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Effects of preterm birth on HIV acquisition risk and antiretroviral prophylaxis safety in HIV-exposed Infants in Botswana

by

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AJBGBO001

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Declaration

I, Gbolahan Ajibola (AJBGBO001), hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Part 0: Preamble

Abstract

Background: The risk of *in-utero* and *peripartum* HIV acquisition in HIV-exposed infants born prior to 37 weeks completed gestational age (preterm), is thought to be high when compared with those delivered at term. However, limited data exist on the differential risk of HIV acquisition among infants born preterm following foetal exposure to HIV versus those born at term. With a reported increase in the prevalence of preterm delivery among infants with *in-utero* HIV and antiretroviral (ARV) drug exposure, it is pertinent to understand the risks associated with mother-to-child-transmission (MTCT) of HIV in infants born preterm versus those born on or after 37 weeks gestational age, as a means of identifying opportunities to eliminate MTCT (eMTCT).

Furthermore, Botswana's current Prevention of Mother-to-Child Transmission of HIV (PMTCT) guidelines recommend a variety of ARV prophylaxis strategies in the first 4 – 6 weeks of life for HIV-exposed infants depending on their risk for HIV acquisition, which ranges from triple antiretroviral (ARV) prophylaxis for infants deemed to be at "high risk" for HIV-acquisition to twice-daily dosing of zidovudine for the first 4-6 weeks of life for infants deemed to have a low HIV acquisition risk. At the time of the MPEPU study (2010 – 2015), Botswana offered 4 weeks of zidovudine (ZDV) alone as post-exposure prophylaxis to all exposed infants, however, participants enrolled in the study were given the option to receive once daily Nevirapine (NVP) as prophylaxis if randomized to a breastfeeding arm for the entire duration of breastfeeding in the absence of maternal 3-drug ART.

Equally important to understanding the risk of HIV acquisition among infants born preterm is an understanding of the hematologic safety of ARV prophylaxis strategies.

Methods: Using data extracted from a Botswana-based placebo-controlled trial evaluating the effect of co-trimoxazole (CTX) on mortality in infants who were HIV-exposed *in-utero* (the Mpepu Study), we describe the prevalence, timing, and risk factors for HIV acquisition in infants born preterm versus those born at term and assessed the hematologic safety of ARV prophylaxis among HIV-exposed preterm infants in the first month of life. Fisher's exact tests were used to compare the prevalence and timing of infants seroconverting at birth or within 72 hours post-delivery (for *in-utero* estimates) and those testing positive at 14-34 days after an initial negative result at birth (for *peripartum* estimates), with prevalence ratios (PR) calculated to ascertain the magnitude and direction of observed associations. The same approach was used to compare the occurrence of severe anaemia and neutropenia between full-term and preterm infants based on ARV prophylaxis received. A multivariable logistic regression model was fit to identify risk factors for HIV acquisition and severe hematologic effects of ARV prophylaxis in the first month of life.

Results: Two thousand eight hundred and sixty-six Mpepu infants, all with *in-utero* HIV exposure, were included in this secondary analysis. Of these, 532 (19%) were born preterm and 2334 (81%) were born at term. There was no significant difference in HIV acquisition rate between preterm and term infants overall (0.8% versus 0.6%, p=0.54, PR=1.33), at birth (0.2% versus 0.3%, p=1.00, PR=0.67) or at 14-34 days post-delivery (0.6% versus 0.3%, p=0.41, PR=2). The only significant predictor of HIV acquisition in both preterm and term infants was associated with the mother's ARV drug regimen as either treatment or prophylaxis. Triple-drug antiretroviral treatment (ART) use in women living with HIV (WLHIV) during the index pregnancy was associated with an observed decrease in odds of HIV acquisition in both preterm and term infants compared to infants born to women who received ZDV only or no ARV prophylaxis at all in the index pregnancy [adjusted Odds Ratio (aOR): 0.003; 95% Confidence Interval (CI): 0.001 – 0.02, p<0.001].

Of a total of 2746 children with haemoglobin results, 23 (0.8%), [10 preterms versus 13 born full term] infants had severe anaemia in the first month of life, while 43 (1.5%) [29 preterms versus 14 full terms] of 2733 children had severe neutropenia. Of 1524 neonates who received twice daily ZDV as prophylaxis, 17 (1.1%) [7 preterms versus 10 full term] had severe anaemia compared to 6 (0.5%) [3 preterms versus 3 full terms] of 1222 neonates who received once-daily dosing of NVP. Twenty-eight 91.8%) [8 preterms versus 20 full term] of 1518 neonates who received twice daily ZDV as prophylaxis and 15 (1.2%) [4 preterms versus 11 full terms] of 1215 neonates who received once-daily dosing of NVP had severe neutropenia. A significantly higher proportion of preterm infants had severe anaemia when compared with those delivered at term (1.9% versus 0.6%, p=0.005, PR=3.16). A similar trend was observed with neutropenia, with a higher proportion of preterm infants with severe neutropenia when compared to term infants (2.5% versus 1.3%, p= 0.070, PR=1.92). Infants with severe anaemia were more likely to have been born preterm (aOR: 2.41; 95% C.I: 0.96 – 6.03; p=0.05) while those with severe neutropenia were more likely to have formula-fed at birth (aOR: 0.37; 95% C.I: 0.19 – 0.73; p=0.004) and younger (14-27 days) at the time of testing (aOR: 0.09; 95% C.I: 0.03 – 0.33; p=<0.001). Infant ARV prophylaxis (twice daily ZDV versus once daily NVP) and randomized treatment arm (CTX versus placebo) did not significantly contribute to the occurrence of severe anaemia and neutropenia.

Conclusions: In settings with low MTCT rates attributable to widespread use of maternal ART in pregnancy, as in Botswana, infants born preterm experienced similar rates of HIV acquisition compared to those delivered at term. Reassuringly, ART use by women in pregnancy significantly reduces the risk of infant HIV acquisition. Infant post-exposure ARV prophylaxis (twice daily ZDV versus once daily NVP) appeared safe in both preterm and term infants and did not contribute to the slight increase in cases of severe hematologic adverse events observed in preterm. However, in an era where triple ARV prophylaxis is now recommended for infants

at high risk of HIV acquisition, understanding the hematologic safety of this approach among preterm infants is of paramount importance.

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List of abbreviations

ACTG	AIDS Clinical Trials Group
ART	Antiretroviral Therapy
ARV	Antiretroviral
BHP	Botswana Harvard AIDS Research Institute Partnership
BMI	Body Mass Index
CBAR	Centre for Biostatistics in AIDS Research
CI	Confidence Interval
CTX	Cotrimoxazole
DAIDS	Division of AIDS
eMTCT	Elimination of mother to child transmission
HIV	Human Immunodeficiency Virus
HPTN	HIV Prevention Trials Network
INSTI	Integrase Strand Transfer Inhibitor
IUGR	Intrauterine Growth Restriction
MoHW	Ministry of Health and Wellness
MTCT	Mother-to-child-transmission
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
NRTIs	Nucleoside reverse transcriptase inhibitors
NVP	Nevirapine
PACTG	Paediatrics AIDS clinical Trials Group
PI	Protease Inhibitors
PMTCT	Prevention of Mother-to-Child-Transmission
PR	Prevalence Ratio
PTB	Preterm birth
RCT	Randomized Controlled Trial
SB	Stillbirth

SGA	Small for Gestational Age
UCT	University of Cape Town
UNAIDS	The Joint United Nations Programme on HIV/AIDS
VPTB	Very Preterm Birth
WHO	World Health Organization
WLHIV	Women Living with HIV
ZDV	Zidovudine

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Structure of Dissertation

This dissertation is structured in 5 parts, each with a separate focus on the research topic. With the exclusion of the preambles and this Introduction chapter, the report contains the following sections:

Structure of dissertation

Part I: Introduction	This section introduces the research topic, discusses its planned aims and objectives, highlights the methodology used and presents a justification for the research work.
Part II: Literature Review	This section investigates and summarizes findings from published research work on the prevalence and timing of mother-to-child transmission of HIV (MTCT) among infants born preterm, as compared to those born at term to WLHIV in the setting of widespread maternal ART use as well as published literature evaluating hematologic effects associated with infant post exposure ARV prophylaxis regimens used for PMTCT among HIV-exposed infants born preterm.
Part III: Manuscripts	In this section, salient findings from the proposed research are presented in the form of 2 manuscripts.
Part IV: Conclusions and Recommendations	Part 4 explores and discusses the potential implications of findings from this study and proffers recommendations for a future direction.
Part V: Appendices	Part 5 presents regulatory documents/approval as well as other relevant tools used for the study.

Part I: Introduction

Effects of preterm birth on HIV acquisition risk and antiretroviral prophylaxis safety in HIV-exposed infants in Botswana

One of the success stories of the Botswana HIV treatment and prevention program lies in the country's prevention of mother-to-child transmission of HIV program (PMTCT). Currently, over 95% of pregnant women living with HIV are accessing antiretroviral (ARV) treatment (for their health) resulting in a mother-to-child (MTCT) transmission rate of <2%, one of the lowest rates in Sub-Saharan Africa. This welcomed achievement, however, means that an increasing number of infants are now exposed to ARV drugs *in-utero*, potentially contributing to increased preterm birth (PTB). Despite the reduced MTCT among HIV-exposed infants, we do not know if the timing or rate of HIV acquisition for infants born preterm differs from infants born on or after 37 weeks gestational age, it is thus imperative to understand if a differential risk of HIV acquisition exists among infants born preterm in contrast to those born at term, as such a finding would warrant national guidelines changes in Botswana. Also, Botswana's current PMTCT guidelines recommend that preterm HIV-exposed infants (lacking any other risk factor), receive a low-risk HIV prophylactic regimen of single-dose nevirapine within 72 hours of birth and twice daily dosing of Zidovudine for the first 28-30 days of life, understanding how effective and safe this regimen is both in preventing infant perinatal HIV acquisition and minimizing hematologic disturbances in early life is of public health importance to ensuring the health, wellbeing and survival of infants born preterm to women living with HIV (WLHIV).

Background

Availability and access to ART for WLHIV have improved globally. As of 2021, UNAIDS [1] estimated that 81% [63–97%] of pregnant women living with HIV had access to antiretroviral medicines to prevent transmission of HIV to their child, resulting in a 52% reduction of new HIV infections among children. However, associations between the use of

ART in pregnancy and adverse birth outcomes, including PTB and very PTB have been reported [2-4].

Botswana, an upper-middle-income country hit hard by the HIV epidemic, stepped forward early in the epidemic providing a robust HIV treatment and prevention program free to its citizens. One of its success stories lies in the fact that Botswana was one of the first high-burden HIV settings to initiate the PMTCT program. Currently, over 95% [5] of pregnant women living with HIV in Botswana are accessing ART, resulting in an MTCT rate of <2% [6]. This welcomed achievement, however, means that an increasing number of infants are now exposed to ARV drugs *in-utero*, with ARV drugs potentially contributing to increased PTB [2, 3, 7-9]. PTB among HIV-exposed infants in Botswana is approximately 21%, in contrast to 13% among HIV-unexposed infants. [10, 11]

Whether or not timing (*in-utero* or *peripartum*) or rate of HIV acquisition for infants born preterm differs from HIV-exposed infants born on or after 37 weeks gestational age has not been well studied. Yet, this represents an important area of research, as currently, the Botswana guidelines recommend deferring HIV testing until 6-10 weeks of life for infants born to WLHIV and the national recommendations for infant ARV prophylaxis regimens for preterm infants do not reflect this group as having a uniquely higher risk of HIV acquisition simply because they were born preterm. In our own work in an early infant treatment study underway in Botswana, which enrols infants who test HIV positive at birth and are at least 35 weeks gestational age at birth, 33.3% of the infants enrolled in the study were born between 35-36 weeks gestational age, a prevalence that exceeds the background preterm birth rate of 21% for infants born to WLHIV, suggesting that preterm HIV-exposed infants may be at higher risk for *in-utero* HIV acquisition. An alternate explanation is that *in-utero* HIV infection may precipitate the onset of labor and PTB, but this potential for reverse causality has not been explored by comparing the gestational ages of *in-utero* and intrapartum events.

At the time of the MPEPU study (2010 – 2015), Botswana's PMTCT program offered 3-drug ART treatment for WLHIV with CD4 cell counts < 250 cells/mm^3, 3-drug ART

prophylaxis for those with CD4 cell count between 250 cells/ mm^3 and ≤350 cells/mm^3 and ZDV-alone for those with CD4 cell count >350 cells/mm^3, while all exposed infants received twice daily ZDV for the first 4-6 weeks of life [12]. However, mothers of infants enrolled in the study were given the option to receive once daily Nevirapine (NVP) as prophylaxis if randomized to a breastfeeding arm for the entire duration of breastfeeding in the absence of maternal 3-drug ART. However, in these guidelines, PTB is not recognized as a "high-risk" characteristic, in and of itself. Hence in Botswana, preterm HIV-exposed infants, lacking any other risk factors, continue to receive a low-risk HIV prophylactic regimen. It is thus imperative to understand the risk of HIV acquisition attributable to being born preterm. ZDV has been shown to interfere with haematopoiesis resulting in anaemia and neutropenia [15-17]. Despite this knowledge, very little data exists that describes the safety and clinical implication of its use for postnatal prophylaxis in preterm neonates with in-utero HIV exposure [18, 19]. Using data from the Mpepu study, we explored the hematologic safety of two different ARV prophylactic regimens, comparing findings between Mpepu infants born preterm to that of those born full-term.

