother-to-child transmission (PMTCT) guidelines in South Africa stipulate that diagnostic testing in infants be performed at 6 weeks of age because this coincides with the 6-week immunization visit and detects virtually all HIV infections acquired during pregnancy (*in utero* [IU]) or during labor and delivery (intrapartum [IP]) (11, 15). Testing is performed with a viral detection assay since HIV antibody detection assays cannot distinguish transplacentally acquired maternal from infant HIV antibodies. Test results are communicated to the infants' caregivers at the 10-week immunization visit. If an infant is HIV infected, additional time is required to prepare the primary caregiver to initiate HAART (11). Initiation of treatment therefore generally occurs at 12 to 14 weeks of age, too late to impact the early peak of HIV-related deaths in South Africa, centered at 8 to 12 weeks of age (1). Diagnostic testing prior to 6 weeks is thus required to initiate HAART earlier, thereby reducing infant mortality.

One of the most widely used assays for early infant diagnosis is the Amplicor HIV DNA PCR assay (Roche Molecular Systems Inc., Branchburg, NJ). Version 1 of this assay was assessed on dried blood spot (DBS) specimens from infants less than 4 days and 10 to 15 days of age (3). The sensitivities in detecting all IU and IP infections were found to be 27.3% and 88.9%, respectively, and the specificities at both ages were 100% (3). This study was subsequently included in a review by Dunn et al. in 1995, together with other studies which used in-house PCR assays on whole blood (5). The overall sensitivities of DNA PCR were found to be 38% at birth, 93% at 14 days, and 96% at 28 days (5). Subsequent studies exclusively assessing the Roche Amplicor assay on whole blood yielded birth sensitivities ranging from 38% to 62% (7, 9, 24) and sensitivities by 1 week, 2 weeks, and 1 month of age of 29%, 67%, and 67 to 92%, respectively (2, 7, 9). The assay was 100% specific using whole blood at birth (excluding cord blood) and at 1 week, 2 weeks, and 1 month of age (2, 7, 24). Antenatal zidovudine (AZT) was provided to selected women in some of these studies (7, 24). The current Roche Amplicor assay, version 1.5, was not assessed on whole blood prior to 6 weeks but has been assessed on DBS samples at 6 weeks of age, yielding a sensitivity and specificity of 100% and 99.6%, respectively (17).

Newer viral detection assays may prove more sensitive for earlier detection of HIV infection in infants. Two such technologies

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have been assessed using DBS samples from pediatric populations, namely, the Roche COBAS AmpliPrep/COBAS TaqMan (CAP/CTM) HIV-1 assay, which detects both HIV-1 RNA and DNA, and the Gen-Probe Aptima HIV-1 screening assay with an automated Tigris analyzer (18, 19). These assays were highly sensitive (99.7% and 100%, respectively) and specific (100% and 97%, respectively) at 6 weeks of age and older (18, 19). The manual version of the Gen-Probe Aptima assay using DBS samples was also sensitive and specific (99.2% and 100%, respectively) for HIV-exposed infants and children (6). Using serum samples, Aptima's specificity was 100% for HIV-exposed, uninfected infants younger than 18 months old (13). However, neither the CAP/CTM nor the Aptima assay has been evaluated for HIV diagnosis in infants before 6 weeks of age.

This study aimed to compare the performances of the Amplicor DNA PCR, CAP/CTM, and Aptima assays using DBS samples to identify IU- and IP-infected infants up to 6 weeks of age.

MATERIALS AND METHODS

A prospective, observational cohort study was conducted at Rahima Moosa Mother and Child Hospital (RMMCH) in Johannesburg, South Africa, from August 2008 to December 2010. HIV-infected women over 18 years of age, admitted to the RMMCH postnatal ward, who were identified during antenatal testing and who planned to attend RMMCH for follow-up PMTCT care were eligible to participate in the study. The Ethics Committee of the University of the Witwatersrand approved the study, and written informed consent was obtained from all mothers prior to enrollment. Mother-infant pairs enrolled in the study continued to receive the routine PMTCT care offered at RMMCH according to national PMTCT guidelines at the time. Initially, single-dose nevirapine (sdNVP) together with maternal AZT from 28 weeks' gestation and infant AZT for 1 week was the recommended PMTCT regimen. If maternal PMTCT care was suboptimal, defined as less than 4 weeks of AZT, the infant received 28 days of AZT. Women with CD4 counts of ≤200 were eligible for HAART. The PMTCT guidelines were updated during 2010 to commence maternal AZT from 14 weeks' gestation and provide HIV-exposed infants with a daily dose of NVP for 6 weeks or for the duration of breast-feeding. Women with CD4 counts of ≤350 were eligible for HAART. At the time of the study, most women at RMMCH elected to exclusively formula feed.

Infants were enrolled at birth in the postnatal ward and seen at 2, 4, 6, and 10 weeks of age. A medical practitioner examined the infant at each visit. The 6- and 10-week visits coincided with routine PMTCT care visits. Infants underwent DNA PCR testing (Amplicor HIV-1 DNA PCR, version 1.5; Roche Molecular Systems Inc., Branchburg, NJ) on peripheral blood at 6 weeks, and test results were provided to the infants' caregivers at the 10-week visit (11). Where the PCR result was negative, the mother was counseled regarding the risk of postnatal transmission of HIV and the infant discharged. Where the PCR result was positive, a confirmatory baseline viral load (VL) test and a CD4 count were performed and the infant was referred for HAART initiation.

For study purposes, DBS samples were obtained from the infants at each visit. At birth and 2 weeks, blood was collected by heel prick onto Schleicher & Schuell 903 (S&S 903 W-041) filter paper cards, and at the 4- and 6-week visits, blood was collected by venesection and spotted onto filter paper cards. Each DBS card was stored at RMMCH at ambient room temperature in a ziplock plastic bag with a desiccant sachet.

Where an infant was identified as being HIV infected by the routine 6-week PCR test, stored DBS samples from birth and 2, 4, and 6 weeks were retrieved and retrospectively tested together with samples from two age-matched, HIV-exposed, uninfected controls at the same time points. Laboratory testing was conducted by accredited hospital-based laboratories using 3 assays: HIV DNA PCR, CAP/CTM HIV-1 (version 1; Roche Molecular Systems, Inc., Branchburg, NJ), and the Aptima HIV-1 screening assay with an automated Tigris analyzer (Gen-Probe Inc., San Diego, CA). Samples were retested where discrepant or equivocal results were obtained at a single time point and additional DBS sample was available; however, only the initial test results were included in the analysis. Infants with at least 2 positive DBS test results on different assays at birth were considered to have an IU infection. IP infection was considered to have occurred where the birth results were negative, followed by at least 2 positive test results on different assays by 6 weeks of age. Postpartum (PP) transmission was documented when the birth and 6-week test results were negative, followed by a positive PCR result in the context of breast-feeding. The possibility that infants infected by early PP transmission are counted in the IP-infected group cannot be excluded.

Where an infant was lost to follow-up before undergoing routine 6-week testing at RMMCH, the National Health Laboratory Service's (NHLS) Laboratory Information System (LIS) was searched to determine whether the infant had accessed PCR testing at another facility. If the surname and date of birth matched, the PCR test result from the LIS was used to assign an HIV infection status at 6 weeks of age. At the end of the study, all birth DBS samples of infants with an unknown HIV status were tested using the Aptima assay to identify all remaining IU-infected infants in the cohort. All positive Aptima tests were confirmed with a CAP/CTM and HIV DNA PCR test. Additionally, all stored DBS samples available for enrolled infants known to have died were submitted for testing.

Statistical analyses were performed using Microsoft Excel 2010. Sensitivity [true-positive results/(true-positive + false-negative results)] and specificity [true-negative results/(true-negative + false-positive results)] were calculated with 95% confidence intervals by following the Wilson score method. Positive predictive values [true-positive results/(true-positive + false-positive results)] and negative predictive values [true-negative results/(true-negative + false-negative results)] were calculated to determine the proportion of infants with positive and negative results, respectively, who were correctly diagnosed.

RESULTS

A convenience sample of 848 mother-infant pairs was enrolled. Ten withdrew during the course of the study. Of the remaining 838 HIV-exposed infants, 128 (15.3%) were excluded from all analyses because they were lost to follow-up or died before an HIV status could be established. Of the remaining 710 mother-infant pairs, 557 (78.5%) received optimal maternal PMTCT care, defined as 4 weeks or more of AZT or HAART. sdNVP with various durations of AZT was administered to 653 (92.0%) infants, 56 (7.9%) infants received daily-dose NVP, and a single infant did not receive any PMTCT prophylaxis. Antenatal CD4 counts were available for 638 women (median count of 354.5×10^6/liter; range, 24×10^6/liter to $1{,}221 \times 10^6$/liter). A CD4 count of $\leq 350 \times 10^6$/liter was recorded for 316 (49.5%) women, accounting for 60.6% of vertical transmissions.

The HIV status of 606 (85%) and 85 (12%) of the 710 infants was determined by the 6-week PCR performed at RMMCH and searching the NHLS LIS, respectively. The remaining 19 (3%) infants had their HIV status established from testing stored DBS samples. Of the 710 infants with a known HIV status, 38 (5.4%) were determined to have been infected either IU ($n = 29$) or IP ($n = 9$) (Table 1). IU transmission accounted for 76.3% of infections detected in the first 6 weeks of life. Positive test results were first documented at the 4- or 6-week visit for 8 of the 9 IP-infected infants. For the remaining infant, who was reportedly exclusively formula fed, the first positive test was at 10.7 weeks of age, when the infant was admitted to RMMCH having defaulted after the birth visit. Daily-dose NVP had been administered to 2 (6.9%) IU- and no IP-infected infants. An additional 5 infants from the study cohort were diagnosed as infected by the routine HIV service but are not included in the analysis. Two of these infants who were

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TABLE 1 Description of study cohort and total number of samples from HIV-uninfected and *in utero*- and intrapartum-infected infants tested with each diagnostic assay

Time of visit	Study cohort			No. of samples tested with each diagnostic assay[b]		
	Median age (wk)	Total no. of infants	No. (%) HIV infected[a]	HIV DNA PCR	CAP/CTM	Aptima
Birth	0.1	710	38 (5.4)	107	107	144[c]
2 wk	2.1	566	25 (4.4)	86	86	86
4 wk	4.6	556	25 (4.5)	88	88	88
6 wk	6.6	595	26 (4.4)[d]	85	84[e]	85
Total		710	38 (5.4)	366	365	403

[a] *In utero* and intrapartum infections. HIV infection status was determined retrospectively where the routine 6-week PCR test identified an infant as HIV infected.
[b] Samples were obtained from infants later determined to be HIV infected, together with two age-matched uninfected controls, as well as from infants who died; birth samples were also obtained from infants of an unknown status.
[c] The screen of birth samples from infants with an unknown HIV status using the Aptima assay accounts for the additional Aptima tests.
[d] Samples were not prepared for 2 HIV-infected infants who attended the 6-week visit.
[e] One 6-week sample produced an invalid CAP/CTM result and could not be retested.

reportedly breast-fed demonstrated PP transmission, as their 6-week PCR tests were negative but repeat PCR tests at 9.4 and 15.6 months of age were positive. Three other infants tested negative at birth, defaulted at 2 weeks of age, and had their first positive test at 6 months or older. Since it was unclear whether HIV transmission had occurred IP or PP, they were excluded from the cohort of IP-infected infants. Two had reportedly been breast-fed, and 1 had reportedly received mixed feeding.