Significance and Rationale

Associations between the use of ART in pregnancy and adverse birth outcomes, including preterm birth (PTB) and very preterm birth (VPTB) have been reported but whether or not the timing of HIV acquisition for infants born before 37 weeks completed gestational age to WLHIV differs from infants born on or after 37 weeks gestational age has not been well studied. It is thus imperative to understand the risk of HIV acquisition attributable to being born preterm as this knowledge has the potential to inform public policy relative to prophylactic strategies for preterm HIV-exposed infants potentially contributing to a reduction in infant HIV acquisition. Furthermore, findings from this study will provide data on the efficacy and haematological safety of prophylactic ARV regimens in infants born preterm.

Aim and Objectives

The primary aim of this study is to describe the differential risk of HIV acquisition in infants born preterm with *in-utero* HIV exposure using full term infants with *in-utero* HIV exposure as the comparator group and to explore any adverse haematologic implications of antiretroviral prophylaxis in the first month of life in these groups, specifically anaemia or neutropenia.

Objectives

1. To describe the prevalence and timing (*in-utero* versus perinatal) of infant HIV acquisition among HIV-exposed infants born prior to 37 weeks completed gestation age compared to those born at term in the Botswana-based Mpepu study.
2. Assess the hematologic safety of ARV prophylaxis in the first month of life among preterm infants compared to those born at term.

Hypothesis

For the first objective, we hypothesize that preterm infants will be more likely to acquire HIV, as measured by HIV infection prevalence, both at birth and at 2-6 weeks of life compared with infants born full-term. Similarly, for our second objective, we hypothesize that infants born preterm with HIV exposure will be more likely to experience anaemia with the use of ZDV compared to NVP prophylaxis, given an earlier physiological nadir of red blood cells compared with infants born at term. However, the occurrence of neutropenia will not differ between the two groups.

Methods

Study Design

The study represents a secondary analysis of data collected from a prospective cohort of children with *in-utero* HIV exposure and their mothers in Botswana enrolled into the "Mpepu study" [20]. The Mpepu study was a randomized controlled trial (RCT) that evaluated

the potential survival benefit of cotrimoxazole (CTX) prophylaxis among children exposed to HIV in the southern districts of Botswana. In the Mpepu study, children were randomly assigned to receive CTX (100 mg/20 mg once daily until age 6 months and 200 mg/40 mg once daily thereafter) or placebo from age 14–34 days to age 15 months. Infants enrolled in the study either received single-dose nevirapine plus 4 weeks of twice-daily zidovudine or 4 weeks of once-daily nevirapine alone for ARV prophylaxis.

A dataset derived from the Mpepu study data that includes maternal pregnancy demographics and delivery details, infant demographics and health status as well as full blood count results at 4 weeks of life was created explicitly for this secondary analysis. The dataset contains information extracted only from participants who consented and agreed to re-use their data/information in future related studies while participating in the Mpepu study.

Study Population

This study utilized data already collected from mother-infant pairs who consented to participation in the Mpepu study and for whom consent was provided to allow their study data to be used for other HIV-related purposes.

Sample Size

With data on 2874 HIV exposed infants (575 delivered preterm, 2299 delivered at term) we had 80% power to detect a 2-fold increase in HIV acquisition prevalence in exposed infants delivered preterm as compared to those delivered ≥37 weeks' gestation, assuming a vertical transmission rate of 2% in the population of infants born full term with in utero HIV exposure (Table 1).

Table 1: Post-Hoc Power Calculation		
Mother-to-Child transmission of HIV Prevalence in term infants	*2%	*2%
Mother-to-Child transmission of HIV Prevalence in preterm infants	*4%	*4%
Estimated Sample size for term infants	*2299	#2334
Estimated Sample size for preterm infants	*575	#532
Alpha	0.05	0.05
Power	80%	74%

* = A priori assumptions, #Observed

Study Duration and Timeline

The entire study duration will be 14 months (Table 2: Gantt Chart).

	Study period in months starting November 2019													
Activities	1	2	3	4	5	6	7	8	9	10	11	12	13	14
IRB Applications and Approvals														
Creation of data set														
Data analysis														
Presentation and publication														

Reporting and Implementation

The results of this study will be submitted to a peer-reviewed journal for publication and presented at appropriate HIV/AIDS conferences and meetings so that they can be maximally accessed by the research community and health workers providing care to infants and children with *in-utero* HIV-exposure.

Statistical Considerations

Primary Endpoints and Primary Analysis

Primary aim #1: To describe the prevalence and timing (in utero versus perinatal) of HIV acquisition among infants born prior to 37 weeks gestation in Botswana.

To compare the prevalence of HIV acquisition among infants delivered preterm, stratified by timing of acquisition (*in-utero* versus *peripartum*) using infant HIV DNA PCR study results. The number of infants seroconverting at birth or within 72 hours post-delivery will be used as the numerator for *in-utero* estimates while testing positive at 2-6 weeks after an initial negative result at birth will be defined as *peripartum* transmission. Overall, in utero, and *peripartum* infant HIV acquisition will be compared between infants born preterm and those born full-term. A Fisher exact test will be used to assess for any significant differences in the prevalence of infant HIV acquisition between preterm and term infants while the prevalence ratio (PR) will be computed to quantify the magnitude and direction of observed associations.

Primary aim #2: Assess the hematologic safety of ARV prophylaxis at one month of life among infants born preterm with *in-utero* HIV exposure. To conduct our analysis of adverse hematologic comparisons between Mpepu preterm and term infants receiving ARV prophylaxis, we will leverage the results of full blood counts with differential counts obtained from blood drawn from Mpepu infants at 1 month of life. We utilized the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Paediatric Adverse Events version 2 (November 2012) with severe anaemia and neutropenia being defined as a lab toxicity grade $\geq$ 3 according to DAIDS tables which present ranges by infant age. We will compare the prevalence of anaemia and neutropenia between Mpepu preterm and term infants using Fisher exact testing and compute the prevalence ratio to quantify the magnitude and direction of observed associations.

For both objectives, logistic regression will be used to assess potential risk factors. To assess risk factors for HIV acquisition among infants born preterm compared to those born at term and those likely to contribute to the occurrence of severe anaemia and neutropenia. Covariates

that explore both maternal and infant factors including maternal age, gravidity, mode of delivery, duration of labor, maternal ART regimen, Infant post-exposure prophylaxis, infant sex, birth weight and gestational age at birth will be included in univariable models. Covariates with either a p-value < 0.10 from the univariable analysis or significantly predictive of in-utero or peripartum HIV acquisition such as duration of labor (as reported in published literature) and our a priori assumption that preterm birth would be associated with higher odds will then be included in a multivariable analysis. Likewise, multivariable analysis for the ARV prophylaxis hematologic safety analysis will be fitted with covariates with either a p-value < 0.10 from the univariable analysis and those likely to contribute to the occurrence of anaemia and neutropenia such as randomized study treatment arm (use of CTX vs Placebo) and infant post-exposure prophylaxis (ZDV vs NVP) which were the main intervention for the Mpepu cohort" [10].

Protection of Human Subjects

Sources of Materials

Data sources was the electronic data capture system used for the Mpepu study. This encrypted database is currently housed at the Centre for Biostatistics in AIDS Research (CBAR) at the Harvard T.H. Chan School of Public Health, with access limited to authorized study personnel only.

Potential Risks to Human Subjects

We believe this to be "no more than minimal risk" to participants as it solely involves de-identified information and is not likely to pose any physical, social, or legal harm to participants.

Potential benefits of the proposed research to participants

There is no likely benefit to the original Mpepu mother-infant pairs to the data that will emerge from this study, as they will not be "active" participants given the retrospective use of data.

Brief Description of Study Procedures

For this study, a dataset created from "Mpepu" containing information extracted only from participants who consented and agreed to re-use of their data/information in future related studies was used. Records from 2874 mother-infant pair who completed the birth and enrolment visits were received from the Mpepu study database housed at the T.H Chan Harvard school of public health. All 2874 maternal and infant records were included in this analysis, this includes 9 infants who died prior to 34 days (8 with a reported gestational age at delivery and 1 without) but were randomized and had safety labs drawn prior to death and are thus included in all analysis.

Data considerations: this report is based on data transferred from Harvard School of Public Health to the Botswana Harvard AID Institute on December 17, 2018. Variables were created based on the proposed prescriptive analysis (Appendix 1) prior to data extraction from the original dataset. Data extraction occurred with Structured query language (SQL).

Data Extraction, security and Storage: data was extracted by a data manager incorporating basic error-checking principles to optimize data quality and to allow rapid querying of illogical or missing data. All data used for this project was de-identified using uniquely generated identifiers. The created dataset was stored in a database locally at the Botswana Harvard AIDS Research Institute Partnership (BHP) with access restricted to the study data manager, study principal investigator and the BHP information technology solutions and security director.

Data cleaning: We employed a multi-step data cleaning and verification process using SQL, Excel and SPSS. We used frequency tables to identify missing data, frequency distributions and SQL programmed logical checks to assess for implausible data (e.g., gestational age of 48 weeks, recorded birth weight of 4.2kg for a neonate delivered at 28 weeks). Missing or illogical data in the database was compared to source documents, specifically scanned medical records, and corrections were made where possible by the data manager only to the dataset created for this sub-study. See Appendix 2 for all corrections made during the data cleaning process.

Importance of the knowledge to be gained

This study has the potential to inform public policy relative to timing of infant HIV testing and ARV prophylaxis strategies for infants born preterm with *in-utero* HIV exposure. With as many as 1 in 5 infants born preterm following HIV exposure in resource-constrained settings with high-burden HIV epidemics, our findings may identify opportunities to tailor the timing of HIV testing based on the gestational age of an infant, as well as the optimal ARV prophylaxis regimen for preterm infants to avoid any hematologic toxicity, recognizing that anaemia can be harmful to the developing neonate's brain and neutropenia can increase risk of infectious morbidity/mortality. [21, 22] As such, any changes in practice arising from this body of work may contribute to improved survival and a reduction in overall neonatal and infant mortality.

Part II: Literature review

Introduction

Botswana provides robust HIV treatment and prevention programs free of charge to its citizens and was one of the first countries in sub-Saharan Africa to offer a comprehensive PMTCT program in 1999 [23]. Before 2016, PMTCT guidelines in Botswana recommended 3-drug ART treatment for WLHIV with CD4 cell counts < 250 cells/mm^3, 3-drug ART prophylaxis for those with CD4 cell count between 250 cells/ mm^3 and ≤350 cells/mm^3 and ZDV-alone for those with CD4 cell count >350 cells/mm^3 [12], while all exposed infants received twice daily ZDV for the first 4-6 weeks of life (Table 1)

TABLE 1: BOTSWANA PMTCT PROPHYLAXIS / TREATMENT ALGORITHM

Time Point (Year)	CD4 Count (cells/mm^3)	Gestational age (weeks)	Regimen
2010 - 2011	< 250	Any	Zidovudine + Lamivudine + Nevirapine/Efavirenz
	≥ 250	14 - 28	Zidovudine + Lamivudine + Nevirapine/Efavirenz*
	≥ 250	≥28	Zidovudine Only~
2012 - 2015	< 350	Any	Tenofovir + Emtricitabine + Efavirenz
	≥ 350	14 - 28	Tenofovir + Emtricitabine + Efavirenz*
	≥ 350	≥28	Zidovudine Only~

*Given as prophylaxis from 14 weeks until delivery
~Given as prophylaxis from 28 weeks until delivery
All exposed infants received twice daily Zidovudine as post exposure prophylaxis for 4-6 weeks

Following updated WHO recommendations in 2016, Botswana scaled up its HIV treatment and prevention program, initially expanding access to ART for pregnant WLHIV to be lifelong, but ultimately offering lifelong ART to all persons with HIV, regardless of their disease stage at the time of diagnosis [24]. These policy changes have been well implemented with over 95% of pregnant WLHIV in Botswana having access to ART [25] contributing to the exceptionally low rates of infant HIV acquisition at <2% [26]. This welcomed achievement, however, means that an increasing number of infants are now exposed to ARV drugs *in-utero*, potentially contributing to increased PTB, or birth prior to 37 weeks completed gestational age, [2-4, 7-9, 25, 27-31]. Preterm birth is associated with increased risk of infectious morbidity, mortality and developmental delays [21, 22].

Currently, PTB among infants with *in-utero* HIV exposure in Botswana is approximately 21% in comparison to 13% among HIV-unexposed infants [10, 11]. This number is expected to rise as ART use in WLHIV increases, and an increasing number of WLHIV conceive while taking ART [32-34]. In anticipation of this increase in the prevalence of PTB amongst pregnant WLHIV, it is imperative to understand if a differential risk of HIV acquisition exists among infants born preterm in contrast to those born at term, as such a finding would warrant national guidelines changes in Botswana, including a recommendation for HIV testing at birth for preterm infants born to WLHIV, for early identification of HIV acquisition and immediate initiation of treatment.

Furthermore, WHO and Botswana's PMTCT guidelines [35, 36] currently recommend a triple ARV prophylaxis strategy in the first 4 – 6 weeks of life for HIV-exposed infants deemed to be at "high risk" of HIV acquisition. In these guidelines, PTB is not recognized as a "high risk" characteristic, in and of itself. Therefore, in Botswana, infants with *in-utero* HIV exposure who are born preterm, lacking any other maternal risk factors, are recommended to receive a "low-risk" HIV prophylactic regimen of either twice daily dosing of ZDV (plus sdNVP within 72 hours of birth) or once-daily dosing of NVP for the first 28-30 days of life. Understanding how effective and safe the current prophylactic regimen is both in preventing infant perinatal HIV acquisition and minimizing hematologic disturbances in early life is of public health importance to ensuring the health, wellbeing and survival of infants born preterm to WLHIV.

Background

This literature review investigates the prevalence and timing of MTCT of HIV among infants born preterm, as compared to those born at term to WLHIV in the setting of widespread maternal ART use. In addition, published literature evaluating hematologic effects associated with infant post-exposure ARV prophylaxis regimens used for PMTCT among HIV-exposed infants born preterm is summarized.

The objectives of the literature review are:

- To identify the prevalence of infant HIV acquisition among infants born prior to 37 weeks completed gestational age
- To explore the timing of HIV acquisition in infants born prior to 37 completed weeks gestational age, comparing rates amongst infants with positive HIV DNA PCR at birth or within 72 hours post-delivery (for in utero transmission) with rates at 4-6 weeks post-delivery (for *peripartum* transmission).
- To describe risk factors associated with HIV acquisition in preterm and term infants
- To compare the prevalence of grade 3 or higher anaemia through 4 weeks of life between preterm and term infants with *in-utero* HIV exposure based on the prophylactic ARV regimen received by infant using the DAIDS Table for Grading the Severity of Adult and Paediatric Adverse Events version 2 (November 2012).
- To compare the prevalence of grade 3 or higher neutropenia through 4 weeks of life between preterm and term infants with HIV exposure based on the prophylactic ARV regimen received by the infant using the DAIDS Table for Grading the Severity of Adult and Paediatric Adverse Events version 2 (November 2012).

Literature search strategy

A search of the Medline bibliographic database using the PubMed interface and the Cochrane Database of Systematic Reviews was performed using the following search terms:

1. Preterm birth and HIV
2. Antiretroviral therapy and preterm birth
3. Prevalence of HIV in preterm HIV-exposed infants
4. Predictors of HIV acquisition in HIV-exposed infants
5. Post exposure prophylaxis in HIV-exposed infants
6. Antiretroviral prophylaxis safety in HIV-exposed infants
7. Anaemia in HIV-exposed infants
8. Neutropenia in HIV-exposed infants

Inclusion criteria

- Population studies that included pregnant WLHIV and their children
- Studies reporting on factors influencing MTCT of HIV in resource-limited settings
- Studies describing pregnancy outcomes in pregnant WLHIV
- Studies describing post-exposure prophylaxis in HIV-exposed children
- Studies outlining the safety of ARV prophylaxis in HIV-exposed children
- Studies with full articles and published in English

Exclusion criteria

- Studies focusing on retention in care, therapeutic drug monitoring, pharmacokinetics and pharmacodynamics of ARVs
- Cost-effectiveness studies

The initial search was restricted to only studies published in English between January 2010 and Dec 2021. Titles and abstracts were screened for relevance, following which 94 articles which met the inclusion criteria were reviewed. Additional 16 studies were identified through searching bibliographies of included articles.

Summary and interpretation of literature

Preterm delivery in pregnant WLHIV

There is a significant body of data reporting increased rate of adverse birth outcomes such as stillbirths (SB) [2], intrauterine growth restriction (IUGR) [4], small for gestational age (SGA) [32], preterm births and neonatal death as pregnancy outcomes among WLHIV when compared to pregnant uninfected women, with maternal HIV and use of ART in pregnancy implicated as independent risk factors for adverse birth outcomes [2, 11, 32].

By far the most common adverse birth outcome in WLHIV is preterm birth [3, 4, 8, 37-43]. Importantly, PTB is associated with increased neonatal morbidity and mortality [44-46], higher risk of neurodevelopment delays, and permanent disabilities in the new-born [47-50]. WLHIV have-a 2-4-fold increase in risk of preterm delivery when compared to their uninfected

counterparts [38]. Regionally, preterm delivery rates in WLHIV remains high ranging from 10.4% in South Africa [39], 21% in Botswana [7], to 24% in Tanzania [40].

Exposure and preterm delivery

The benefits of ART in preventing infant HIV acquisition are undisputed. However, the potential mechanisms of PTB from *in utero* exposure to HIV and/or ART must be elucidated in order to ensure that the regimens recommended in pregnancy optimize the health of the mother, foetus and child. It is estimated that for every 100 HIV transmissions prevented through the use of maternal ART, 22 additional preterm deliveries occur, highlighting the significance of this issue [30]. Overall, findings for the presumed association between *in-utero* exposure to ARV drugs and PTB are highly heterogeneous; some studies found no evidence of associations [3, 41-43], while others found increased risk of PTB with exposure to ARVs [31, 32, 51-54]. Factors implicated for this observed heterogeneity include differences in ARV drug classes, the timing of ART initiation in pregnant WLHIV (preconception versus 1st trimesters versus late pregnancy), inadequately powered studies, poor methodology for gestational age dating of pregnancies, and study designs which only enrolled women after 36 weeks gestation, thus potentially understating the prevalence of PTB due to selection bias [30, 55].

Current guidelines recommend initiation of ART consisting of at least 3 drugs from two classes of ARV drugs for all persons living with HIV, including pregnant WLHIV. The usual regimen recommended includes a combination of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) along with a non-nucleoside reverse transcriptase inhibitor (NNRTIs), a protease inhibitor (PI) or, more recently, an integrase strand transfer inhibitor (INSTI). Among these ARV drug classes, some (more than others) have been associated with increased PTB, with P.I's implicated more often than others [7, 31, 56]. Studies in Botswana have further demonstrated higher risk of PTB among pregnant WLHIV on older NRTIs, such as the combination of zidovudine and lamivudine, compared to the use of newer NRTIs such as tenofovir and emtricitabine. Similarly, with regimens containing NNRTIs, there has been a trend towards increased PTB risk among women receiving nevirapine in pregnancy compared

to efavirenz [2]. More recently, data suggests that the use of dolutegravir, an INSTI, as part of an ART regimen may reduce the prevalence of PTB in WLHIV to those reported in women without HIV [57-59].

Regarding associations between timing of ART initiation and PTB, several studies have reported mixed results, with some studies showing increased rates of PTB with 1st rather than 2nd or 3rd-trimester maternal initiation of ART, particularly PI-based regimen [3, 7, 41] and others showing no difference in the rates of PTB regardless of whether ART was initiated prior to conception or during pregnancy [9, 30, 32]. In Botswana, evidence suggests an increase prevalence of PTB in pregnant WLHIV with pre-conception use of a PI-based ART regimen [2, 7].

PTB and HIV acquisition risk

Studies have consistently reported higher rates of vertical transmission with *in-utero* exposure to HIV in infants who are born preterm compared to those born full term [37, 60, 61]. Preterm HIV acquisition rates varied in the literature, ranging from 1.6% – 31% [60, 62-64]. However, only a handful of studies have attempted to postulate the mechanism between PTB and the risk of HIV acquisition. Candyce et al [60], linked the increased vulnerability of HIV acquisition in preterm infants to the thin and friable skin and mucous membranes, especially when infants are exposed to conditions such as chorioamnionitis, prolonged rupture of membranes, or vaginal birth. Kuhn et al [63] added a possibility of increased vulnerability to HIV acquisition among preterm infants being associated with a failure to acquire protective levels of neutralizing antibodies, since the passive transfer of maternal antibodies across the placenta may occur late in pregnancy.

Similarly, very few studies exist that describe the timing of vertical HIV transmission among infants born preterm. Kuhn et al [63], reported a strong association between PTB and intrapartum transmission (“relative risk (RR)” 3.7; “95% confidence interval (CI)”, 2.2–6.1), and concluded that preterm infants may be more vulnerable to HIV acquisition at delivery than earlier in the intrauterine environment.

It thus appears from literature that gaps remain in our understanding of the timing and risk of HIV acquisition among infants born preterm compared to infants born full-term. Additionally, much of the published literature dates back to an era when HIV care guidelines only advocated for ART in pregnancy for WLHIV with low CD4 cell counts or AIDS-defining illnesses. It is thus imperative to conduct more studies to disentangle this seemingly complex association, particularly in cohorts where women are receiving currently recommended and newer regimens, such as INSTI-based ART.

Post-exposure prophylaxis in HIV-exposed children

Since results from the AIDS Clinical Trials Group (ACTG) 076 trial were published in 1994 [65], maternal use of ARV drugs, as well as, neonatal ARV prophylaxis represent the cornerstone in preventing infant HIV acquisition. Several studies have quantified value of reducing infant HIV acquisition through dosing of infant ARV post-exposure prophylaxis to protect infants against HIV acquisition in PMTCT programs, with findings of a reduction of HIV acquisition by 25%–42% in the absence of maternal use of ARV drugs, to < 2% in settings where maternal ART is used in pregnancy, with infant prophylaxis initiated at birth and continued for 4 to 6 weeks. The studies provide evidence-based support of guidelines advocating for infant ARV prophylaxis as an important integral component of PMTCT of HIV [35, 66].

Currently, WHO recommends once-daily dosing of NVP or twice-daily ZDV started within 72 hours of birth and continued for 4-6 weeks of life as options for infant post-exposure prophylaxis [13]. The effectiveness of this approach was first shown in a study conducted by the New York State Department of Health. This study showed that early initiation of ZDV in the first 48 hours of life (as compared with the use of no ZDV prophylaxis) was significantly associated with a reduction in the risk of HIV transmission (RR - 0.30, C.I - 0.10–0.96) [67]. Since then, more studies have corroborated this finding amongst which are HPTN 040/PACTG 1043 [68], the NVAZ trial [69], and a randomized control trial conducted in South Africa [70].

In all these studies, adding neonatal prophylaxis as a component of PMTCT was more efficacious in preventing MTCT than when infant post-exposure prophylaxis was not employed.

Taking things a step further, many more studies like the Prevention of Milk-Borne Transmission of HIV-1 in Botswana (Mashi) [71], Six Weeks of Extended Nevirapine (SWEN) [72], the Post-Exposure Prophylaxis of Infant (PEPI) [73], Prevention of mother-to-child transmission of HIV-1 through breast-feeding by treating infants prophylactically with lamivudine in Dar es Salaam, Tanzania (MITRA) [74] and more recently the Effect of co-trimoxazole on mortality in HIV-exposed but uninfected children in Botswana (the Mpepu Study) [20] and the Promoting Maternal and Infant Survival Everywhere Study. Poster (PROMISE) [75] studies evolved. Findings from these studies have continued to validate the value of infant prophylaxis and provided evidence to support the use of extended prophylaxis as a feasible approach that could further reduce rates of vertical transmission in breastfed infants of WLHIV. Though these studies varied greatly in their approach in terms of regimens compared and duration of administration, most concluded that NVP was a slightly more effective regimen than ZDV as monotherapy for post-exposure prophylaxis in extended use [20, 73, 75]. In addition, and perhaps more significant, findings from these studies added to our understanding on key maternal and infant risk factors associated with vertical transmission, introducing the concept of HIV acquisition risk stratification [76]. Under the concept or risk stratification guide , infants were broadly assigned into 2 categories; a “high risk” category comprising infants with identifiable risk factors for HIV acquisition such as maternal lack of ART use during pregnancy, maternal ART initiation late in pregnancy, or presence of a detectable maternal viral load in the third trimester of pregnancy for women initiating treatment in pregnancy or a detectable viral load at any time in pregnancy for women who conceive on ART, and a “low risk” category made up of all other HIV exposed infants. With these new recommendations, infants at highest risk for vertical transmission should be receiving a three-drug prophylactic ARV regimen, while those with low risk of vertical transmission receive the

once daily dosing of NVP or twice daily dosing of ZDV started within 72 hours of birth, with either option continued for the first 4-6 weeks of life or longer.

Put together, evolving WHO guidelines on post exposure prophylaxis has been guided by the results of randomized controlled clinical trials. Current WHO recommendations call for once-daily NVP for 6 weeks among breastfeeding infants and 4–6 weeks of once-daily NVP or twice-daily ZDV among non-breastfeeding infants for post-exposure prophylaxis [13].

Neonatal and Infant post-exposure prophylaxis safety

Safety data on ZDV and NVP used in the neonatal period for prevention of infant HIV acquisition are reassuring [19, 77, 78]. However, most of the reassuring data has been from studies that excluded infants born preterm. ZDV is the ARV agent with the most safety data in the neonatal population. The most common adverse events associated with ZDV prophylaxis in the new-born period includes transient and mild anaemia and neutropenia, which usually resolves by 12 weeks of age [18]. Less common are reports of mitochondrial toxicities associated with postnatal use of ZDV (and other NRTIs). While some studies such as the Perinatal AIDS Collaborative Transmission Study [79] and a review of 5 US cohorts with a combined population of over 10,000 children exposed to NRTIs [80], have not found perinatal ARV exposure to be associated with mitochondrial dysfunction others have upheld this possibility, especially with ZDV [81].