Only 26 (68%) of the 38 IU- and IP-infected infants presented for routine DNA PCR testing at 6 weeks as part of the study (Table 1). Of these 26 infants, 18 were IU infected. Of the 12 infants who did not attend the 6-week visit, 11 (92%) were IU infected. By 14 weeks of age, 23 (61%) of the 38 IU- and IP-infected infants were in care to commence treatment. Of these 23 infants, 16 were IU infected. Of the 29 IU- and 9 IP-infected infants identified, 13 (45%) IU- and 2 (22%) IP-infected infants had died or were lost to follow-up before HAART could be commenced.

The sensitivities of the HIV DNA PCR, CAP/CTM, and Aptima assays in identifying IU- and IP-infected infants are depicted in Table 2. Equivocal test results were obtained only with the HIV DNA PCR assay and were omitted from this analysis. At birth, the CAP/CTM and Aptima assays identified 76.3% of all infants with early infections detectable by 6 weeks of age. The DNA PCR assay demonstrated a lower sensitivity (68.4%), missing 3 IU-infected infants. At 2 weeks, all the assays showed reduced sensitivity. Four HIV-infected infants had positive tests at birth that became negative at 2 weeks of age and became positive again at 4 weeks of age. All 4 infants had received sdNVP at birth. Two infants tested negative at 2 weeks on a single assay (*viz.*, one each on the Aptima and CAP/CTM assays), and 2 infants tested negative on 2 assays (CAP/CTM and Aptima assays in one instance and DNA PCR and CAP/CTM assays in the second). By 4 weeks, the CAP/CTM and Aptima assays demonstrated 96.0% sensitivity, missing 1 IP-infected infant who was only identifiable by any assay from 6 weeks of age. This infant could have undergone an early PP transmission but was reportedly exclusively formula fed. Furthermore, the infant received sdNVP prophylaxis, so the delay in diagnosis could not be attributed to viral suppression as a result of daily-dose NVP. At 6 weeks, all the assays demonstrated 100% sensitivity; however, the diagnosis of 2 HIV-infected infants would have been delayed with the DNA PCR assay due to equivocal results that would have required retesting.

CAP/CTM was the only assay to demonstrate 100% specificity for identifying HIV-uninfected infants of all ages (Table 2). The Aptima assay yielded 3 false-positive results that were all negative upon repeat testing. DNA PCR demonstrated the poorest specificity at all ages except birth, with 5 of the 7 false-positive results occurring on a single day. All false-positive and equivocal DNA PCR results that were retested yielded true-negative results.

DISCUSSION

The Amplicor HIV DNA PCR, CAP/CTM, and Aptima assays were assessed in infants born to a population of women predominantly infected by subtype C, with 50% having antenatal CD4 counts over 350×10^6/liter and 79% having received at least 4 weeks of antenatal PMTCT prophylaxis. Over 90% of infants in this cohort received sdNVP at birth. In this setting, 29 (76%) of the 38 HIV-infected infants diagnosed at 6 weeks of age were identifiable at birth.

The CAP/CTM and Aptima assays were more sensitive and specific than DNA PCR for identifying IU- and IP-infected and HIV-uninfected infants at any age up to 6 weeks using DBS samples. The World Health Organization (WHO) recommends testing of all HIV-exposed infants at 4 to 6 weeks of age and requires that viral detection assays have a minimum sensitivity of 95% and a specificity of 98% (23). At 4 weeks of age, the CAP/CTM and Aptima assays were 96% sensitive and 100% specific, fulfilling WHO requirements, yet failed to identify one HIV-infected infant. The sensitivity of the DNA PCR assay complied with the WHO standards only at 6 weeks of age. There were a number of false-positive DNA PCR results at all ages besides birth, yielding specificities below the WHO-recommended threshold of 98% (23). This is likely a result of contamination, which can be a consequence of the manual nature of the DNA PCR assay. However, these results are not necessarily representative, since higher specificities have previously been reported (17). The Aptima test also demonstrated false-positive results, most likely due to low-level contamination which may have occurred during the initial sample preparation, which is performed manually. CAP/CTM was the only assay with a specificity of 100% at all ages and would be the best confirmatory assay for infants under 6 weeks of age.

At birth, the CAP/CTM and Aptima assays identified 76.3% of all HIV-infected infants detectable at 6 weeks. This is higher than previous descriptions of 27.3% to 62% using earlier viral detection

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TABLE 2 Performance of the HIV DNA PCR, CAP/CTM, and Aptima assays in identifying *in utero*- and intrapartum-infected and HIV-uninfected infants in the first 6 weeks of life[a]

Infection status and time of visit	HIV DNA PCR				CAP/CTM				Aptima			
	Total no. of infants tested[b]	No. with true-positive result	% Sensitivity (95% CI)	PPV (%)	Total no. of infants tested	No. with true-positive result	% Sensitivity (95% CI)	PPV (%)	Total no. of infants tested	No. with true-positive result	% Sensitivity (95% CI)	PPV (%)
Infected												
Birth	38	26	68.4 (52.5–80.9)	100	38	29	76.3 (60.8–87.0)	100	38	29	76.3 (60.8–87.0)	93.5
2 wk	25[1]	16	64.0 (44.5–79.8)	84.2	25[3]	15	60.0 (40.7–76.6)	100	25[3]	16	64.0 (44.5–79.8)	100
4 wk	24	21	87.5 (69.0–95.7)	91.3	25	24[c]	96.0 (80.5–99.3)	100	25	24[c]	96.0 (80.5–99.3)	100
6 wk	22	22	100 (85.1–100)	91.7	24	24	100 (86.2–100)	100	24	24	100 (86.2–100)	96.0
Uninfected												
	Total no. of infants tested[d]	No. with true-negative result	% Specificity (95% CI)	NPV (%)	Total no. of infants tested	No. with true-negative result	% Specificity (95% CI)	NPV (%)	Total no. of infants tested	No. with true-negative result	% Specificity (95% CI)	NPV (%)
Birth	67	67	100 (94.6–100)	84.8	69	69	100 (94.7–100)	88.5	106	104	98.1 (93.4–99.5)	92.0
2 wk	60	57	95.0 (86.3–98.3)	86.4	61	61	100 (94.1–100)	85.9	61	61	100 (94.1–100)	87.1
4 wk	62	60	96.8 (89.0–99.1)	95.2	63	63	100 (94.3–100)	98.4	63	63	100 (94.3–100)	98.4
6 wk	61	59	96.7 (88.8–99.1)	100	60	60	100 (94.0–100)	100	61	60	98.4 (91.3–99.7)	100

[a] Superscripts denote numbers of HIV-infected infants with positive tests at birth but negative 2-week results. CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.
[b] Excluding 3 equivocal tests (n = 1 at 4 weeks and n = 2 at 6 weeks).
[c] Same IP-infected infant missed by CAP/CTM and Aptima assays at 4 weeks.
[d] Excluding 4 equivocal tests (n = 2 at birth, n = 1 at 2 weeks, and n = 1 at 4 weeks).

assays in populations receiving little, if any, PMTCT prophylaxis (3, 5, 7, 9, 24). Possible explanations include a reduction in IP transmission due to enhanced PMTCT prophylaxis, resulting in a proportionate increase of IU-infected infants in the first 6 weeks of life, and/or improved sensitivity of newer viral detection assays. Version 1.5 of the DNA PCR assay using DBS samples achieved a better sensitivity at birth than earlier versions of the assay (3) but is not as sensitive as CAP/CTM or Aptima at this age.

The benefits of a birth diagnosis need to be considered in the context of the high rate of loss to follow-up in routine settings where infants do not return for 6-week testing or test results (16). At RMMCH, the current 6-week testing program identified 26 HIV-infected infants, whereas testing at birth would have identified 29 HIV-infected infants without the need for an additional health care visit or risking the demise of infants prior to testing. Of the 12 (32%) HIV-infected infants who did not return for testing at 6 weeks, 11 (92%) infants could have been identified through birth testing. Whether IU-infected infants are at higher risk of rapid disease progression than IP-infected infants remains controversial (8, 12), but if true, then early identification and HAART initiation in this group of infants would be especially important in reducing infant mortality. The disadvantage of birth testing is the need for a second test for early identification of IP-infected infants, substantially increasing the costs of early infant diagnosis programs. Considering the 2010 WHO infant feeding policy of exclusive breast-feeding (22), which requires additional testing 6 weeks after weaning, IP-infected infants would be identified later in life but delayed testing will put them at increased risk of morbidity and mortality.

A limitation of this study is that the findings are skewed in favor of identifying IU- compared with IP-infected infants. *All* IU-infected infants in the cohort were identified, since birth DBS samples were available for testing. In contrast, the LIS lookup could identify only IP-infected infants who accessed diagnostic testing after defaulting from the study, and the true number of IP-infected infants is therefore unknown. However, this mimics the reality in routine settings, where IP-infected infants who default by 6 weeks do not access an early diagnosis, whereas IU-infected infants who are in the health care system at the time of birth represent missed opportunities for early identification. This assumes that the majority of births occur in health care facilities, as is the case in South Africa (20).

The lowest sensitivity in all 3 assays occurred at 2 weeks of age. We speculate that this is a consequence of the sdNVP prophylaxis administered to the infants at birth. Daily NVP, as recommended by current South African PMTCT guidelines (10), was administered to 2 IU-infected infants but not to any of the IP-infected infants in this cohort. Delayed diagnosis as a result of this regimen was not demonstrated, but the study was not designed to assess this possibility. However, the possibility that daily NVP may suppress viral replication and delay the diagnosis of IP infections beyond 6 weeks of age cannot be excluded and requires further investigation. The 2-week results may be improved with the newer version of the quantitative CAP/CTM assay (Roche CAP/CTM, version 2), which has improved sensitivity and viral load quantification compared to version 1 (4, 14). This remains to be assessed in future studies.

In conclusion, the CAP/CTM and Aptima technologies demonstrated superior performance to the HIV DNA PCR assay at all ages, detecting 96% of all early HIV infections by 4 weeks and 76%

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at birth. Infant diagnosis at 4 weeks is an option but will lead to earlier treatment only if turnaround times improve to guarantee the availability of test results by 6 weeks. Testing at 4 weeks, however, may not be viable in the context of daily-dose NVP that could potentially delay the diagnosis of IP infections; this requires further study. Alternatively, birth testing can be conducted to identify IU-infected infants who are in the health care system at the time of birth, with IP infections being detected by a later test. Birth testing would ensure that more HIV-infected infants access testing and would enable treatment to be initiated early enough to improve outcomes and reduce infant mortality.

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My colleagues and I recently published an article in JCM, detailed below, of which I am first author. I would like to please request written permission to reproduce this work in my MSc (Med) dissertation entitled "Identifying Interventions to Improve Outcomes of the South African Prevention of Mother-to-Child Transmission Programme" to be submitted to the University of the Witwatersrand for examination.

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Many thanks.