As with ZDV, NVP use for post exposure prophylaxis (PEP) has also been found to be largely safe, with very few cases of mild and transient anaemia and neutropenia reported [82, 83]. Although there is an increased risk of hepatotoxicity, as well as rash and hypersensitivity reactions observed in adults upon NVP initiation, this has not been an issue among infants receiving NVP for PEP [84-86]. However, concerns still exist about the development of resistance among infants exposed to NVP, should vertical transmission occur given the low barrier of resistance of this NNRTI [87, 88].

There are limited safety data for multiple-agent ARV prophylaxis regimens for HIV-exposed infants, particularly for the recommended triple ARV regimen used in infants deemed to be at high risk for HIV acquisition. These data, are so far reassuring for neonates born at term with reports suggesting that they are generally well tolerated despite a higher incidence of non-specific signs and symptoms and early treatment discontinuation [89, 90]. Little is known on whether or not these findings apply or can be replicated in neonates born preterm, as limited data to this effect exists. Importantly, many guidelines do not recommend use of various ARVs in infants born less than 35 weeks gestational age.

Needs for future research

Overall, and despite the mixed data and ambiguity, more evidence points towards increase rates of PTB in WLHIV with pre-conception use of a PI based ART. Furthermore, due to study design and the evolving landscape of preferred ART regimens for use among pregnant WLHIV, the literature around timing and prevalence of HIV-acquisition for infants born preterm does not yet provide consistent findings. It is thus imperative to conduct well designed studies that enrol women sufficiently early in pregnancy to document the occurrence of preterm birth, that use sound methods of pregnancy dating, are adequately powered to detect clinically meaningful difference in pregnancy outcomes by timing of maternal ART initiation, either prior to conception or by trimester of pregnancy, and with robust measurement of known confounders such as prior history of preterm birth, pre-pregnancy body mass index (BMI), gestational weight gain and concomitant use of alcohol or illicit substance in pregnancy in order to understand if ART regimens currently recommended for use by national and WHO guidelines provide adequate protection to prevent infant HIV acquisition whether an infant is born prior to or after 37 weeks gestational age. Also, despite the presence of sound evidence on the safety and efficacy of infant post exposure prophylaxis with ZDV and / or NVP (even when used for extended periods), most of the data has been published by studies evaluating HIV exposed infants delivered at term ($\geq$ 37 weeks' gestation). With the increased prevalence of preterm birth associated with maternal use of ART, research is needed to establish the safety

and efficacy of ZDV and NVP when used for infants born preterm. Lastly, as the use of triple ARV prophylaxis in exposed infants deemed to be high risk gains momentum, there is need to evaluate the immediate and long-term safety profile of this approach particularly when used in infants born preterm.

Conclusion

The variability of findings and opinions from the literature highlights the gaps in our understanding of the timing and risk of HIV acquisition among infants born preterm, bringing to light the need to conduct more studies to disentangle this seemingly complex association. Such studies would need to ensure that women are recruited early in pregnancy to aid the documentation of preterm birth and adequately powered to detect clinically meaningful difference in pregnancy outcomes by timing of maternal ART initiation.

Also, more research is required to gain a better understanding on infant post exposure prophylaxis safety particularly with evolving World Health Organization (WHO) guidelines.

Part III: Manuscripts

Manuscript 1: No Increased In-utero and Peripartum HIV acquisition risk in HIV-exposed infants delivered preterm

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Abstract

Background: Limited data exists on the differential risk of HIV acquisition between infants born preterm (< 37 weeks gestation) versus those born at term (≥ 37 weeks' gestation) to women living with HIV (WLHIV). With a reported increase in preterm delivery among pregnant WLHIV, it is important to understand risks associated with vertical transmission of HIV in infants born preterm versus those born full term as this would inform timing of early infant diagnosis testing recommendations and antiretroviral (ARV) prophylaxis strategies.

Methods: Using data extracted from a placebo-controlled randomized trial designed to assess the survival benefit of cotrimoxazole (CTX) use among HIV-exposed infants in Botswana (the Mpepu Study), we describe the prevalence, timing and risk factors for HIV acquisition in infants born preterm versus those born term. Fisher exact testing was used to test for difference in prevalence and timing of infants acquiring HIV overall in the first 34 days of life, as well as at birth or within 72 hours post-delivery (for *in-utero* estimates) and between 14-34 days after an initial negative result at birth (for *peripartum* estimates), with prevalence ratios (PR) calculated to ascertain the magnitude and direction of observed associations. A multivariable logistic regression model was used to assess risk factors for infant HIV acquisition.

Results: Two thousand eight hundred and sixty-six HIV infants born to women living with HIV in the Mpepu study were included in this secondary analysis. Of these, 532 (19%) were born preterm, while 2334 (81%) were born full-term. There was no observed difference in the prevalence of HIV acquisition among infants born preterm versus term overall (0.8% versus 0.6%, p=0.54, PR=1.33), at birth (0.2% versus 0.3%, p=1.00, PR=0.67) or between 14-34 days post-delivery (0.6% versus 0.3%, p=0.41, PR=2). The only significant predictor of HIV acquisition in both preterm and term infants was absence of maternal antiretroviral use during pregnancy, with risk of HIV acquisition reduced by 96% among infants whose mothers were taking antiretroviral treatment (ART) during pregnancy (aOR: 0.003, C.I: 0.01 - 0.02, p<0.001).

Conclusions: In our cohort with widespread use of ART in pregnancy, there was no observed increase of *in-utero* and *peripartum* HIV acquisition among infants born preterm following foetal exposure to HIV compared to those born at term.

***Word count**: 347*

Introduction

Botswana, provides a robust HIV treatment and prevention programming free of charge to its citizens and was one of the first countries in sub-Saharan Africa to offer comprehensive Prevention of Mother-to-Child Transmission of HIV (PMTCT) programming [1, 2]. Before 2016, PMTCT guidelines in Botswana recommended 3-drug ART (Zidovudine/Lamivudine/Nevirapine modified to Tenofovir/Emtricitabine/Efavirenz in 2012) for pregnant women with CD4 cell count 200 - ≤350 cells/mm^3 and ZDV-alone for those with CD4 cell count >350 cells/mm^3 from 28 weeks gestational age till delivery and end of breastfeeding period for those opting to breastfeed [3], however, participants enrolled in the study were given the option to receive once daily NVP as prophylaxis if randomized to a breastfeeding arm for the entire duration of breastfeeding in the absence of maternal 3-drug ART. Following updated World Health Organization (WHO) recommendations in 2016, Botswana scaled up its HIV treatment and prevention programming, initially expanding access to antiretroviral treatment (ART) for pregnant women living with HIV (WLHIV) to be lifelong, but ultimately offering lifelong ART to all persons with HIV, regardless of their disease stage at time of diagnosis [4]. These policy changes have been well implemented with over 95% of pregnant WLHIV in Botswana having access to ART [5] resulting in a low vertical transmission rate of <2% [6]. This welcomed achievement, however, means that an increasing number of infants are now exposed to antiretroviral drugs (ARVs) *in-utero*, with ARVs potentially contributing to increased birth prior to 37 weeks gestational age, or preterm birth (PTB), [7-17].

Currently, PTB among HIV-exposed infants in Botswana is approximately 22% in comparison to 13% among HIV-unexposed infants [18-20]. This figure is expected to rise as ART use in WLHIV increases, and an increasing number of WLHIV conceive while taking ART. In anticipation of this increase in PTB rates amongst pregnant WLHIV, it is imperative to understand if a differential risk of HIV-acquisition exist among HIV-exposed infants born preterm in contrast to those born at term, as such a finding might warrant birth testing of all preterm infants born to women living with HIV.

Furthermore, WHO and Botswana's PMTCT guidelines [4, 21] currently recommend a triple ARV prophylaxis strategy in the first 4 – 6 weeks of life for HIV-exposed infants deemed to be at "high risk" of HIV-acquisition. In these guidelines, PTB is not recognized as a "high risk" characteristic, in and of itself. Therefore, in Botswana, preterm HIV-exposed infants, lacking any other risk factor, receive a low-risk HIV prophylactic regimen of single-dose nevirapine within 72 hours of birth and twice daily dosing of zidovudine for the first 28-30 days of life. The primary aim of this paper is to describe the differential risk of HIV acquisition in infants born preterm with *in-utero* HIV exposure using infants born at term with *in-utero* HIV exposure as the comparator group

Methods

This study represents a secondary analysis of data collected from a prospective cohort of HIV-exposed children and their mothers in Botswana enrolled into the "Mpepu study". The Mpepu study was a randomized controlled trial (RCT) evaluating the potential survival benefit of cotrimoxazole (CTX) prophylaxis among HIV-exposed but uninfected (HEU) children in Botswana [22]. Per the Botswana government's Prevention of Mother-to-Child Transmission of HIV (PMTCT) programming at the time, guidelines advocated that pregnant WLHIV receive three-drug ART during pregnancy and breastfeeding. All neonates with HIV exposure received single dose Nevirapine (sdNVP) at birth plus Zidovudine (ZDV) for prophylaxis in the first 4 weeks of life. Infant HIV DNA PCR testing was performed at the birth and

randomization study visit, per protocol. For infants with an initial positive HIV DNA PCR test, repeat confirmatory testing was performed. A positive confirmatory test was the criteria for infant HIV diagnosis. *In-utero* transmission was deemed to have occurred if positive PCR results were obtained from samples tested at birth coupled with a positive confirmatory test and presumed *peripartum* HIV acquisition if the positive PCR result was obtained at randomization (14-34 days of life) or later in life after an initial negative result at birth.

From the curated dataset, we describe the prevalence, timing and risk factors for HIV acquisition in infants born preterm versus those born term. Fisher exact testing was used to compare the prevalence and timing of infants seroconverting at birth or within 72 hours post-delivery (for *in-utero* estimates) and those testing positive at 14-34 days after an initial negative result at birth (for *peripartum* estimates), while risk for HIV acquisition was assessed using logistic regression. Preterm was defined as birth earlier than 37 weeks gestation.

Statistical Considerations

To compare the prevalence of HIV acquisition among infants delivered preterm, stratified by timing of acquisition (*in-utero* versus *peripartum*) using infant HIV DNA PCR study results, the number of infants seroconverting at birth or within 72 hours post-delivery was used as the numerator for *in-utero* estimates while those testing positive at 2-6 weeks after an initial negative result at birth was used for *peripartum* transmission. Overall, *in-utero*, and *peripartum* infant HIV acquisition was compared between infants born preterm and those born full-term. Fisher exact test was used to assess for any significant differences in the prevalence of infant HIV acquisition between preterm and term infants while the prevalence ratio was computed to quantify the magnitude and direction of observed associations.

Logistic regression was used to assess potential risk factors for HIV acquisition among infants delivered preterm. Covariates that explore both maternal and infant factors including maternal age, gravidity, mode of delivery, duration of labor, maternal ART regimen, Infant post-exposure prophylaxis, infant sex, birth weight and gestational age at delivery were

included in univariable models. Covariates with either a p-value ≤ 0.10 from the univariable analysis or significantly predictive of *in-utero or peripartum* HIV acquisition were then included in a multivariable analysis adjusting for our a priori assumption that preterm birth would be associated with higher odds.

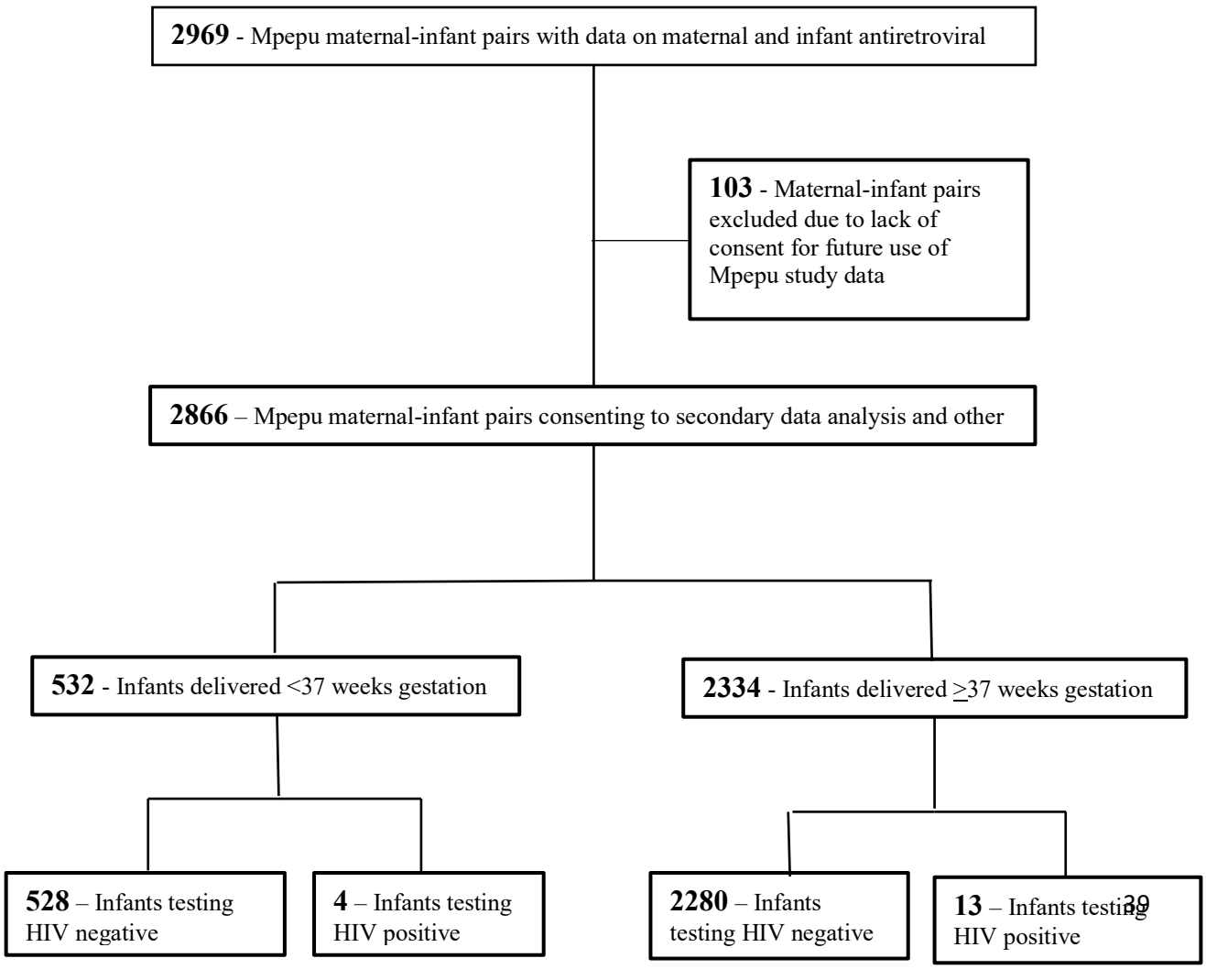
Ethical Considerations

The Health Research Development Committee of the Botswana Ministry of Health and Wellness (HPDME 13/18/1) and the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (HREC 591/2020) provided regulatory overview and approval for this secondary analysis. Consent verification for use of data in future studies occurred for all participants included in this analysis prior to use of their data.

Results

Data on 2866 of 2969 mother infants' pairs consenting to secondary use of data while participating in the Mpepu study was used for this analysis (Fig 1).

Figure 1: Study flowchart



Of the 2866 included in this analysis, 2334 (81%) were born at ≥37 weeks' gestation, while 532 (19%) were born prior to 37 weeks completed gestational age. One thousand four hundred and sixty-one (51%) were females while 1405 (49%) were males. Median birth weight was 2.9kg (Range 1, 4.6 kg), with 493 (17.2%) weighing < 2.5kg (Low birth weight) at birth. Equal proportions of children were randomized to receive either CTX or placebo [1432 (50%) and 1434 (50%)] respectively, with randomization occurring at a median age of 28 days (Range 14, 34). Most infant's formula fed (79.1%), with only 20.6% breastfed from birth, 0.3% mixed fed. Average maternal age was 30 years (Range 18, 47 years) and most mothers (82.5%) received a 3-drug ART regimen either for prophylaxis or treatment during pregnancy, while 353 (12.3%) received just Zidovudine and 149 (5.2%) did not receive any treatment (Table 1).

Overall, 17 infants became HIV-infected giving an MTCT rate of 0.6%. There was no observed difference in the prevalence of HIV between infants born preterm versus those born term overall (0.8% versus 0.6%, p=0.54, PR=1.33), at birth (0.2% versus 0.3%, p=1.00, PR=0.67) and at 14-34 days post-delivery (0.6% versus 0.3%, p=0.41, PR=2).

Table 1: Demographics by Infant HIV Status at birth and 14-34 days post-delivery in children enrolled in the Mpepu study Botswana

		HIV status (Overall)				HIV status at birth			HIV status at 14-34 days		
		Total N = 2866	Negative N = 2849	Positive N = 17	p-value	Negative N = 2859	Positive N = 7	p-value	Negative N = 2849	Positive N = 10	p-value
Enrolment site	Gaborone	1698 (59.2)	1689 (59.3)	9 (52.9)	0.306	1694 (59.3)	4 (57.1)	0.697	1689 (59.3)	5 (50.0)	0.418
	Lobatse	214 (7.5)	214 (7.5)	0 (0.0)		214 (7.5)	0 (0.0)		214 (7.5)	0 (0)	
	Molepolole	954 (33.3)	946 (33.2)	8 (47.1)		951 (33.3)	3 (42.9)		946 (33.2)	5 (50.0)	
Infant Sex	Female	1461 (51.0)	1453 (51.0)	8 (47.1)	0.811	1459 (51.0)	2 (28.6)	0.279	1453 (51.0)	6 (60.0)	0.754
	Male	1405 (49.0)	1396 (49.0)	9 (52.9)		1400 (49.0)	5 (71.4)		1396 (49.0)	4 (40.0)	
Birth weight category	Low birth weight (<2.5 kg)	493 (17.2)	490 (17.2)	3 (17.6)	1	493 (17.2)	0 (0.0)	0.612	490 (17.2)	3 (30.0)	0.391
	Normal birth weight (≥2.5 kg)	2373 (82.8)	2359 (82.8)	14 (82.4)		2366 (82.8)	7 (100)		2359 (82.8)	7 (70.0)	
Infant prophylaxis	ZDV	1566 (54.7)	1553 (54.5)	13 (76.5)	0.341	-	-	-	1553 (54.5)	7 (70.0)	0.795
	NVP	1259 (43.9)	1255 (44.0)	4 (23.5)		-	-		1255 (44.1)	3 (30.0)	
	Unknown	41 (1.4)	41 (1.4)	0 (0.0)		-	-		41 (1.4)	41 (1.4)	
Gestational age at delivery category*	<37 weeks	532 (18.8)	528 (18.8)	4 (23.4)	0.544	531 (18.8)	1 (14.3)	1	528 (18.8)	3 (30.0)	0.411
	≥37 weeks	2293 (81.2)	2280 (81.2)	13 (76.5)		2287 (81.2)	6 (85.7)		2280 (81.2)	7 (70.0)	
Maternal Age	Mean (years)	30	30	27	0.01	30	27	0.109	30	27	0.042
	S. D (years)	6	6	4.5		6	6		6	4	
	Min, Max (years)	18, 47	18, 47	18, 34		18, 47	18, 34		18, 47	18, 33	
Delivery Mode	Elective caesarean section	128 (4.5)	128 (4.5)	0 (0.0)	0.245	128 (4.5)	0 (0)	0.561	128 (4.5)	0 (0)	0.437
	Emergent caesarean section	277 (9.7)	277 (9.7)	0 (0.0)		277 (9.7)	0 (0)		277 (9.7	0 (0)	

	Vaginal delivery	2461 (85.9)	2444 (85.8)	17 (100)		2454 (85.8)	7 (100)		2444 (85.8)	10 (100)	
Duration of labour	0-<6 hrs	755 (31.5)	751 (31.5)	4 (28.6)	0.188	754 (31.5)	1 (20.0)	0.032	751 (31.5)	3 (33.3)	0.27
	6-12 hrs	986 (41.1)	978 (41.0)	8 (57.1)		984 (41.1)	2 (40.0)		978 (41.0)	6 (66.7)	
	12-24 hrs	613 (25.5)	612 (25.6)	1 (7.1)		612 (25.5)	1 (20.0)		612 (25.6)	0 (0)	
	>24 hours	46 (1.9)	45 (1.9)	1 (7.1)		15 (1.9)	1 (20.0)		45 (1.9)	0 (0)	
Gravidity	Grand Multigravida	77 (2.7)	77 (2.7)	0 (0.0)	0.728	77 (2.7)	0 (0)	0.495	77 (2.7)	0 (0)	0.807
	Multigravida	2391 (83.4)	2377 (83.4)	14 (82.4)		2386 (83.5)	5 (71.4)		2377 (83.4)	9 (90.0)	
	Primigravida	398 (13.9)	395 (13.9)	3 (17.6)		396 (13.9)	2 (28.6)		395 (13.9)	1 (10.0)	
Delivery regimen	None	149 (5.2)	133 (4.7)	16 (94.1)	<0.001	142 (5.2)	7 (100)	<0.001	133 (4.7)	9 (90.0)	<0.001
	ZDV	353 (12.3)	353 (12.4)	0 (0.0)		353 (12.3)	0 (0)		353 (12.4)	0 (0)	
	HAART	2364 (82.5)	2363 (82.9)	1 (5.9)		2364 (82.5)	0 (0)		2363 (82.9)	1 (10.0)	
Maternal level of education	None	54 (1.9)	53 (1.9)	1 (5.9)	0.413	54 (1.9)	0 (0)	0.905	53 (1.9)	1 (10.0)	0.178
	Primary	410 (14.3)	409 (14.4)	1 (5.9)		409 (14.3)	1 (14.3)		409 (14.4)	0 (0)	
	Junior Secondary	1627 (56.8)	1616 (56.7)	11 (64.7)		1622 (56.7)	5 (71.4)		1616 (56.7)	6 (60.0)	
	Senior Secondary	556 (19.4)	552 (19.4)	4 (23.5)		555 (19.4)	1 (14.3)		552 (19.4)	3 (30.0)	
	Tertiary	219 (7.6)	219 (7.7)	0 (0.0)		219 (7.7)	0 (0)		219 (7.7)	0 (0)	
House hold electricity	No	1329 (46.4)	1319 (46.3)	10 (58.8)	0.337	1324 (46.3)	5 (71.4)	0.261	1319 (46.3)	5 (50.0)	1
	Yes	1537 (53.6)	1530 (53.7)	7 (41.2)		1535 (53.7)	2 (28.6)		1530 (53.7)	5 (50.0)	

* priori assumption that preterm birth would be associated with higher odds of HIV acquisition

In univariable analysis, we observed a trend toward increased odds of infant HIV acquisition (*in-utero and peripartum*) based on mothers' age at the time of delivery (OR: 0.90, p = 0.010) and ART use during pregnancy (OR: 0.004, p <0.001) in WLHIV (Table 2). Odds of an infant being HIV positive by 34 days of life decreased as maternal age increased by a year (OR: 0.89, p = 0.05) and in infants born to WLHIV who were on ART for prophylaxis or treatment during the index pregnancy (OR: 0.006, p <0.001). Stratified by timing of HIV acquisition (*in-utero* versus *peripartum*), infants born to WLHIV with a duration of labour lasting over 24 hours had increased odds of acquiring HIV *in-utero* (OR: 10.42, p = 0.03) (Table 2).

In multivariable analysis that included maternal age, delivery regimen, duration of labor (covariates with either a p value $\leq$ 0.10 from the univariable analysis or significantly predictive of *in-utero or peripartum* HIV acquisition) and adjusting for our a priori assumption that preterm birth would be associated with higher odds of infant HIV acquisition, maternal receipt of triple antiretroviral treatment in pregnancy was significantly protective against infant HIV acquisition, decreasing the odds by approximately 96% (aOR: 0.003, C.I: 0.001 - 0.02, p<0.001) (Table 2). Thus, absence of antiretroviral regimens received by women during pregnancy was the only measured covariate in multivariable modelling that was independently predictive of HIV acquisition in children born to WLHIV in the first month of life.

Table 2: Predictors of Positive HIV Status

Overall				
Predictor	**Univariable analysis**		**Multivariable analysis**	
	OR (95% C.I),	**p-value**	**aOR, (95% C.I)**	**p-value**
Maternal Age	0.90 (0.82– 0.98)	0.010	0.94, (0.85 – 1.04)	0.210
Delivery regimen (No ART = Ref)	0.004 (0.001 – 0.03)	<0.001	**0.003, (0.001 – 0.02)**	**<0.001**
Duration of labour (0 – 24 hours = Ref)	3.89, (0.51 – 30.0)	0.190	4.97, (0.35 – 70.43)	0.240
Gestational age category (<37 = Ref)	0.75, (0.24 – 2.32)	0.620	2.09, (0.63 – 6.92)	0.230
***In-utero* HIV acquisition**				
Duration of labour (0 – 24 hours = Ref)	10.42, (1.23 – 88.35)	0.031	**10.17, (1.20 – 86.31)**	**0.031**
Gestational age category (<37 = Ref)	1.39, (0.17 – 11.59)	0.756	1.35, (0.16 – 11.28)	0.783
***Peripartum* HIV acquisition**				
Maternal Age	0.89, (0.80 – 0.99)	0.051	0.93, (0.82 – 1.06)	0.272
Delivery regimen (No ART = Ref)	0.006, (0.001 – 0.05)	<0.001	**0.006, (0.001 – 0.05)**	**<0.001**
Gestational age category (<37 = Ref)	0.54, (0.14 – 2.10)	0.374	1.55, (0.38 – 6.42)	0.542

NB: Only covariates with either a p-value < 0.10 from the univariable analysis or significantly predictive of in-utero or peripartum HIV acquisition and gestational age at birth were included in the multivariable analysis.

Discussion

From our analysis, we found no difference in the prevalence of HIV in HIV-exposed infants delivered preterm when compared to those delivered at term in the first month of life (0.8% versus 0.6%), and when stratified by timing of infection [at birth (0.2% versus 0.3%) and at 14-34 days post-delivery (0.6% versus 0.3%)] as postulated by the very few studies that have looked at this [23-26]. Though we observed an increase trend in HIV acquisition rate from

0.2% *in-utero* to 0.6% in the *peripartum* period among infants delivered preterm as against no increase at all in those delivered term, this observed increase was non-significant. We however, acknowledge that this non-significant increase observed could have been due to the very low vertical transmission rate in our cohort and cautiously interpret this finding to be a true reflection of things in similar settings with widespread use of ART for PMTCT such as ours.