Kind regards,
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Birth Diagnosis of HIV Infection on Infants to Reduce Infant Mortality and Monitor for Elimination of Mother-to-Child Transmission

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ABSTRACT

Background: Early initiation of antiretroviral therapy (ART) depends on an early infant diagnosis (EID) and is critical to reduce HIV-related infant mortality. We describe the implementation of a routine Prevention of Mother-to-Child Transmission (PMTCT) program and focus on EID to identify opportunities to improve outcomes.

Methods: HIV-exposed infants and their mothers were enrolled in a prospective, observational cohort study at a routine, hospital-based PMTCT and HIV treatment service in Johannesburg, South Africa. Infant HIV status was determined by testing samples collected between birth and 6 weeks and searching the national laboratory information system for PCR results of defaulting infants who accessed testing elsewhere.

Results: Of 838 enrolled infants, HIV status was determined for 606 (72.3%) by testing at the study site, 85 (10.1%) by accessing test results from other facilities, 19 (2.3%) by testing stored samples and remained unknown in 128 (15.3%) infants. In total, 38 perinatally HIV-infected infants were identified. Thirty (79%) HIV-infected infants accessed 6-week testing and initiated ART at a median age of 16.0 weeks, but only 14 were in care a median of 68 weeks later and 4 had died. Eight (21%) HIV-infected infants, 2 of whom died, escaped identification by routine testing. Their mothers were younger, more likely to be foreign and accessed less optimal antenatal care.

Conclusions: Six-week testing delayed ART initiation beyond the time of early HIV-related infant mortality and missed one-fifth of perinatally HIV-infected infants. Earlier diagnosis and improved retention in care are required to reduce infant mortality and accurately measure elimination of MTCT.

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The Human Immunodeficiency Virus (HIV) epidemic in South Africa contributes significantly to pediatric morbidity and mortality. Infants who are infected with HIV via mother-to-child transmission (MTCT) are at significantly increased risk of mortality,[1] with 20% of untreated perinatally infected infants dying by 3 months of life and 48% dying by 1 year.[2] HIV-related infant mortality peaks at 2 to 3 months of age[3] and can only be curbed with effective prevention of MTCT (PMTCT) programs that limit vertical transmission and provide early treatment for infants who are infected with HIV.

Globally, only 28% of eligible children receive antiretroviral therapy (ART), half the treatment coverage achieved in adults (54%),[4] with similar trends evident in South Africa.[5] Initiation of ART is critically dependent on an accurate and timely diagnosis of HIV-infection. According to South African guidelines, HIV-exposed infants should undergo diagnostic testing at 6 weeks of age using an HIV DNA polymerase chain reaction (PCR) test.[6,7] The rationale behind this recommendation is that a single PCR test at 6 weeks of age is able to detect virtually all *in utero* (IU) and intrapartum (IP) infections,[8] maximizing the number of infected infants that could be identified by a complex and costly test in a low resource setting.[9] Six weeks also coincides with an established immunization visit and was therefore practical from a programmatic perspective. At the time, single dose nevirapine (sdNVP) was the only available prophylaxis, exclusive formula feeding was recommended and ART was initiated only once immunological deterioration occurred.

However, since the recommendation for 6-week diagnostic testing was made, several critical factors have changed. The CHER Study demonstrated the importance of early

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ART initiation at a median age of 7 weeks to reduce infant morbidity and mortality.[10] A peak in HIV-related infant mortality at 2 to 3 months of age was recognized,[3] further emphasizing the need for early ART initiation to reduce infant deaths. PMTCT guidelines were expanded to incorporate zidovudine (AZT) as well as NVP,[6,7] providing prophylactic cover at time points associated with high rates of vertical transmission (viz. during late pregnancy and the intrapartum period), thus proportionately reducing IP infections. Initial estimates of early vertical or IU infections detectable at birth of 38%[11] are therefore no longer relevant. With more extensive PMTCT prophylaxis and the availability of more sensitive assays, the proportion of IU relative to IP infections has increased to ±75%[12,13] and more perinatally infected infants are detectable at birth than at 6 weeks of age.[12] Furthermore, the policy of exclusive breastfeeding for HIV-exposed infants[14,15] requires a second PCR test in addition to the 6-week test to exclude postnatal infections following weaning.[6,7] Despite considerable evolution of the early infant diagnosis (EID) environment, EID algorithms have remained unchanged.

EID is not only essential to identify HIV-infected infants for timely initiation of ART to reduce infant mortality, but is also required for monitoring the PMTCT program as we aspire to elimination of MTCT (eMTCT) by 2015.[16] EID services need to be re-assessed to optimize infant outcomes and accurately monitor PMTCT program performance. This study describes the implementation of a routine PMTCT program at an academic hospital in Johannesburg, South Africa to identify opportunities for improved outcomes in HIV-exposed infants, particularly with respect to EID services.

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MATERIALS AND METHODS

A prospective, observational cohort study was conducted at Rahima Moosa Mother and Child Hospital (RMMCH) in Johannesburg, South Africa. Women over 18 years of age, who were aware of their HIV positive status antenatally and planned to attend the hospital for postnatal PMTCT care were eligible to participate. Mother-infant pairs were recruited from August 2008 to July 2010 and follow-up continued until December 2010. The University of the Witwatersrand's Ethics Committee approved the study and written informed consent was obtained from mothers prior to enrolment.

All mother-infant pairs enrolled in the study received the routine PMTCT care available at RMMCH as per national guidelines at the time.[6] Women with CD4 counts below 200 initiated ART; those with higher CD4 counts received AZT from 28 weeks gestation and sdNVP at the time of delivery.[6] Infants received sdNVP at birth and 7 days of AZT; 28 days was administered if a mother had received less than 4 weeks of antenatal AZT.[6] Exclusive formula feeding was supported at the time. The 2010 PMTCT guidelines, released toward the end of the study, stipulated ART eligibility for HIV-infected women with CD4 counts below 350, the administration of AZT from 14 weeks gestation and provision of daily dose NVP to infants for 6 weeks;[7] exclusive breastfeeding was recommended.[14] All HIV-exposed infants were to undergo HIV DNA PCR testing on peripheral blood at 6 weeks of age (Amplicor HIV-1 DNA PCR, version 1.5; Roche Molecular Systems Inc, Branchburg, NJ).[6,7] Results of the routine 6-week PCR test defined the infants' HIV status and were communicated to caregivers at the 10-week immunization visit.[17] PCR negative infants were discharged from the program. PCR positive infants had a confirmatory viral load and baseline CD4 test performed and were referred to the treatment clinic to

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prepare for ART initiation.[6,17] The treatment and diagnostic services at RMMCH operate from the same facility but run on different days. Viral suppression was achieved when an infant's viral load was less than 400 copies/ml.[17]

As part of the study, infants were seen at birth in the postnatal ward and at 2-, 4-, 6- and 10-weeks of age. A medical practitioner examined the infants at each visit and collected and stored dried blood spot (DBS) samples. These samples were tested using the standard of care HIV DNA PCR and 2 newer assays (CAP/CTM HIV-1 version 1, Roche Molecular Systems, Inc., Branchburg, NJ and Aptima HIV-1 screening assay, Gen-Probe Inc., San Diego, CA) to determine whether vertically infected infants could be identified prior to 6 weeks of age.[12] Infants were classified as IU-infected where birth samples tested positive using 2 different assays. Intra-partum-infected infants had negative results at birth but tested positive on 2 different assays by 6 weeks of age. HIV-infected infants accessing care at RMMCH were followed up until the end of the study. Infants were considered to have remained in care if they attended RMMCH in the last 9 months of the study.

Where an infant was lost to follow-up (LTFU) from RMMCH before routine 6-week testing could be performed, the National Health Laboratory Service's Laboratory Information System (LIS) was searched to determine whether the infant had accessed PCR testing at another health facility. Where the surname and date of birth matched, the PCR result on the LIS was used to assign an HIV status for the infant. At the end of the study, all birth DBS samples from infants with an unknown HIV status were tested to identify remaining IU-infected infants in the cohort. All available 4-week study samples were also tested.

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Statistical analyses were performed with Microsoft Excel 2010, Statistica 10 (StatSoft Inc., Tulsa, OK) and STATA 12.0 (StataCorp LP, College Station, TX). A Chi^2 test was used to detect differences between categorical variables. Mann Whitney U test or Kruskal Wallis ANOVA were used to compare 2 or more continuous variables, respectively. $p<0.05$ was considered significant. Where multiple comparisons between groups were performed, a Bonferroni correction was applied to adjust the significance level.

RESULTS

Over a 2-year period, a convenience sample of 848 mother-infant pairs was enrolled (Figure 1). Ten withdrew during the course of the study. Since the remaining 838 infants included 9 sets of twins, a total of 829 HIV-infected women were enrolled. The nationality of 804 of these women was known and 43% were foreign, 86% of whom were from Zimbabwe. The infants in this cohort have been previously described.[12] Briefly, 606 (72%) of the 838 enrolled infants accessed diagnostic testing at RMMCH and 85 (10%) underwent testing at other facilities (Figure 1). An HIV status was established for an additional 19 infants who defaulted from the study by HIV testing of DBS samples collected for study purposes. An HIV status was therefore available for 85% (710) of enrolled infants. IU-infection was excluded in the remaining 128 defaulting infants by virtue of negative birth DBS results, but no samples were available to determine their HIV status at 6-weeks. In total, 38 HIV-infected infants were identified in the cohort, comprising 29 (76%) infants who tested positive at birth (IU infections) and 9 who were negative at birth but positive by 6 weeks (IP infections) (Figure 1). An additional 5 HIV-infected infants were later

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identified. Two infants who were HIV-uninfected at 6 weeks of age later tested PCR positive in the context of breastfeeding (postnatal transmission). An additional 3 infants tested negative at birth, defaulted from the study at 2 weeks of age and had positive PCR tests at 6 months or older. Since it is unclear whether these infants were infected intra-partum or later as a result of breastfeeding, they are excluded from the cohort of IP-infected infants.

All but 1 of the 838 enrolled infants (99.9%) received NVP at birth (Figure 2). Majority (92%) of these infants received sdNVP according to the 2008 guidelines. Exclusive breastfeeding, only recommended toward the end of the study,[14] was reported in 89 (11%) infants in the cohort. The 2 infants with postnatal transmissions received sdNVP. Data regarding cotrimoxazole administration were not collected. Of the 606 infants who accessed routine diagnostic testing at RMMCH, 565 (93%) returned to receive their results and 26 (4.3%) were HIV infected (Figure 2). Of the infected infants identified at RMMCH, 25 (96%) received PCR results and 23 (88%) initiated ART. Two of the 3 infants who did not initiate treatment were LTFU and one moved to his rural home before commencing ART. The 23 infants who initiated ART were followed for a median of 51.6 weeks (range 0.0–112.1 weeks). Ten (43%) of these infants were not in care at the end of the study because 7 infants, 2 of whom had achieved viral suppression on treatment, were LTFU and 3 infants died shortly after initiating ART. Of the 13 infants in care, 12 were virologically suppressed and 1 was in the process of achieving suppression. Retention in care of HIV-infected infants was reasonable until the first clinic visit for care, but decreased dramatically between ART initiation (88%) and achieving viral suppression. In addition to the 26 HIV-infected infants who were diagnosed at RMMCH, 4 infected infants defaulted from the study and were diagnosed elsewhere (Figure 2). One of these 4 infants died in hospital at 4.0 weeks of age, 1 returned to RMMCH for treatment at 20.1 months of age and was in care at the end of the study and the outcomes of the remaining 2 infants are unknown. Therefore, of the 30 infants who accessed a diagnosis through the PMTCT program, 14 were in care at the end of the study, having been followed up for a median of 67.7 weeks, and a total of 4 (13%) infants died. An additional 8 infected infants defaulted from the study and did not access routine testing at another facility (Figure 2). All 8 infections were IU transmissions and therefore detectable at birth. Two (25%) of these 8 infants died at 5.0 and 13.1 weeks of age; the outcomes of the remaining 6 are unknown.