Our finding also suggests non-use of antiretroviral by women during pregnancy being the only measured covariate in our cohort that was independently predictive of HIV acquisition in children born to WLHIV re-affirms several other similar findings in literature [27-30]. This also confirms that scaling up of cART within programs could help eliminate MTCT. Though lower than the reported 95% ART coverage from the Botswana national program [31, 32], use of ART during pregnancy remained high at 82.5% in our cohort, this can be attributable to the fact that our study was conducted during the initial phase of the transitioning to triple ART use for PMTCT in Botswana. We reported a much lower MTCT rate of 0.6% in our cohort compared to the <2% reported nationally [6] confirming the effectiveness of triple ART in PMTCT as reported by several other studies globally and within the region [33-36]. Similarly, we observed a high preterm delivery rate of 19% in WLHIV in a setting of high ART coverage consistent with reports from several other studies [16, 17, 37-40] and corroborates findings suggesting *in-utero* exposure to HIV and ART as possible drivers of the increased preterm delivery rates observed in WLHIV.

As mentioned above, the main limitation of this study is the very low MTCT rate observed, this could have reduced our power in detecting a true difference in risk of HIV acquisition between HIV exposed infants delivered preterm versus those delivered term. Also, our analysis was limited to 34 days post-delivery which could have led to underestimation of *peripartum* HIV acquisition rates in our cohort. This however is unlikely as participants in the Mpepu study were followed up for a period of 18 months and our review of the 18-month infant HIV status within the study did not report any new seroconversion occurring.

Conclusion

In settings with low MTCT rates attributable to widespread use of triple ART regimen in pregnancy as in Botswana, there was no observed increase HIV acquisition risk overall, *in-utero* and *peripartum* among HIV-exposed infants born preterm versus those born at term. However, as MTCT rates decreases across most program with a potential rise in preterm delivery rates, it might me more prudent to look at findings from pooled data across several programs which will provide more power to detect any differential risk of HIV-acquisition.

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Manuscript 2: **Zidovudine and Nevirapine Post-Exposure Prophylaxis Safety in Preterm HIV-Exposed Uninfected Neonates**

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Abstract

Background: Neonatal antiretroviral (ARV) prophylaxis is an important integral component of PMTCT of HIV and represents the cornerstone in preventing infant HIV acquisition. Safety data on ARVs used in the neonatal period for prevention of infant HIV acquisition are reassuring, however, most of the reassuring data has been from studies that excluded infants born preterm. Understanding how standard ARV prophylaxis regimens impact the hematologic profile of infants born preterm is of great public health importance.

Methods: Using data extracted from a placebo-controlled randomized trial designed to assess the survival benefit of CTX use among HIV-exposed infants in Botswana (the Mpepu Study), we compared the occurrence of anaemia and neutropenia between full-term and preterm neonates in the first month of life (4–6 weeks) based on ARV prophylaxis received. A multivariable logistic regression model was fit to identify risks for severe anaemia and neutropenia.

Results: Of a total of 2746 children with haemoglobin results, 23 (0.8%), infants had severe anaemia in the first month of life, while 43 (1.5%), of 2733 children had severe neutropenia. A significantly higher proportion of preterm infants had severe anaemia and neutropenia when compared with those delivered at term 1.9% versus 0.6%, p=0.005 and 2.5% versus 1.3%, p= 0.070 respectively. Preterm infants were more prone to severe anaemia (aOR: 2.41; 95% C.I: 0.96 – 6.03; p=0.05) while infants with severe neutropenia were more likely to have formula-fed at birth (aOR: 0.37; 95% C.I: 0.19 – 0.73; p=0.004) and younger (14-27 days) at the time of testing (aOR: 0.09; 95% C.I: 0.03 – 0.33; p=<0.001). Infant antiretroviral prophylaxis (twice daily ZDV versus once daily NVP) and randomized treatment arm (CTX versus placebo) did not significantly contribute to the occurrence of severe anaemia and neutropenia in preterm and term infants.

Conclusions: While low rates of haematological abnormalities were observed overall, infants born preterm were more likely to have a haematological abnormality as compared to infants

born at term. Infant post-exposure ARV prophylaxis (twice daily ZDV versus once daily NVP) appeared safe in both preterm and term infants and did not contribute to the slight increase in cases of severe hematologic adverse event observed in preterm. However, in an era where triple ARV prophylaxis is now recommended for infants at high risk of HIV acquisition, understanding the hematologic safety of this approach among preterm infants is of paramount importance.

***Word count**: 383*

Introduction

Several studies have quantified the value of reducing infant HIV acquisition through dosing of infant ARV post-exposure prophylaxis to protect infants against HIV acquisition in PMTCT programming, with findings of a reduction of HIV acquisition by 25%–42% in the absence of maternal use of ARVs, to < 2% in settings where maternal ART is used in pregnancy, with infant prophylaxis initiated at birth and continued for 4 to 6 weeks. These studies provide evidence-based support of guidelines advocating for infant ARV prophylaxis as an important integral component of PMTCT of HIV [1, 2] and represent the cornerstone in preventing infant HIV acquisition.

Safety data on ZDV and NVP used in the neonatal period for prevention of infant HIV acquisition are reassuring [3-5] with the most common adverse events associated with both agents when used for prophylaxis in the newborn period being transient and mild anaemia and neutropenia, which usually resolves by 12 weeks of age [6]. However, most of the reassuring data has been from studies that excluded infants born preterm hence understanding how standard ARV prophylaxis regimens impact the hematologic profile of infants born preterm is of great public health importance.

Using data extracted from a placebo-controlled randomized trial designed to assess the survival benefit of CTX use among HIV-exposed infants in Botswana (the Mpepu Study),

we compared the occurrence of anaemia and neutropenia between full-term and preterm neonates in the first month of life based on ARV prophylaxis received. A multivariable logistic regression model was fit to identify risks for severe anaemia and neutropenia in the first month of life among infants born preterm.

Methods

This study represents a secondary analysis of data collected from a prospective cohort of HIV-exposed children and their mothers in Botswana enrolled into the "Mpepu study". The Mpepu study was a randomized controlled trial (RCT) that evaluated the potential survival benefit of CTX prophylaxis among HIV-exposed but uninfected (HEU) children in Botswana [7]. Enrolled neonates were randomized to either receive twice daily dosing of Zidovudine (ZDV) per the Botswana government's Prevention of Mother-to-Child Transmission of HIV (PMTCT) programming at the time or once daily Nevirapine per study protocol as prophylaxis in the first 4 weeks of life. Consent verification for the use of data in future studies occurred for all participants included in this analysis before use of their data.

Statistical Analysis

We conducted our analysis of adverse hematologic comparisons between preterm and term infants receiving ARV prophylaxis using the Statistical Package for the Social Sciences version 29 software. We compared the results of full blood counts with differential counts obtained from blood drawn at 1 month of life, grading the severity of events using the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Paediatric Adverse Events version 2 (November 2012). Severe anaemia and neutropenia were defined as a toxicity grade ≥ 3 according to DAIDS tables which present ranges of each event by infant age. Prevalence of anaemia and neutropenia between preterm and term infants was done using Fisher exact testing while a multivariable logistic regression model was fit to identify risks for severe anaemia and neutropenia. Preterm was defined as birth earlier than 37 weeks' gestation.

Ethical Considerations

The Health Research Development Committee of the Botswana Ministry of Health and Wellness (HPDME 13/18/1) and the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (HREC 591/2020) provided regulatory overview and approval for this secondary analysis.

Results

Haemoglobin results on 2746 infants were used for the anaemia analysis. Of these, 511 (18.6%) infants were born preterm while 2235 (81.4%) were born full term. Three hundred and eight (60.3%) of infants born preterm vs 1216 (54.4%) of those born full term received twice-daily ZDV as post-exposure prophylaxis, while 203 (39.7%) of preterm vs 1019 (45.6%) born full term received once-daily dosing of NVP for post-exposure prophylaxis. Similarly, neutrophil results from 2733 infants were used for the assessment of neutropenia. Of these, 511 (18.7%) infants were born preterm while 2222 (81.3%) were born full term. Three hundred and eight (60.3%) of those born preterm vs 1210 (54.5%) of those born full term received twice-daily ZDV as post-exposure prophylaxis, while 203 (39.7%) infants born preterm and 1012 (45.5%) born full term received once-daily dosing of NVP for post-exposure prophylaxis.

Severe Anaemia: Overall, 23 infants (0.8%) had severe anaemia in the first month of life. A significantly higher proportion of preterm infants had severe anaemia when compared with those delivered at term 1.9% versus 0.6%, p=0.005. Of 1524 (308 born preterm versus 1210 born at term) neonates who received twice daily ZDV as post-exposure prophylaxis, 17 (1.1%) had severe anaemia compared to 6 (0.4%) of 1215 (203 delivered preterm versus 1012 delivered at term) neonates who received once-daily dosing of NVP. Amongst infants who received ZDV with severe anaemia, 7 (2.3%) were born preterm versus 10 (0.8%) delivered at term, while amongst those receiving NVP, 3 (1.5%) were preterm versus 3 (0.3%) born at term. The proportion of infants with severe anaemia by post-exposure prophylaxis regimen (ZDV &

NVP) did not differ significantly amongst those delivered preterm versus those born at term (p=0.06 for each group) see Table 1.

Table 1: Proportion of infants with severe anaemia and neutropenia by post-exposure prophylaxis regimen

		Severe Anaemia			Severe Neutropenia		
		<37 weeks	≥37 weeks	p-value	<37 weeks	≥37 weeks	p-value
Gestational age at delivery	ZDV Prophylaxis	7 (2.3%)	10 (0.8%)	0.060	8 (2.6%)	19 (1.6%)	0.232
	NVP Prophylaxis	3 (1.5%)	3 (0.3%)	0.061	4 (2.0%)	10 (1.0%)	0.270

Severe Neutropenia: Similarly, only 43 (1.5%) of 2733 children had severe neutropenia with a higher proportion born preterm (2.5% versus 1.3%, p= 0.070). One thousand five hundred and eighteen (308 born preterm versus 1210 born at term) received twice daily ZDV as prophylaxis while 1215 (203 delivered preterm versus 1012 born at term) received NVP. Amongst infants who received ZDV with severe neutropenia, 8 (2.6%) were born preterm versus 20 (1.6%) delivered at term, while amongst those receiving NVP, 4 (2.0%) were preterm versus 11 (1.0%) born at term. The proportion of infants with severe neutropenia by post-exposure prophylaxis regimen (ZDV & NVP) did not differ significantly amongst those delivered preterm versus those born at term (p=0.23 and 0.27 respectively) see Table 1.

Preterm infants were more prone to severe anaemia (aOR: 2.41; 95% C.I: 0.96 – 6.03; p=0.05) while infants with severe neutropenia were more likely to have formula-fed at birth (aOR: 0.37; 95% C.I: 0.19 – 0.73; p=0.004) and younger (14-27 days) at the time of testing (aOR: 0.09; 95% C.I: 0.03 – 0.33; p=<0.001) Table 2. Infant antiretroviral prophylaxis (twice daily ZDV versus once daily NVP) and randomized treatment arm (CTX versus placebo) did not significantly contribute to the occurrence of severe anaemia and neutropenia in preterm and term infants.

Table 2: Predictors of Severe anaemia and neutropenia

Severe Anaemia				
Predictor	**Univariable analysis**		**Multivariable analysis**	
	OR (95% C.I)	**p-value**	**aOR, (95% C.I)**	**p-value**
Gestational age category (<37 = Ref)	3.37, (1.47 – 7.73)	0.004	2.41, (0.96 – 6.03)	0.052
Infant Prophylaxis (ZDV = Ref)	-	-	0.49, (0.19 – 1.26)	0.141
Randomized treatment group (Placebo = Ref)	-	-	1.97, (0.83 – 4.67)	0.134
Severe Neutropenia				
Feeding method at birth (Breastfeeding = Ref)	0.34, (0.18 – 0.64)	<0.001	0.37, (0.19 – 0.73)	0.004
Age at randomization category (28-34 = Ref)	0.11, (0.03 - 0.35)	<0.001	0.09, (0.03 – 0.33)	< 0.001
Infant Prophylaxis (ZDV = Ref)	-	-	1.37, (0.64 – 2.95)	0.424
Randomized treatment group (Placebo = Ref)	-	-	1.38, (0.73 – 2.57)	0.320

NB: Only covariates with either a p-value < 0.10 from the univariable analysis or significantly predictive of severe anaemia or neutropenia were included in the multivariable analysis shown.

Discussion

Our findings show that the occurrence of severe anaemia and neutropenia following exposure to ZDV and NVP used for prophylaxis post-delivery is safe in children delivered preterm as well as in those delivered at term. Only 0.8% and 1.5% of children who received ZDV and NVP in our cohort had severe anaemia and neutropenia respectively. Though we reported lower rates than those observed in other studies [4-6, 8], our findings like those of others demonstrate that ZDV and NVP use for post-exposure prophylaxis in HIV-exposed infants is safe with clinically insignificant anaemia and neutropenia. Our reported lower rates might be attributable to our approach of reporting on just occurrences of severe anaemia and neutropenia compared to other studies where a more liberal approach reporting on all occurrences of anaemia and neutropenia was deployed.