Mothers of infants who were LTFU from the PMTCT program differed significantly from mothers of infants who were diagnosed at RMMCH, in that fewer presented for antenatal care before 20 weeks gestation ($p = 0.005$), accessed antenatal CD4 testing ($p<0.001$) or received optimal antenatal PMTCT care from 28 weeks gestation ($p = 0.006$) (Table 1). The mothers of infants who defaulted were younger ($p = 0.002$) and were less likely to be South African ($p<0.001$) (Table 1). Thus, infants born to younger, foreign mothers with poor antenatal health seeking behaviors were at greatest risk of being LTFU from RMMCH's PMTCT program. These mothers accounted for 8 (67%) of the 12 infected infants who were LTFU from RMMCH and all 8 infections were IU transmissions.

The timing of the implementation of the PMTCT program among HIV-infected infants identified at RMMCH is depicted in Figure 3. Infected infants presented for PCR testing and the result visit at median ages of 6.6 and 10.6 weeks, respectively.

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HIV-uninfected infants underwent testing at a comparable median age of 6.4 weeks (range 1.7–11 weeks) and received results at a median age of 10.6 weeks (range 6.7–20.0 weeks). The 23 infants who initiated ART did so at a median age of 16.0 weeks – a median delay of 10.1 weeks from the time of PCR testing and 6.1 weeks from receipt of results (Figure 3). Delays occurred when infants were referred from the diagnostic clinic to the treatment clinic, with a median time interval of 2.1 weeks from the result visit to first presentation for care. Once infants had presented to the treatment clinic, a median of 2.5 weeks lapsed before ART was initiated. By 12 weeks of age, only 4 infants had initiated ART. Two symptomatic infants who were admitted to hospital were fast-tracked onto treatment at 6.3 and 8.6 weeks of age and were in care at the end of the study. The other 2 infants initiated treatment at 9.4 and 10.9 weeks of age but died shortly thereafter. Virological suppression occurred in 14 (54%) infected infants at a median age of 36.5 weeks; a median of 18.4 weeks lapsed from the time treatment was initiated to virological suppression (Figure 3).

DISCUSSION

Eighty two percent of HIV-exposed infants in this cohort received an HIV status by virtue of routine testing at 6 weeks of age. This EID coverage is equivalent to that reported for Gauteng province, the region where RMMCH is located.[18] It is therefore likely that all HIV-infected and uninfected infants in the cohort who would have accessed routine testing were identified. Of the 38 perinatally HIV-infected infants, 30 (79%) were tested for HIV-infection at RMMCH or other facilities and 8 (21%) were completely missed by the routine program. Although only 14 (37%) infants were retained in care at a median of 68 weeks, all but two were virologically suppressed. By the end of the study, 18 (47%) infected infants were LTFU and 6 (16%) had died. The loss of HIV-infected infants was maximal after ART initiation and further investigation to understand the factors responsible is required.

The 8 HIV-infected infants who did not access routine testing were all IU-infected and therefore diagnosable at birth. Two (25%) of these infants were known to have died, almost double the death rate of 13% among the 30 infants who accessed diagnostic testing through the PMTCT program. The outcomes of the remaining 6 defaulting infants are unknown. These infants represent critical missed opportunities for reducing infant mortality and measuring perinatal transmission of HIV when monitoring for eMTCT.[16]

Maternal characteristics can identify infants at high risk of loss from the PMTCT program. Young or foreign mothers, who present late for antenatal care with no CD4 results, should alert healthcare workers, as their infants are at increased risk of not returning at 6 weeks for PCR testing. These findings concur with other studies which report increased LTFU of infants born to mothers who present late for antenatal care,[19] as well as positive associations between PCR testing uptake and older maternal age[20,21] and maternal adherence to PMTCT prophylaxis.[21] Young women are perhaps less experienced and less aware of the available antenatal facilities. Furthermore, foreign nationals tend to be a vulnerable group who may face difficulties accessing antenatal care in South Africa, whether through lack of knowledge, financial constraints, cultural differences, language barriers or prejudice. Increased maternal counseling and support, as well as pro-active follow-up of these infants, are required. This is in line with the call by UNAIDS to invest extra efforts to improve coverage of maternal and pediatric health services for vulnerable groups.[4]

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Initiation of ART in HIV-infected infants at RMMCH only occurred at a median age of 16.0 weeks. If the EID program could be optimized by halving the time intervals from testing PCR positive to the result visit (4 weeks) and from the result visit to ART initiation (6 weeks), then age at ART initiation could be reduced to ±11 weeks. However, this is 4 weeks later than the median age of ART initiation in the CHER study[10] and is too late to ensure that infected infants initiate treatment in time to impact the peak of infant mortality at 8 to 12 weeks of age[3] or the 20% of perinatally infected infants who demise by 13 weeks (3 months) of age[2]. Reducing early infant mortality related to HIV requires earlier ART initiation[10] which requires an earlier diagnosis.

Due to the fragmentation of the South African healthcare system and incomplete or misplaced patient-held maternal and infant health records, it is not always possible to identify HIV-exposed infants for PCR testing at the 6-week immunization visit. HIV-exposed infants should be easier to identify at birth, as all HIV-infected mothers should be identified in maternity wards to receive peripartum PMTCT prophylaxis.[6,7] In South Africa, majority (89%) of women deliver in healthcare facilities,[22] therefore a birth PCR should achieve higher EID coverage than the 6-week test. Performing PCR testing at birth would identify ±75% of perinatally HIV-infected infants[12,13] early enough for ART to impact on early mortality, ensure identification of those infected infants who are lost by 6 weeks with the current program and provide more accurate monitoring of transmission for eMTCT.[16] HIV-infected infants identified by birth testing could be traced and linked to care should they subsequently be LTFU from the PMTCT program. If IU-infection is associated with rapid progression and increased morbidity and mortality,[1,23] then a birth PCR would identify the infants at highest risk of early mortality for ART initiation. However, birth testing would not identify infants infected IP or postnatally and a second PCR test for infants who test negative at birth would be required. Diagnosis of infected infants receiving multi-drug or extended prophylaxis may be delayed beyond 4 to 6 weeks[24-26] and 6-week testing may therefore be too early to detect all IP-infected infants. Optimally timed additional tests after birth will be required to ensure detection of all vertically infected infants. Algorithms may differ depending on resource constraints, but rapid HIV tests to exclude HIV-infection in exposed infants should be considered to reduce the cost of PCR testing and the number of clinic visits required.[27,28] Since South African guidelines already recommend 2 PCR tests for HIV-exposed infants, at 6 weeks and after cessation of breastfeeding,[6,7] birth testing together with a second PCR test to detect IP infections and rapid testing to exclude postnatal infections may be feasible.

There are a number of limitations to this study. It was a single center, hospital-based study with a high proportion of foreign nationals and may therefore not be representative of outcomes in the country or even the hospital, as only those patients who planned to return to RMMCH for postnatal care were eligible for enrolment. Importantly, enrolled mothers were aware of their status antenatally; since these mothers are more likely to return for follow-up than women first diagnosed as HIV-infected at delivery,[29] follow-up rates may be favorably skewed. Losses to follow-up may be higher in other settings where antenatal, delivery and EID services occur at different sites. Additionally, an HIV status could not be determined for 15% of enrolled infants. Although efforts were made to identify infants who accessed EID at other facilities by searching the national LIS, it is possible that additional IP-infected

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infants were missed. ART coverage for infected infants may be higher than reported, as some defaulting infants may have accessed ART elsewhere.

On-going evaluation of management strategies, including EID practices, is required to optimize outcomes in an evolving HIV epidemic. In order to identify all perinatally HIV infected infants for ART in time to reduce early infant mortality, EID testing should commence at birth. Birth testing will also enable more accurate monitoring of eMTCT. However, additional testing later in infancy and lack of retention in care, particularly following ART initiation, will need to be addressed to reduce HIV-related infant mortality.

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Table 1. Antenatal PMTCT care and maternal characteristics by method of infant diagnosis*.

	1. Mothers of infants diagnosed at RMMCH	2. Mothers of infants diagnosed at facilities other than RMMCH	3. Mothers of infants who defaulted from the PMTCT program	p-value†
n				
Mothers	602	82	145	
Infants	606	85	147	
HIV-infected infants	26	4	8	
Age (years)	28.3 (18.2-44.8; n=602)	27.4 (18.6-42.1; n=82)	26.5 (16.2-41.1; n=145)	**0.002**
Nationality: local	354/584 (60.6)	47/82 (57.3)	58/138 (42.0)	**<0.001**
Antenatal clinic visit ≤20 weeks gestation	148/588 (25.2)	15/79 (19.0)	18/140 (12.9)	**0.005**
Accessed antenatal CD4 testing	551/602 (91.5)	68/82 (82.9)	98/145 (67.6)	**<0.001‡•**
Accessed any antenatal PMTCT prophylaxis	555/602 (92.2)	71/82 (86.6)	115/144 (79.9)	**<0.001**
Antenatal PMTCT from ±28 weeks gestation	243/602 (40.4)	34/82 (41.5)	38/144 (26.4)	**0.006**
Peripartum viral load (log)	3.73 (0.00-5.72; n=259)	4.16 (0.00-5.48; n=43)	4.84 (4.38-5.59; n=8)	**<0.001‡**

CD4, Cluster of Differentiation 4; HIV, Human Immunodeficiency Virus; PMTCT, Prevention of Mother-to-child Transmission; RMMCH, Rahima Moosa Mother and Child Hospital

* Data are n/total (%) for categorical variables and median (range; n) for continuous variables. The total number of mothers varies due to missing data.

† Overall comparison of all 3 groups. All significant results in the overall comparison were significantly different between groups 1 and 3.

‡ Significant difference between groups 1 and 2.

• Significant difference between groups 2 and 3.

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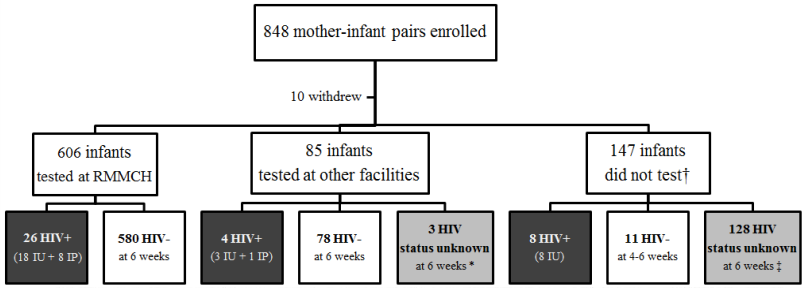
FIGURE LEGENDS

Figure 1. Determination of the HIV status of infants in the study cohort.

Figure 2. PMTCT care cascade for HIV-exposed infants born at Rahima Moosa Mother and Child Hospital (RMMCH).

The 565 infants who attended the result visit represent only 67% of the total 838 infants in the cohort. Compared to the total number of perinatally infected infants (n = 38), 68% were diagnosed at RMMCH, 61% initiated treatment and 34% remained in care. Assuming the 2 HIV-infected infants with unknown outcomes that were diagnosed at other facilities did access treatment and remained in care, then a maximum 42% of infected infants would have been in care at the end of the study. Of the 14 infants remaining in care, 12 of those diagnosed at RMMCH were virologically suppressed and the other 2 infants were in the process of achieving suppression.

Figure 3. Median age at PCR testing, receipt of results and treatment for HIV-infected infants identified at Rahima Moosa Mother and Child Hospital.



HIV+, Human Immunodeficiency Virus positive; HIV-, Human Immunodeficiency Virus negative; IP, Intrapartum; IU, *In Utero*; RMMCH, Rahima Moosa Mother and Child Hospital
† HIV status established by testing available dried blood spot samples collected for study purposes.
* Tested negative at birth, defaulted from the study at 2 weeks of age and only had positive PCR tests at 6 months or older.
‡ Status at 6 weeks of age unknown, but no IU-infection as dried blood spot samples collected at birth tested negative.

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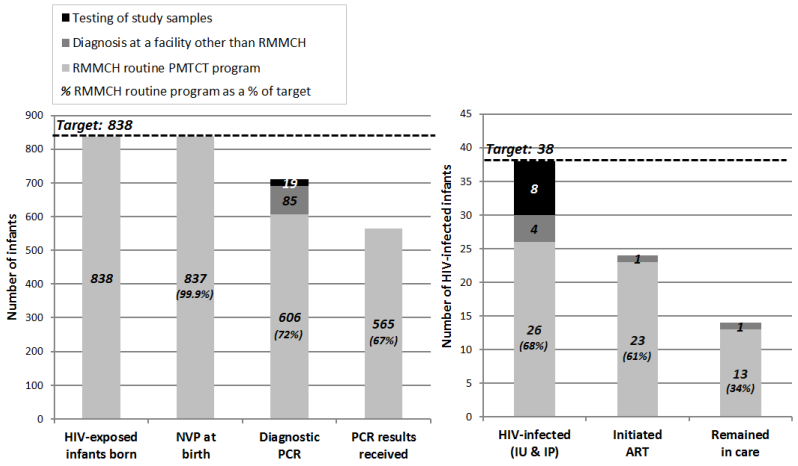
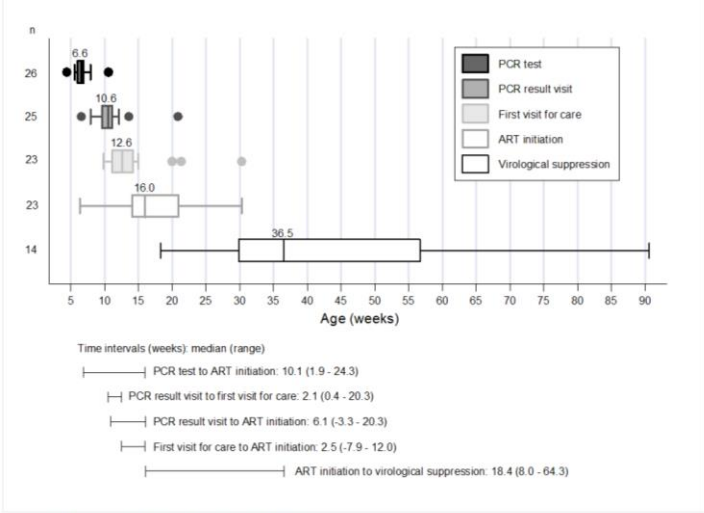


ART, Antiretroviral Therapy; HIV, Human Immunodeficiency Virus; IP, Intrapartum; IU, *In Utero*; NVP , Nevirapine; PCR, Polymerase Chain Reaction; PMTCT, Prevention of Mother-to-Child Transmission; RMMCH, Rahima Moosa Mother and Child Hospital



ART, Antiretroviral Therapy; PCR, Polymerase Chain Reaction

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APPENDIX D

Permission to include Pediatric Infectious Disease Journal publication (Appendix C) in dissertation.

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APPENDIX E

Variables in the Clinical E-database: Variables that were analysed are indicated in blue text. New variables that were added into the database during the analysis are highlighted in pink.

1	Mother clinic identification number
2	Date interviewed
3	Blood taken on mother at birth? (yes / no)
4	DBS taken on infant at birth? (yes / no)
	MATERNAL DEMOGRAPHICS, OBSTETRIC/CLINICAL HISTORY
5	Mother date of birth
6	Mother age at delivery (years)
7	Race (Black / Coloured / Indian / White / unknown)
8	Area living
9	Location of area living (province, district)
10	Area originating from
11	Nationality (local / foreign)
12	Antenatal clinic viz. booking clinic
13	Location of antenatal clinic (province, district)
14	Booking date at antenatal clinic
15	Number of weeks before delivery woman booked
16	Gestational age at booking (wks)
17	Status disclosed to partner (yes / no / unknown)
18	Partner tested (yes / no / unknown)
19	Feeding intention (breast / formula / mixed / unknown)
20	Parity
21	Gravidity
22	Number of children alive
23	Previous miscarriage (yes / no / unknown)
24	Previous premature baby (yes / no / unknown)
25	Was pregnancy planned (yes / no / unknown)
26	Syphilis (positive / negative / unknown)
27	Alcohol (yes / no / unknown)
28	Smoking (yes / no / unknown)
29	Diabetes(yes / no / unknown)
30	Hypertension (yes / no / unknown)
31	Other illnesses (yes / no / unknown)
32	Tuberculosis ever (yes / no / unknown)
	MATERNAL PMTCT / HAART
33	Nevirapine given in a previous pregnancy (yes / no / unknown)
34	Maternal PMTCT (coded as per Appendix F)
35	Maternal PMTCT - rank
36	Maternal PMTCT - ordinal categorisation with 10 week cut-off (suboptimal / intermediate / optimal)
37	Maternal PMTCT - sdNVP (yes / no)
38	Maternal PMTCT - number of weeks of antenatal AZT
39	Maternal PMTCT - number of weeks of antenatal HAART
40	Maternal PMTCT - number of weeks of antenatal AZT or HAART
41	WHO stage at initiation of HAART
42	Comment on stage
43	Date of maternal HAART initiation
44	1st regimen
45	Changed regimen? (yes / no)
46	Date of regimen change
47	Reason for regimen change (Side effect / teratogen / failing / other)
48	New regimen
49	Experienced side effects? (yes / no)
50	1st side effect
51	Date 1st side effect
52	2nd side effect
53	Date 2nd side effect
54	HAART stopped (yes / no)
55	HAART interrupted (yes / no)
56	Date HAART stopped
57	Date HAART restarted

58	Clinic providing HAART antenatally
59	Clinic providing HAART antenatally - decode
60	Clinic providing HAART postnatally
61	Clinic providing HAART postnatally - decode
	MATERNAL TESTING
62	CD4 nadir: count
63	CD4 nadir: date of test
64	Last recorded antenatal CD4 count prior to delivery: count
65	Last recorded antenatal CD4 count: category (≤200 / 200-350 / 350-500 / 500-750 / >750)
66	Last recorded antenatal CD4 count: # of weeks before delivery count performed
67	Last recorded antenatal CD4 count: date of test
68	Second last antenatal CD4 count prior to delivery: count
69	Second last antenatal CD4 count: category (≤200 / 200-350 / 350-500 / 500-750 / >750)
70	Second last antenatal CD4 count: # of weeks before delivery count performed
71	Second last antenatal CD4 count: date of test
72	Earliest antenatal CD4 count prior to delivery: count
73	Earliest antenatal CD4 count: category (≤200 / 200-350 / 350-500 / 500-750 / >750)
74	Earliest antenatal CD4 count: # of weeks before delivery count performed
75	Earliest antenatal CD4 count: gestational age at test (wks)
76	Earliest antenatal CD4 count: date of test
77	1st postnatal CD4 count: count
78	1st postnatal CD4 count: category (≤200 / 200-350 / 350-500 / 500-750 / >750)
79	1st postnatal CD4 count: # of weeks after delivery count performed
80	1st postnatal CD4 count: date of test
81	2nd postnatal CD4 count: count
82	2nd postnatal CD4 count: category (≤200 / 200-350 / 350-500 / 500-750 / >750)
83	2nd postnatal CD4 count: # of weeks after delivery count performed
84	2nd postnatal CD4 count: date of test
85	1st recorded haemoglobin
86	Date of 1st recorded haemoglobin
87	2nd recorded haemoglobin
88	Date of 2nd recorded haemoglobin
89	Plasma VL (copies/mL)
90	VL log
91	Valid VL? (yes / no)
92	Date of VL test
	INFANT BIRTH
93	Infant clinic identification number
94	Infant date of birth
95	Gender (male / female)
96	Place of birth
97	Gestation (term / preterm / postterm)
98	Gestational age (wks)
99	Birth defects
100	Mode of delivery (vaginal delivery / caesarean section)
101	Premature rupture of membranes (yes / no / unknown)
102	Infant PMTCT (coded as per Appendix F)
103	Infant PMTCT groups (suboptimal / sdNVP & 1-2 weeks AZT / sdNVP & 4 weeks AZT / daily-dose NVP)
104	PMTCT Comment
105	Birth weight (kg)
106	Birth length (cm)
107	Birth head circumference (cm)
108	Apgar comment
109	Apgar - 1 minute
110	Apgar - 5 minutes
111	Apgar - 10 minutes
	INFANT FINAL / TESTING
112	Final status (infected / uninfected / unknown but no IU infection)
113	Method of infant diagnosis (tested during study / found result on NHLS LIS / other)
114	Status positive: timing of transmission (IU / IP / PP / IP,PP)
115	Final outcome (in care, discharged, LTFU, transferred out, died)
116	Alive/dead at end of 2010 (alive / dead / unknown)
117	Alive/dead at 10-14 weeks (alive / dead / unknown)

118	Date of death
119	Age at death (weeks)
120	LTFU status (yes / no)
121	Details of LTFU
122	Date of LTFU
123	Hospital admissions (yes / no)
124	Antibiotics (yes / no)
125	WHO stage of infant if positive
126	Date 1st PCR test
127	Age at 1st PCR test (weeks)
128	1st PCR result (positive / negative / error)
129	Gave 1st PCR result (yes / no / unknown)
130	Needed 2nd PCR test (yes / no)
131	Reason for 2nd PCR test (rejected / clinical concern / post breastfeeding / other)
132	Date 2nd PCR test
133	Age at 2nd PCR test (weeks)
134	2nd PCR result (positive / negative / error)
135	Date 3rd PCR test
136	Age at 3rd PCR test (weeks)
137	3rd PCR result (positive / negative / error)
138	Date of infant CD4 test (incomplete)
139	Result of infant CD4 - absolute (incomplete)
140	Result of infant CD4 - percentage (incomplete)
141	Final feeding practice (exclusive breast / exclusive formula / mixed / unknown)
	INFANT VISIT 1 (BIRTH, 0 DAYS)
142	Date of visit
143	Age at visit (weeks)
144	Feeding practice (breast / formula / mixed / unknown)
145	Visit number according to access database
146	Weight (kg)
147	Length (cm)
148	Head circumference (cm)
149	Weight for age z-score
150	Height for age z-score
151	Weight-for-height z-score
152	Head circumference for age z-score
153	Clinical health (well / mild symptoms / sick)
154	Clinical impression (positive / negative / unsure)
155	Wasted (yes / no)
156	Eczema (yes / no)
157	Hepatomegaly (yes / no)
158	Splenomegaly (yes / no)
159	Significant lymphadenopathy (yes / no)
160	Localised lymphadenopathy (yes / no)
161	Asymptomatic thrush (yes / no)
162	Symptomatic thrush (yes / no)
163	Signs (yes / no)
164	Comment
165	DBS taken (yes / no)
166	Blood taken (yes / no)
	INFANT VISIT 2 (2 WEEKS, 14 DAYS)
167-191	As per Infant Visit 1 (variables *142-166*)
	INFANT VISIT 3 (4 WEEKS, 28 DAYS)
192-216	As per Infant Visit 1 (variables *142-166*)
	INFANT VISIT 4 (6 WEEKS, 42 DAYS)
217-241	As per Infant Visit 1 (variables *142-166*)
242	Visit date match to PCR test date? (yes / no)
	INFANT VISIT 5 (10 WEEKS, 70 DAYS)
243-267	As per Infant Visit 1 (variables *142-166*)
	INFORMATION ONLY (DATES DO NOT INDICATE VISITS)
268	Date
269	Comment
270	Date 2
271	Comment 2

APPENDIX F

Original coding of maternal and infant PMTCT regimens in the Clinical E-database.