Additionally, our findings showed that occurrence of severe anaemia and neutropenia was more common in HIV-exposed infants born preterm than those born at term. Our comparison showed that a higher proportion of preterm infants had severe anaemia and neutropenia when compared with those delivered at term (1.9% versus 0.6%, p=0.005 and 2.5% versus 1.3%, p= 0.070 respectively). We were unable to compare this trend with any previous publications or findings as most studies that has looked and reported on the hematologic adverse effect of ZDV and NVP previously either excluded infants born preterm or did not report on such findings. We however believe this to attributable to an earlier physiological nadir of red blood cells in preterm infants when compared with infants born at term [9, 10]. Furthermore, and contrary to as we postulated, the proportion of infants with severe anaemia by post-exposure prophylaxis regimen did not differ significantly despite the observed trend of a higher proportion of preterm infants on ZDV versus NVP with severe anaemia (2.3% versus 1.5%, p=0.06).

Lastly, we found that the odds of severe anaemia increased 2-fold in infants born preterm as compared to those born at term while the likelihood of the occurrence of severe neutropenia increased in neonates who were formula-fed at birth and younger (14-27 days) at the time of testing. Infant antiretroviral prophylaxis (twice daily ZDV versus once daily NVP) and randomized treatment arm (CTX versus placebo) did not significantly contribute to the occurrence of severe anaemia and neutropenia in preterm and term infants.

Conclusion

Low rates of haematological abnormalities were observed overall, infants born preterm were more likely to have a haematological abnormality as compared to infants born at term. Infant post-exposure ARV prophylaxis (twice daily ZDV versus once daily NVP) was safe in both preterm and term infants and did not contribute to the slight increase in cases of severe hematologic adverse events observed in preterm. However, in an era where triple ARV

prophylaxis is now recommended for infants at high risk of HIV acquisition, understanding the hematologic safety of this approach among preterm infants is of paramount importance.

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Part IV: Discussions and Conclusions

Discussions

Prevalence of HIV in HIV exposed infants born preterm: Results from this analysis showed no difference in the prevalence of HIV in HIV-exposed infants delivered preterm when compared to those delivered at term in the first month of life (0.8% versus 0.6%), and when stratified by timing of infection [at birth (0.2% versus 0.3%) and at 14-34 days post-delivery (0.6% versus 0.3%)] as postulated by the very few studies that have looked at this [37, 60-62]. Though we observed an increase trend in HIV acquisition rate from 0.2% *in-utero* to 0.6% in the *peripartum* period among infants delivered preterm as against no increase at all in those delivered term, this observed increase was non-significant. We, however, acknowledge that this non-significant increase observed could have been due to the very low vertical transmission rate in our cohort and cautiously interpret this finding to be a true reflection of things in similar settings with widespread use of ART for PMTCT such as ours.

Timing of HIV acquisition in infants born preterm: When stratified by timing of infection, we observed an increased trend in HIV acquisition in the *peripartum* period among infants delivered preterm as against no increase at all in those delivered term, and believe this to be a signal of increased vulnerability in infants delivered preterm. We however, cautiously interpret this finding to be a true reflection of things in similar settings with widespread use of ART for PMTCT such as ours due to the very low vertical transmission rate observed in our cohort.

Risk for HIV acquisition in preterm infants: Our finding also suggests non-use of antiretroviral by women during pregnancy being the only measured covariate in our cohort that was independently predictive of HIV acquisition in children born to WLHIV, this re-affirms several other similar findings in literature [100-103]. This also confirms that scaling up of cART within programs could help eliminate MTCT. Though lower than the reported 95% ART coverage from the Botswana national program [104, 105], use of ART during pregnancy remained high at 82.5% in our cohort, this can be attributable to the fact that our study was conducted during the initial phase of the transitioning to triple ART use for PMTCT in

Botswana. We reported a much lower MTCT rate of 0.6% in our cohort compared to the <2% reported nationally [26] confirming the effectiveness of triple ART in PMTCT as reported by several other studies globally and within the region [106-109]. Similarly, we observed a high preterm delivery rate of 19% in WLHIV in a setting of high ART coverage consistent with reports from several other studies [30-32, 51-53] and corroborates findings suggesting *in-utero* exposure to HIV and ART as possible drivers of the increased preterm delivery rates observed in WLHIV.

Safety of prophylactic ARV regimens used in HIV-exposed infants born preterm: Our analysis provides details on adverse hematologic comparisons between preterm and term infants receiving ARV prophylaxis in the first month of life. Our finding shows that infant post-exposure ARV prophylaxis in the form of twice daily ZDV or once daily NVP, as currently recommended by W.H.O, appears safe in both preterm and term infants despite the slight increase in cases of severe anaemia and neutropenia observed in infants delivered preterm. Our findings show that the occurrence of severe anaemia and neutropenia following exposure to ZDV and NVP used for prophylaxis post-delivery is safe in children delivered preterm as well as in those delivered at term. Only 0.8% and 1.5% of children who received ZDV and NVP in our cohort had severe anaemia and neutropenia respectively. Though we reported lower rates than those observed in other studies [18, 77, 78, 81], our findings like those of others demonstrates that ZDV and NVP use for post exposure prophylaxis in HIV exposed infant is safe with clinically insignificant and transient anaemia and neutropenia. Our reported lower rates might be attributable to our approach of reporting on just occurrences of severe anaemia and neutropenia compared to other studies where a more liberal approach reporting on all occurrences of anaemia and neutropenia was deployed. Additionally, our result showed that the occurrence of severe anaemia and neutropenia was more common in HIV-exposed infants born preterm than those born at term. Our comparison showed that a higher proportion of preterm infants had severe anaemia and neutropenia when compared with those delivered at term (1.9% versus 0.6%, p=0.005 and 2.5% versus 1.3%, p= 0.070 respectively).

We were unable to compare this trend with any previous publications as most studies that have looked at and reported on the hematologic adverse effect of ZDV and NVP previously either excluded infants born preterm or did not report on such findings. We however believe this to be attributable to an earlier physiological nadir of red blood cells in preterm infants when compared with infants born at term [110, 111]. Furthermore, and contrary to as we postulated, the proportion of infants with severe anaemia by post-exposure prophylaxis regimen did not differ significantly despite the observed trend of a higher proportion of preterm infants on ZDV versus NVP with severe anaemia (2.3% versus 1.5%, p=0.06).

Risk for severe anaemia and neutropenia in preterm infants: we found that the odds of severe anaemia increased 2-folds in infants born preterm as compared to those born at term while infants the likelihood of the occurrence of severe neutropenia increased in neonates who were formula-fed at birth and younger (14-27 days) at the time of testing. Infant antiretroviral prophylaxis (twice daily ZDV versus once daily NVP) and randomized treatment arm (CTX versus placebo) did not significantly contribute to the occurrence of severe anaemia and neutropenia in preterm and term infants.

As mentioned above, the main limitation of this study is the very low MTCT rate observed, this could have reduced our power in detecting a true difference in risk of HIV acquisition between HIV exposed infants delivered preterm versus those delivered term. Also, our analysis was limited to 34 days post-delivery which could have led to underestimation of *peripartum* HIV acquisition rates in our cohort. This however is unlikely as participants in the Mpepu study were followed up for a period of 18 months and our review of the 18-month infant HIV status within the study did not report any new seroconversion occurring.

Needs for future research: Overall, and despite the mixed data, ours and evidence from other studies points towards increase rates of PTB in WLHIV. We however are of the opinion that due to the evolving landscape of preferred ART regimens for use among pregnant WLHIV, there still exist a knowledge gap around the timing and prevalence of HIV-acquisition for

infants born preterm. It is thus imperative to conduct well designed studies that enrol women sufficiently early in pregnancy to document the occurrence of preterm birth, that use sound methods of pregnancy dating, are adequately powered to detect clinically meaningful difference in pregnancy outcomes by timing of maternal ART initiation, either prior to conception or by trimester of pregnancy, and with robust measurement of known confounders. Also, despite the presence of sound evidence on the safety and efficacy of infant post exposure prophylaxis with ZDV and NVP (even when used for extended periods), most of the data has been published by studies evaluating HIV exposed infants delivered at term (≥ 37 weeks' gestation). With the increased prevalence of preterm birth associated with maternal use of ART, more research is needed to establish the safety and efficacy of ZDV and NVP when used for infants born preterm. Lastly, as the use of triple ARV prophylaxis in exposed infants deemed to be high risk gains momentum, there is need to evaluate the immediate and long-term safety profile of this approach particularly when used in infants born preterm.

Conclusions

The purpose of this research was to understand if the prevalence of HIV, timing of HIV acquisition and the attributable risk for HIV acquisition differs in HIV-exposed infants born preterm when compared to those born at term. Furthermore, we sought to provide data on the efficacy and haematological safety of prophylactic ARV regimens used in HIV-exposed infants born preterm. For both set objectives, we believe our findings as presented in this dissertation and summarized below adds to the body of knowledge already existing in this field and perchance has provided new insight into better understanding the risk of HIV acquisition being born preterm confers.

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Part V: Appendices

Appendix 1: Variables

Predictor Variables	Variable Name	Label
Maternal gravidity	Prev_pregnancies	Number of previous pregnancies
Maternal ART at delivery	Delivery_regimen	Regimen at delivery
Mode of delivery	Delivmode	Delivery mode
Gestational age at birth	Gestage_deliv	Gestational age at delivery
Gestational age category	gestagecat	Gestational age by category
Sex of the infant	Sex	Sex
Birth weight	b_weight	Weight at birth
Birth length	b_length	Infant's length
Presence/absence of sepsis at birth	pre_rando_sepsis	Diagnosis of sepsis at birth
ZDV ARV prophylaxis	r_coverage	ZDV use as prophylactic coverage at time of randomization
NVP ARV prophylaxis	r_coverage1	NVP used as prophylactic coverage at time of randomization
Infant feeding method at birth	Feeding1	Feeding status at birth
Infant feeding method at 2-4 weeks(rando)	Feedrand	Feeding status at randomization
Birth weight category	Bwtcat	Birth weight by category
Days of life at randomization by category	Daysoflifecat	Days of life at randomization by category
Money earned personally by mother	Earnings	Money earned personally by mother
Highest education level completed	edlevel	Highest education level completed

Electricity in home	electric	Electricity in home
Labor duration	labordur	Labor duration
Maternal age at enrolment	Momage_enroll	Mother's age at enrolment
Enrolment site	sitecd	Enrolment site
CTX vs Placebo use	temp_rx	Original, partially encoded treatment arm
Age at randomization by category	Rand_days	Days of life at randomization
Haemoglobin grading at 2-4 weeks	Hgb0grade	Calculated haemoglobin severity grade
Absolute neutrophil grading at 2-4 weeks	Neutr0grade	Calculated neutrophil severity grade
Infant HIV status at birth	PCR_birth	DNAPCR at birth
Infant HIV status at 2-weeks(rando)	rand_dnapcr	DNA PCR at randomization

Appendix 2: Data Cleaning Summary

b_weight	
056-1995296-2-10	birth weight modified to 2.9 per source doc
056-3981150-2-10	birth weight modified to 3.4 per source doc
bwtcat	
056-1995262-3-10	bwtcat changed to Normal birth weight based on provided bw
056-1984769-3-10	bwtcat changed to Normal birth weight based on provided bw
056-1984502-2-10	bwtcat changed to Normal birth weight based on provided bw
056-1980572-5-10	bwtcat changed to Normal birth weight based on provided bw
056-498797-6-10	bwtcat changed to Normal birth weight based on provided bw
gestage_deliv	
056-1980131-5-10	gestage_deliv changed to 42 weeks per source doc and gestagecat updated accordingly
056-1981799-1-10	gestage_deliv changed to 37 weeks per source doc
056-1982005-4-10	gestage_deliv changed to 43 weeks per source doc and gestagecat updated accordingly
056-1982085-0-10	gestage_deliv changed to 39 weeks per source doc and gestagecat updated accordingly
056-1984588-4-10	gestage_deliv changed to 35 weeks per source doc and gestagecat updated accordingly
056-1995047-5-10	gestage_deliv changed to 33 weeks per source doc
056-1995501-4-10	gestage_deliv changed to 40 weeks per source doc and gestagecat updated accordingly
056-3980387-2-10	gestage_deliv changed to 28 weeks per source doc and gestagecat updated accordingly
056-3981240-0-10	gestage_deliv changed to 34 weeks per source doc
056-398960-1-10	gestage_deliv changed to 33 weeks per source doc
056-4980441-0-10	gestage_deliv changed to 39 weeks per source doc and gestagecat updated accordingly
056-4980488-5-10	gestage_deliv changed to 31 weeks per source doc and gestagecat updated accordingly
056-4981282-2-10	gestage_deliv changed to 35 weeks per source doc
056-4981739-3-10	gestage_deliv changed to 40 weeks per source doc and gestagecat updated accordingly
056-4982067-6-10	gestage_deliv changed to 40 weeks per source doc and gestagecat updated accordingly
056-4982075-0-10	gestage_deliv changed to 40 weeks per source doc and gestagecat updated accordingly
056-498733-5-10	gestage_deliv changed to 37 weeks per source doc
056-4995041-2-10	gestage_deliv changed to 32 weeks per source doc and gestagecat updated accordingly
ga	
056-1980131-5-10	ga changed to 42 weeks per source doc
056-1980465-3-10	ga changed to 43 weeks per source doc
056-1981799-1-10	ga changed to 37 weeks per source doc
056-1982005-4-10	ga changed to 43 weeks per source doc
056-1982085-0-10	ga changed to 39 weeks per source doc