Table F1: Original coding of maternal PMTCT.

	CODE	*MATERNAL PMTCT REGIMEN*
1	1	none
2	2	sdNVP only
3	3	AZT stat only
4	4	sdNVP and stat AZT
5	4.1	sdNVP and 2 days AZT
6	4.2	sdNVP and 4 days AZT
7	4.3	sdNVP and 5 days AZT
8	4.4	sdNVP and 1 wk AZT
9	4.41	sdNVP and 10 days AZT
10	4.5	sdNVP and 2 wks AZT
11	4.6	sdNVP and 3 wks AZT
12	5	sdNVP and 4 wks AZT
13	5.1	sdNVP and 5 wks AZT
14	5.11	AZT 4 wks; no NVP
15	5.12	4 wks AZT then 3 wks nothing
16	5.2	sdNVP and 6 wks AZT
17	5.3	sdNVP and 7 wks AZT
18	5.4	sdNVP and 8 wks AZT
19	5.41	AZT 8 wks; no NVP
20	5.5	sdNVP and 9 wks AZT
21	5.6	sdNVP and 10 wks AZT
22	5.7	sdNVP and 11 wks AZT
23	5.8	sdNVP and 12 wks AZT
24	5.81	AZT 12 wks; no NVP
25	5.82	AZT 18 wks; no NVP
26	5.9	sdNVP and 13 wks AZT
27	5.91	sdNVP and 16 wks AZT
28	5.92	sdNVP and 20 wks AZT
29	5.93	sdNVP and 22 wks AZT
30	6	HAART 2 days
31	6.1	HAART 4 days
32	6.2	HAART 5 days
33	6.3	HAART 1 wk
34	6.4	HAART 2 wks
35	6.5	HAART 3 wks
36	6.6	HAART 4 wks
37	6.7	HAART 5 wks
38	6.8	HAART 6 wks
39	6.9	HAART 7 wks
40	7	HAART 8 wks
41	7.1	HAART 9 wks
42	7.2	HAART 10 wks
43	7.3	HAART 11 wks
44	7.4	HAART 12 wks
45	7.41	HAART 12 wks then stopped 4 wks
46	7.5	HAART 13 wks
47	7.51	HAART 14 wks
48	7.52	HAART 15 wks
49	7.6	HAART 16 wks
50	7.7	HAART 17 wks
51	7.8	HAART 20 wks
52	7.81	HAART 24 wks
53	7.9	HAART 28 wks
54	8	HAART 36 wks
55	9	4 wks AZT then 6 wks HAART
56	9.1	4 wks AZT then 2 wks HAART
57	9.2	6-9 wks AZT then 4 wks HAART
58	12	HAART preconception
59	13	unknown

Table F2: Original coding of infant PMTCT.

	CODE	*INFANT PMTCT REGIMEN*
1	1	none
2	2	sdNVP
3	2.1	sdNVP and stat AZT
4	2.2	sdNVP and 2 days AZT
5	2.4	sdNVP and 4 days AZT
6	3	sdNVP and 1 wk AZT
7	3.1	sdNVP and 10 days AZT
8	4	sdNVP and 2 wks AZT
9	5	sdNVP and 3 wks AZT *(n = 0)*
10	6	sdNVP and 4 wks AZT
11	7	NVP 6 wks
12	7.1	NVP 5 wks
13	8	NVP while breast feeding

APPENDIX G

The adapted mathematical model described below was designed to assess several PCR testing scenarios for the diagnosis of perinatal HIV-infection in infants. Only birth, six-week, ten-week and 14-week diagnostic testing in the context of selected sensitivity analyses were investigated for this MSc.

Description of Early Infant Diagnosis model

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February 2013

The approach adopted here is to simulate the outcomes in a cohort of 240,000 infants born to HIV-infected mothers, under a number of different PCR testing scenarios. Each mother-infant pair is randomly assigned a different set of characteristics, based on the observed distribution of characteristics in the South African population. The outcomes in which we are interested are those that are needed to calculate the total survival time of the infant (over their whole life) and the length of time each infant spends in different HIV disease stages if they become infected. This enables us to calculate the total life years saved and quality-adjusted life years (QALYs) saved when comparing different PCR testing strategies, as well as the number of QALYs saved per PCR test.

Demographic assumptions

A sex is randomly assigned to each infant at the point of birth, with the proportion of male births being set at 0.5039, the proportion assumed in the ASSA2008 AIDS and Demographic model [1]. A time to non-HIV death is also randomly assigned to each infant, at the point of birth. The age at death is determined to be the lesser of the time to non-HIV death and the time to AIDS death (if the child acquires HIV). The time to non-HIV death is randomly assigned based on life tables calculated from HIV-negative mortality rates in South Africa in 2005, which are obtained from the ASSA2008 AIDS and Demographic model.

Model of mother-to-child transmission

The assumptions about mother-to-child transmission of HIV are adapted from those in a previously-published model [2]. We consider intrauterine transmission and intrapartum transmission; for the purpose of this analysis, postnatal transmission rates have been set to zero. Table G1 summarizes the assumptions regarding intrauterine and intrapartum transmission, under different forms of antiretroviral prophylaxis. The assumed proportions of women who are receiving different forms of prophylaxis are based on a South African survey of women who were interviewed and tested for HIV at immunization clinics, conducted in 2010 [3]. The assumed combined intrauterine and intrapartum transmission risk is based on various studies that have evaluated the efficacy of different forms of antiretroviral prophylaxis, reviewed elsewhere [4, 5]. The fraction of this transmission that is intrapartum is greatest in the absence of antiretroviral prophylaxis, which is relatively more effective in preventing intrauterine transmission than in preventing intrauterine transmission.

Table G1: Rates of intrauterine and intrapartum transmission, under different forms of antiretroviral prophylaxis

	% of mothers accessing	Combined intrauterine and intrapartum transmission	% of combined transmission that is intrapartum
No prophylaxis	8%	20%	62%
Single-dose nevirapine	5%	12%	35%
Single-dose nevirapine + AZT	54%	4%	24%
Highly active ART (HAART)	33%	2%	20%

Although postnatal transmission is not considered in this analysis, assumptions about the duration of breastfeeding determine the proportion of infants who are receiving extended nevirapine prophylaxis and hence the sensitivity of the PCR (discussed below). It is assumed that 20% of pregnant women who are diagnosed HIV-positive choose not to breastfeed, that 55% choose to breastfeed exclusively and that 25% of women practise mixed feeding (these assumed rates of breastfeeding are higher than those observed historically [6, 7], because of the current phasing out of free formula milk in public sector clinics). The duration of exclusive breastfeeding (EBF) is randomly assigned, in those women who choose this feeding strategy, by sampling from an exponential distribution with a median of 2 months and resetting any sampled values greater than 6 months to 6 months (the assumed maximum duration of EBF). Similarly, the duration of mixed feeding is randomly assigned by sampling from an exponential distribution with a median of 7 months and limiting the maximum duration of mixed feeding to 36 months. In those women who elect to practise EBF initially, 30% are assumed to practise abrupt weaning at the end of the simulated EBF period and the remaining 70% combine breastfeeding with other feeds (mixed feeding), with the duration of the remaining mixed feeding period being simulated in the same way as in women who practise mixed feeding from birth. These breastfeeding assumptions for women who are diagnosed HIV-positive are based on various South African data sources, reviewed elsewhere [4].

It is assumed that all infants born to HIV-diagnosed mothers receive at least 6 weeks of nevirapine prophylaxis, in line with recent South African guidelines [8]. (In a sensitivity analysis we will consider the effect of reducing this proportion to 50%, to assess the possible effect of imperfect compliance with guidelines.) The proportion of breastfeeding women who receive HAART is assumed to be the same as the proportion of women who receive HAART antenatally (33%). If breastfeeding women are not on HAART but do receive the standard 6 weeks of nevirapine prophylaxis, the assumed probability that they will continue prophylaxis for the entire duration of breastfeeding is set at 40% (this is an arbitrary assumption, as we do not currently have data on the proportions of breastfeeding women who are receiving extended nevirapine prophylaxis). We also consider a sensitivity analysis in which the 40% assumption is replaced with 90%.

The assumptions have been set to be consistent with the South African PMTCT programme prior to December 2012. However, it has recently been announced that South Africa will switch from WHO option A to WHO option B in future, meaning that triple-drug regimens will be recommended for all HIV-positive pregnant women (regardless of their CD4 count) and continued throughout breastfeeding [9]. We therefore consider a sensitivity analysis in which it is assumed that 92% of pregnant women initiate HAART during pregnancy (and remain on HAART throughout breastfeeding) and the remaining 8% receive no prophylaxis.

Model of HIV survival

The assumptions about survival in HIV-infected children are the same as those in a previously-described model [10]. Briefly, children are assumed to progress through two disease stages prior to dying from AIDS, in the absence of ART; the first stage represents children who have not yet met

the criteria for ART eligibility specified in the 2006 WHO paediatric ART guidelines [11], and the second stage represents children who have met the eligibility criteria. The rates at which children progress from the first stage to the second stage are assumed to be very high during the first few months of life, but drop to lower levels thereafter. Similarly, the rates of untreated mortality in the second stage are assumed to be highest during the first few months of life but drop to relatively low levels at later ages. Children who start ART when in the second stage of HIV disease are assumed to be at a high risk of AIDS mortality during the first 3 months of ART, but then experience a 90% lower mortality rate once they have stabilized on ART. Perinatally-infected children who start ART when in the first stage of HIV disease are assumed not to go through an initial 'high risk' phase and are also assumed to have a lower mortality rate during the 'low risk' phase than that in children who started ART while in the second HIV stage, with these parameters being set to match the observed difference in mortality between early ART and deferred ART groups in the CHER trial [12]. The extent of this difference reduces as the age at early ART initiation increases, in line with the results of the PREDICT trial [13].

The default assumption is that children infected intrauterine and children infected intrapartum have the same mortality rates, based on a pooled analysis of African cohorts [14]. However, there is some evidence to suggest that children infected intrauterine may have higher rates of mortality than children infected intrapartum [15]. This is potentially important to consider, as PCR testing at birth would have more benefit (relative to testing at other durations) if the infants with detectable HIV at birth have a relatively high mortality risk. In a sensitivity analysis, we therefore consider the effect of setting the relative rate of progression to ART eligibility, comparing intrapartum-infected children to intrauterine-infected children, to 0.7, consistent with the observed cumulative mortality rates at 6 months of 0.29 and 0.42 in intrapartum- and intrauterine-infected infants respectively [15].

Although the modelled rates of disease progression and mortality in untreated HIV-infected children are consistent with South African data sources [12, 16, 17], the resulting estimates of survival are substantially higher than those observed in other African cohorts [18]. We therefore include a sensitivity analysis in which the rates of progression to the ART-eligible state and the rates of mortality are all multiplied by a factor of 1.5, which yields overall estimates of survival more consistent with those estimated by Marston et al in a pooled analysis of African paediatric HIV data [18].

Model of PCR screening

We consider 6 possible times for PCR screening with a single PCR test: at birth, 6 weeks after birth, 10 weeks after birth, 14 weeks after birth, 6 months after birth and 9 months after birth. These correspond to the infant ages at which South African women routinely visit health centres for immunization and postnatal care. We also consider routine PCR screening with 2 tests, the first at birth and the second 6 weeks after birth, 10 weeks after birth or 14 weeks after birth. In addition, it is assumed that HIV testing can occur 6 weeks after weaning (with either a PCR or an antibody test if testing is performed after the age of 18 months). It is further assumed that if ART is initiated prior to 18 months (without the child having being diagnosed at one of the standard screening visits), PCR testing would have been performed 1 month previously.

Table G2 summarizes the model assumptions regarding test uptake, test collection and test sensitivity, for each of the possible screening times. We assume that if screening is performed at birth, all women would receive the test. However, if screening is performed at 6 weeks, we assume that only 80% of HIV-diagnosed mothers would bring their children for testing [19-21]. We assume that the fraction of women bringing their infants for testing would decrease the longer the time to the screening visit, although there are no local data to support this assumption. Based on a study conducted in KwaZulu-Natal [22], we assume that HIV-positive test results are received in 66% of cases; this proportion is roughly consistent with proportions observed in other African settings [23]. In most situations the PCR is assumed to have 100% sensitivity in detecting

paediatric HIV [24]. However, if the test is performed immediately after birth, it is assumed that it would not detect HIV that has just been acquired intrapartum. In addition, it is assumed that the test would have reduced sensitivity if it is performed in children who are receiving nevirapine prophylaxis, based on (a) unpublished data relating to intrauterine-infected infants in South Africa and (b) data relating to intrapartum-infected infants in a multicentre study, who received forms of prophylaxis other than nevirapine [25]. It is assumed that sensitivity would be lower in intrapartum infections than in intrauterine infections (since the latter are more established at the time that nevirapine is initiated) and that the sensitivity increases as the time since starting nevirapine increases (the longer the time since prophylaxis was started, the greater is the likelihood that the virus will have mutated and become detectable). We also consider a sensitivity analysis in which the PCR sensitivity in children receiving nevirapine prophylaxis is assumed to be the same as that in children who are not receiving prophylaxis.

Table G2: Assumed rates of PCR uptake and PCR sensitivity

Time of PCR screening	Birth	6 weeks	10 weeks	14 weeks	6 months	9 months
% of children who get tested	100%	80%	75%	70%	65%	60%
% of caregivers receiving test results, if test is performed	66%	66%	66%	66%	66%	66%
PCR sensitivity: intrauterine infection						
Child is receiving NVP prophylaxis	100%	85%	91%	95%	98%	100%
Child not receiving NVP prophylaxis	100%	100%	100%	100%	100%	100%
PCR sensitivity: intrapartum infection						
Child is receiving NVP prophylaxis	0%	70%	80%	89%	95%	98%
Child not receiving NVP prophylaxis	0%	100%	100%	100%	100%	100%

There is little current data on the proportion of HIV-positive mothers who take their children for HIV testing after ceasing breastfeeding, although the proportion is believed to be low. In light of this uncertainty, we have conservatively assumed that only 10% of breastfeeding HIV-positive mothers take their children for HIV testing following weaning, but we also consider a sensitivity analysis in which it is optimistically assumed that all HIV-positive mothers take their children for testing following weaning. As with routine PCR screening, we assume that only 66% of mothers who get their children tested after weaning actually receive the test results. PCRs are assumed to be conducted only if the child is brought for testing before the age of 18 months; at older ages children would be tested using rapid tests, which are assumed to be 100% sensitive.

Model of timing of ART initiation

There is assumed to be a 2-week delay between the collection of PCR specimens and the return of test results. In addition, there is assumed to be a 2-week delay between the return of a positive test result and the initiation of ART (assuming that the child is HIV-positive and still alive). We optimistically assume that if the caregiver receives the HIV-positive diagnosis, the infant would always be started on ART. However, we also consider a scenario in which it is more conservatively assumed that only 50% of the children who receive an HIV diagnosis actually start ART, more in line with data from other African countries [23]. Guidelines for paediatric ART initiation in South Africa have recently changed and it is now recommended that all children under the age of 5 years should be started on ART as soon as they are diagnosed HIV-positive.

In addition to HIV diagnosis through screening, the model allows for HIV diagnosis following the development of HIV-related symptoms. In the interests of simplicity, we assume that if ART is initiated as a result of the diagnosis of HIV-related symptoms, this occurs after the child has progressed to the second HIV stage (ART-eligible according to 2006 WHO criteria), at a rate that is proportional to their AIDS mortality risk. It is assumed that the probability of ART initiation following the diagnosis of HIV-related symptoms would be 0.5 if there was no routine PCR screening. For each HIV-infected child we simulate the time to ART initiation following screening

(if the child receives a positive screening result) and the time to ART initiation following diagnosis of HIV-related symptoms (if the symptoms lead to HIV diagnosis). The actual time of ART initiation is the minimum of the two, or is undefined if neither of the conditions for ART initiation are met.

The assumption that there is a 50% chance of ART initiation following the diagnosis of HIV-related symptoms is based on previous modelling work in South Africa [10], but this assumption may be too conservative in light of recent evidence of high rates of ART uptake in South African adults and children [26]. We therefore consider a sensitivity analysis in which the assumed chance of starting ART following the diagnosis of HIV-related symptoms is increased to 90%.

QALY calculations

Based on the review of Tengs and Lin [27], we assign a quality of life weight of 0.94 to children who are untreated and HIV-positive, who have not yet met the 2006 WHO criteria for ART eligibility, and a weight of 0.7 to untreated HIV-positive children who have met the 2006 WHO criteria for eligibility. The same weight is assumed to apply to children who are in the 'high risk' phase following ART initiation, but a higher weight of 0.85 is assumed to apply to children once they have stabilized on ART [28]. A discount rate of 3% per annum is used in calculating QALYs saved. In calculations of life years saved, we do not apply any quality of life weights or use any discounting.

Summary of sensitivity analyses

The scenarios that we consider in the sensitivity analysis can be summarized as follows:

- Scenario 1 (Baseline): This represents current South African policy in November 2012, prior to the announcement of the switch to WHO option B.
- Scenario 2 (Lower uptake of 6-week course of NVP): The fraction of HIV-positive mothers who provide nevirapine prophylaxis to their infants is reduced from 100% to 50%.
- Scenario 3 (Higher uptake of extended NVP): The fraction of breastfeeding HIV-positive mothers initiating the 6-week course of nevirapine, who continue the nevirapine prophylaxis after 6 weeks, is increased from 40% to 90%.
- Scenario 4 (WHO option B): The proportion of HIV-positive mothers who receive HAART during pregnancy is increased to 92% (and the same proportion continue HAART during breastfeeding). The remaining 8% of women are assumed to receive no antenatal prophylaxis.
- Scenario 5 (Lower mortality in children infected intrapartum): The rate of progression to the ART-eligible state in children infected intrapartum is assumed to be 0.7 times that in children who are infected intrauterine.
- Scenario 6 (Higher HIV-related mortality): The rates of progression to the ART-eligible state and the rates of mortality are assumed to be 1.5 times those in the baseline scenario.
- Scenario 7 (No loss in PCR sensitivity due to NVP): The PCR in children receiving nevirapine prophylaxis is assumed to be the same as that in children who are not receiving prophylaxis.
- Scenario 8 (Higher uptake of PCR screening after weaning): The assumed proportion of mothers who take their children for PCR testing 6 weeks after weaning is increased from 10% to 100%.
- Scenario 9 (Low linkage to care): The assumed proportion of HIV-diagnosed children who start ART 2 weeks after diagnosis is reduced from 100% to 50%.
- Scenario 10 (Higher ART access in absence of screening): The assumed fraction of HIV-infected children who would start ART in the absence of routine PCR screening is increased from 50% to 90%.

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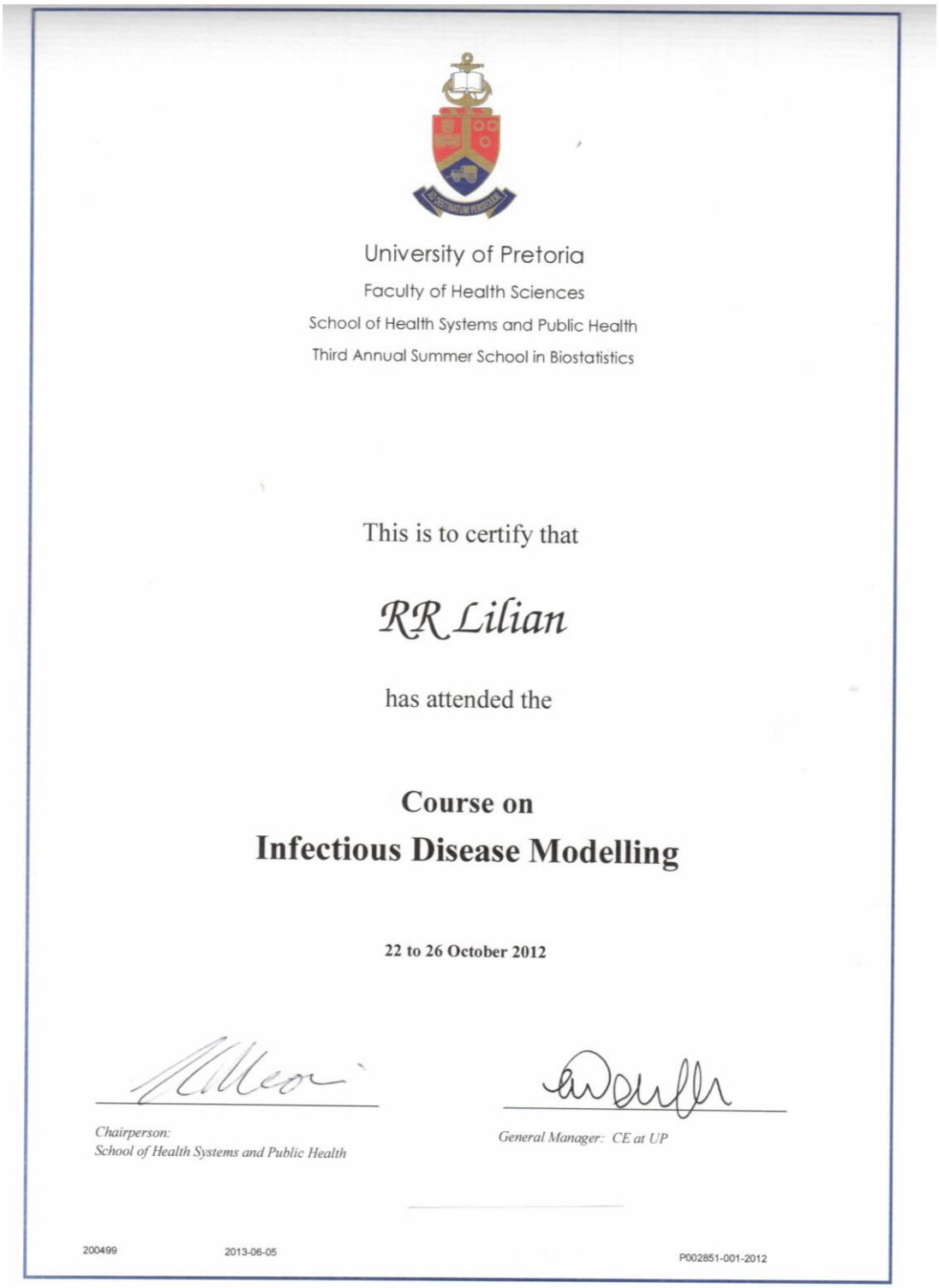
APPENDIX H

Certificate of attendance, Infectious Disease Modelling Course: In addition to collaborating with Dr Johnson, a one-week Infectious Disease Modelling Course was attended to gain a greater understanding of the modelling process.



University of Pretoria

Faculty of Health Sciences

School of Health Systems and Public Health

Third Annual Summer School in Biostatistics

This is to certify that

RR Lilian

has attended the

Course on

Infectious Disease Modelling

22 to 26 October 2012

Chairperson:
School of Health Systems and Public Health

General Manager: CE at UP

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APPENDIX I

Ethics approval to perform the Earlier Infant Diagnosis Study at Rahima Moosa Mother and Child Hospital.

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Sherman

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M080626**
PROJECT	Diagnosing Human Immunodeficiency Virus (HIV) infection earlier than six weeks of age with a single HIV PCR test
INVESTIGATORS	Dr G Sherman
DEPARTMENT	Paediatric Haematology
DATE CONSIDERED	08.06.27
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 08.08.04 **CHAIRPERSON** .....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor :

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX J

Ethics approval to perform a secondary data analysis for degree purposes using data from the Earlier Infant Diagnosis Study.

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Ms Rivka Lilian

CLEARANCE CERTIFICATE	M10366
PROJECT	Identifying Interventions to Improve Outcomes of the South African Prevention of Mother-to-Child Transmission Programme (revised title)
INVESTIGATORS	Ms Rivka Lilian
DEPARTMENT	Molecular Medicine & Haematology
DATE CONSIDERED	26/03/2010
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 04/05/2012 **CHAIRPERSON** .....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof G Sherman

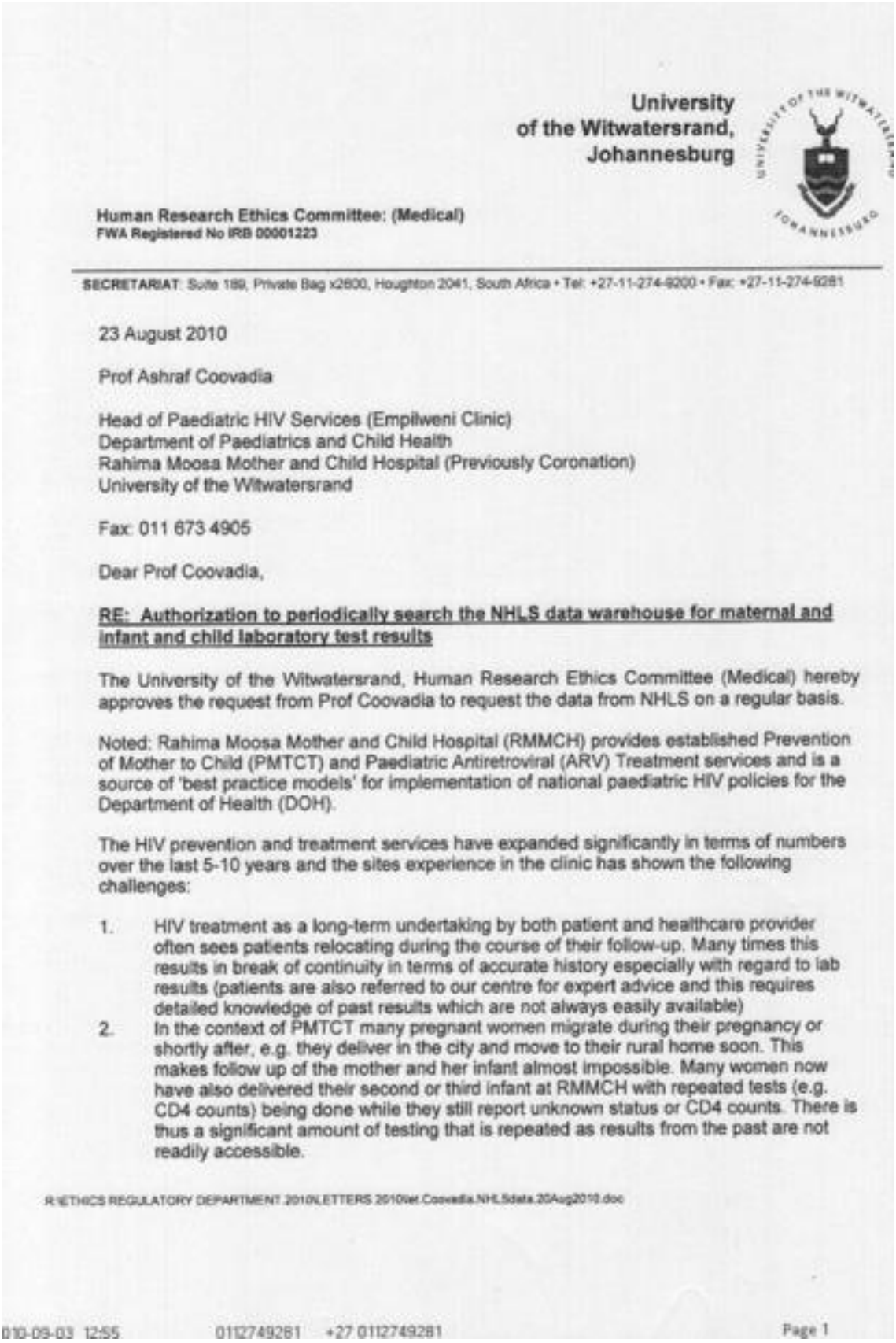
DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX K

Ethics approval for Rahima Moosa Mother and Child Hospital's PMTCT programme to search the National Health Laboratory Service's Laboratory Information System for maternal and infant laboratory test results.



University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee: (Medical)
FWA Registered No IRB 00001223

SECRETARIAT: Suite 189, Private Bag x2600, Houghton 2041, South Africa • Tel: +27-11-274-9200 • Fax: +27-11-274-9281

23 August 2010

Prof Ashraf Coovadia

Head of Paediatric HIV Services (Empilweni Clinic)
Department of Paediatrics and Child Health
Rahima Moosa Mother and Child Hospital (Previously Coronation)
University of the Witwatersrand

Fax: 011 673 4905

Dear Prof Coovadia,

RE: Authorization to periodically search the NHLS data warehouse for maternal and infant and child laboratory test results

The University of the Witwatersrand, Human Research Ethics Committee (Medical) hereby approves the request from Prof Coovadia to request the data from NHLS on a regular basis.

Noted: Rahima Moosa Mother and Child Hospital (RMMCH) provides established Prevention of Mother to Child (PMTCT) and Paediatric Antiretroviral (ARV) Treatment services and is a source of 'best practice models' for implementation of national paediatric HIV policies for the Department of Health (DOH).

The HIV prevention and treatment services have expanded significantly in terms of numbers over the last 5-10 years and the sites experience in the clinic has shown the following challenges:

1. HIV treatment as a long-term undertaking by both patient and healthcare provider often sees patients relocating during the course of their follow-up. Many times this results in break of continuity in terms of accurate history especially with regard to lab results (patients are also referred to our centre for expert advice and this requires detailed knowledge of past results which are not always easily available)
2. In the context of PMTCT many pregnant women migrate during their pregnancy or shortly after, e.g. they deliver in the city and move to their rural home soon. This makes follow up of the mother and her infant almost impossible. Many women now have also delivered their second or third infant at RMMCH with repeated tests (e.g. CD4 counts) being done while they still report unknown status or CD4 counts. There is thus a significant amount of testing that is repeated as results from the past are not readily accessible.

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The nature of long-term HIV care and treatment as well as prevention from the individual patient point of view would be assisted by putting in place an easier mechanism of tracing past results. Currently the process of tracking individual patients' results is very difficult as there is no system in place for looking for individuals.

The patient population attending RMMCH services is dynamic in that antenatal care is accessed at surrounding primary health care facilities or at RMMCH; babies are delivered at RMMCH; postnatal care occurs either at RMMCH or at a primary health care clinic and ongoing well baby care usually occurs at clinics unless children become acutely ill requiring admission to RMMCH. Furthermore, patients seek healthcare from various clinics and in a variety of provinces depending on their circumstances. It is therefore difficult to collate longitudinal laboratory (let alone clinical) data on women and children because testing is performed in different facilities.

Prof Coovadia has become aware that it is possible to extract these data from the National Health Laboratory Service's data warehouse on a regular basis e.g. monthly. This may involve submitting a list of patients (with patient identifying details such as patient name, surname, hospital number, date of birth) to the NHLS requesting specific test results for a specific time period whereupon an automated search of the warehouse will be done and matching patients with their specified test results returned to the site. The site will update their electronic patient database with this information. There may be other options for accessing the data from NHLS that can be explored with the NHLS data warehouse manager. As is current practice, only data capturers/managers and clinicians working in the PMTCT and ARV services clinic will have access to this strictly confidential data which is kept in a secure and locked area, with restricted access.

This is not a research project but an attempt to improve the sites record keeping and ultimately their services to patients and reporting to Department of Health. Reports will be on aggregated data and not contain any patient identifying details whatsoever. In Prof Coovadia's position as head of the HIV programme at RMMCH, it is important to document the impact of these services and identify shortcomings requiring improvement. As Chair of the Gauteng PMTCT working group, it is apparent that Provincial and National Departments of Health require assistance in accurately monitoring and evaluating the outcomes of these services.

Please liaise with Prof Wadee regarding your application to the NHLS.

cc – Professor Ahmed Wadee – Head of the School of Pathology, University of the Witwatersrand.

Kind regards

PROF PETER CLEATON-JONES
For and on behalf of the Human Research Ethics Committee: (Medical)

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