056-1984588-4-10	ga changed t0 35 weeks per source doc
056-1984769-3-10	ga changed to 31 weeks per souce doc. Gestage_deliv and gestagecat updated accordingly
056-1994911-2-10	ga changed to 40 weeks per source doc
056-1995047-5-10	ga changed to 33 weeks per source doc
056-1995095-4-10	ga changed to 38 weeks per source doc. Gestage_deliv updated accordingly
056-1995501-4-10	ga changed to 40 weeks per source doc
056-3980387-2-10	ga changed to 28 weeks per source doc
056-3980494-4-10	ga changed to 36 weeks per source doc
056-3981233-1-10	ga changed to 36 weeks per source doc
056-3981240-0-10	ga changed to 34 weeks per source doc
056-398791-0-10	ga changed to 37 weeks per source doc. Gestage_deliv and gestagecat update accordingly
056-398960-1-10	ga changed to 33 weeks per source doc
056-4980441-0-10	ga changed to 39 weeks per source doc
056-4980488-5-10	ga changed to 31 weeks per source doc
056-4981282-2-10	ga changed to 35 weeks per source doc
056-4981717-1-10	ga changed to 36 weeks per source doc
056-4981739-3-10	ga changed to 40 weeks per source doc
056-4982017-5-10	ga changed to 31 weeks per source doc. Gestage_deliv and gestagecat updated accordingly
056-4982067-6-10	ga changed to 40 weeks per source doc
056-4982075-0-10	ga changed to 40 weeks per source doc
056-498733-5-10	ga changed to 37 weeks per source doc
056-4995041-2-10	ga changed to 32 weeks per source doc
056-4995303-5-10	ga changed to 40 weeks per source doc. Gestage_deliv updated accordingly
gestagecat	
056-1984769-3-10	updated to $\leq$ to 37 weeks per source doc
056-1980572-5-10	updated to > 37 weeks per source week
056-1984502-2-10	updated to > 37 weeks per source week
056-1995262-3-10	updated to > 37 weeks per source week
056-498797-6-10	updated to > 37 weeks per source week
momage_enroll	
056-1995262-3-10	updated to 28 years per source doc
056-1980572-5-10	updated to 30 years per source doc
056-1984502-2-10	updated to 35 years per source doc

056-498797-6-10	updated to 27 years per source doc
056-1984769-3-10	updated to 21 years per source doc
daysoflifecat	
056-1980572-5-10	updated to version 1.0 and 28-34 days
056-1984502-2-10	updated to version 2.0 and 14-27 days
056-1984769-3-10	updated to version 1.0 and 28-34 days
056-1995262-3-10	updated to version 2.0 and 14-27 days
056-498797-6-10	updated to version 1.0 and 28-34 days
anc0	
056-1980166-5-10	updated to 1590 per source doc
056-1980168-0-10	updated to 1480 per source doc
056-1980238-0-10	updated to 2010 per source doc
056-1980703-3-10	updated to 4380 per source doc
056-1981020-5-10	updated to 2380 per source doc
056-1982045-2-10	updated to 2660 per source doc
056-1984387-6-10	updated to 1920 per source doc
056-1984447-3-10	updated to 1800 per source doc
056-1984477-5-10	updated to 3140 per source doc
056-1984724-0-10	updated to 4980 per source doc
056-198888-6-10	updated to 5580 per source doc
056-1994883-2-10	updated to 4670 per source doc
056-1995160-6-10	updated to 2000 per source doc
056-1995226-2-10	updated to 2170 per source doc
056-1995314-6-10	Updated to 2720 per source doc
056-1995316-1-10	updated to 1540 per source doc
056-3981144-3-10	updated to 2840 per source doc
056-398728-0-10	updated to 2050 per source doc
056-398933-2-25	updated to 1260 per source doc
056-398960-1-10	updated to 3020 per source doc
056-398992-5-10	updated to 1080 per source doc
056-4980198-2-10	updated to 1040 per source doc
056-4980265-6-10	updated to 1490 per source doc
056-4980307-6-10	updated to 2540 per source doc
056-4980636-6-10	updated to 1900 per source doc
056-4980708-1-10	updated to 490 per source doc
056-4981861-3-10	updated to 1590 per source doc

056-4981953-4-10	updated to 2550 per source doc
056-4982532-2-10	updated to 1220 per source doc
056-4984217-0-10	updated to 2260 per source doc
056-4984230-6-10	updated to 1660 per source doc
056-4984424-4-10	updated to 2640 per source doc
056-4984441-0-10	updated to 1110 per source doc
056-4984484-1-10	updated to 3280 per source doc
056-4984735-0-10	updated to 1500 per source doc
056-4984785-1-10	updated to 11030 per source doc
056-4984789-5-10	updated to 3430 per source doc
056-4994957-2-10	updated record to 3200 per source doc
056-4995013-2-10	updated record to 2290 per source doc
056-4995452-0-10	updated record to 2240 per source doc
neut0grade	
056-1980166-5-10	updated to G0 per source doc
056-1980168-0-10	updated to G0 per source doc
056-1980238-0-10	updated to G0 per source doc
056-1980703-3-10	updated to G0 per source doc
056-1981020-5-10	updated to G0 per source doc
056-1982045-2-10	updated to G0 per source doc
056-1984387-6-10	updated to G0 per source doc
056-1984447-3-10	updated to G0 per source doc
056-1984477-5-10	updated to G0 per source doc
056-1984724-0-10	updated to G0 per source doc
056-198888-6-10	updated to G0 per source doc
056-1994883-2-10	updated to G0 per source doc
056-1995160-6-10	updated to G0 per source doc
056-1995226-2-10	updated to G0 per source doc
056-1995314-6-10	updated to G0 per source doc
056-1995316-1-10	updated to G0 per source doc
056-3981144-3-10	updated to G0 per source doc
056-398728-0-10	updated to G0 per source doc
056-398933-2-25	updated to G1 per source doc
056-398960-1-10	updated to G0 per source doc
056-398992-5-10	updated to G1 per source doc
056-4980198-2-10	updated to G1 per source doc
056-4980265-6-10	updated to G0 per source doc

056-4980307-6-10	updated to G0 per source doc
056-4980636-6-10	updated to G0 per source doc
056-4980708-1-10	updated to G4 per source doc
056-4981861-3-10	updated to G0 per source doc
056-4981953-4-10	updated to G0 per source doc
056-4982532-2-10	updated to G1 per source doc
056-4984217-0-10	updated to G0 per source doc
056-4984230-6-10	updated to G0 per source doc
056-4984424-4-10	updated to G0 per source doc
056-4984441-0-10	updated to G1 per source doc
056-4984484-1-10	updated to G0 per source doc
056-4984735-0-10	updated to G0 per source doc
056-4984785-1-10	updated to G0 per source doc
056-4984789-5-10	updated to G0 per source doc
056-4994957-2-10	updated to G0 per source doc
056-4995013-2-10	updated to G0 per source doc
056-4995452-0-10	updated to G0 per source doc
hgb0grade	
056-1980166-5-10	updated to G0 per source doc
056-1980168-0-10	updated to G0 per source doc
056-1980238-0-10	updated to G2 per source doc
056-1980703-3-10	updated to G0 per source doc
056-1981020-5-10	updated to G0 per source doc
056-1982045-2-10	updated to G1 per source doc
056-1984387-6-10	updated to G0 per source doc
056-1984447-3-10	updated to G0 per source doc
056-1984477-5-10	updated to G2 per source doc
056-1984724-0-10	updated to G0 per source doc
056-198888-6-10	updated to G0 per source doc
056-1994883-2-10	updated to G0 per source doc
056-1995160-6-10	updated to G0 per source doc
056-1995226-2-10	updated to G0 per source doc
056-1995314-6-10	updated to G2 per source doc
056-1995316-1-10	updated to G1 per source doc
056-3981144-3-10	updated to G0 per source doc
056-398728-0-10	updated to G2 per source doc
056-398933-2-25	updated to G0 per source doc

056-398960-1-10	updated to G0 per source doc
056-398992-5-10	updated to G1 per source doc
056-4980198-2-10	updated to G1 per source doc
056-4980265-6-10	updated to G0 per source doc
056-4980307-6-10	updated to G0 per source doc
056-4980636-6-10	updated to G0 per source doc
056-4980708-1-10	updated to G0 per source doc
056-4981861-3-10	updated to G0 per source doc
056-4981953-4-10	updated to G2 per source doc
056-4982532-2-10	updated to G0 per source doc
056-4984217-0-10	updated to G0 per source doc
056-4984230-6-10	updated to G0 per source doc
056-4984424-4-10	updated to G0 per source doc
056-4984441-0-10	updated to G0 per source doc
056-4984484-1-10	updated to G0 per source doc
056-4984735-0-10	updated to G0 per source doc
056-4984785-1-10	updated to G1 per source doc
056-4984789-5-10	updated to G0 per source doc
056-4994957-2-10	updated to G0 per source doc
056-4995013-2-10	updated to G0 per source doc
056-4995452-0-10	updated to G1 per source doc
rand_dnapcr	
056-3981144-3-10	updated PCR to NEG
basehivstat	
056-1980572-5-10	updated to no positive result
056-1995262-3-10	updated to no positive result
056-1984502-2-10	updated to no positive result
056-1984769-3-10	updated to no positive result
056-498797-6-10	updated to no positive result

Appendix 3: Ethics Approvals

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

30 November 2020

HREC REF: 591/2020

Prof L Myer
School of Public Health & Family Medicine
Falmouth Building-FHS
Email: - Landon.myer@uct.ac.za
Student: gbolajibola@gmail.com

Dear Prof Myer

PROJECT TITLE: EFFECTS OF PRETERM BIRTH ON HIV ACQUISITION RISK AND ANTIRETROVIRAL PROPHYLAXIS SAFETY IN HIV-EXPOSED INFANTS IN BOTSWANA. (PERHAPS STUDY)-MSC CANDIDATE DR GBOLAHAN AJIBOLA

Thank you for your response letter, addressing the issues raised by the Faculty of Health Sciences Human Research Ethics Committee (HREC).

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

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020 & 06 July 2020.

Approval is granted for one year until the 30 November 2021.

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

The HREC acknowledge that the student: - Dr Gbolahan Ajibolan will also be involved in this study.

Please quote the HREC REF in all your correspondence.

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

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

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS017: Annual Progress Report / Renewal

Record Reviews/Audits/Collection of Biological Specimens/Repositories/Databases/Registries

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30/6/2024
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee	Signed by candidate	Date Signed	22/6/23

Note: Please note that incomplete submissions will not be reviewed.
Please email this form and supporting documents (if applicable) in a combined ~~pdf file to~~ hrec-enquiries@uct.ac.za.

HUMAN RESEARCH ETHICS COMMITTEE
22 JUN 2023
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

Please clarify your plan for research-related activities during COVID-19 lockdown

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	June 12, 2023		
HREC REF Number	591/2020	Current Ethics Approval was granted until	30 November 2021
Protocol title	EFFECTS OF PRETERM BIRTH ON HIV ACQUISITION RISK AND ANTIRETROVRAL PROPHYLAXIS SAFETY IN HIV-EXPOSED INFANTS IN BOTSWANA. (PERHAPS STUDY)		
Principal Investigator	Prof. Landon Myer		
Department / Office Internal Mail Address	Public Health		
1.1 Does this protocol receive US Federal funding?		☐ Yes	☐ No

2. Protocol status (tick ✓)

☐	Research-related activities are ongoing
☐	Data collection is complete, data analysis only
Please indicate (in the block below) the titles and HREC reference numbers of any projects currently making use of the Database/registry/repository.	
None	

3. Protocol summary

Total number of records or specimens collected, reviewed or stored since the original approval		2866 records
Total number of records or specimens collected, reviewed or stored since last progress report		2866 records
Have any research-related outputs (e.g. publications, abstracts, conference presentations) resulted from this research? If yes, please list and attach with this report.	☐ Yes	☐ No

TELEPHONE: 363 2500
FAX: 317 0155
TELEGRAMS: RABONGAKA
TELEX: 2818 CARE BD

Republic of Botswana

MINISTRY OF HEALTH
PRIVATE BAG 0038
GABORONE

REFERENCE NO: HPRD: 6/14/1 **24th August 2022**

Health Research Development Division

Principal Investigator: Gbolahan Ajibola
Notification of IRB Review: **Continuing Review**

PROTOCOL TITLE: EFFECTS OF PRETERM BIRTH ON HIV ACQUISITION RISK AND ANTIRETROVIRAL PROPHYLAXIS SAFETY IN HIV-EXPOSED INFANTS IN BOTSWANA (PERHAPS STUDY)

Review Type:	Expedited/Health Research Development Division
Review Date:	24th August 2022
Approval Date	24th August 2022
Effective Date:	25th August 2022
Expiration Date:	24th August 2023

This certifies that the continuing review request for the protocol above was reviewed under review procedures. Approval is valid for a period of 1 year.

___ Study is continuing.
___ Open for enrollment
___ Accrual complete with treatment intervention and /or participant interviews/ surveys continuing.
___ Subject interventions/data collection ended on (date): _____
__X__ Open for analysis only. Expected end date: **Dec 2022**
___ Competed (including all analysis). Dated completed: _____
___ Cooperative Review
___ Other, Please describe: _____

Attachment (s)

- HRDC continuing review application form dated August 18, 2022.
- Current HRDC Approval (dated 19 August 2021)
- Protocol document version 1.0 dated April 2021.

If you have any questions please do not hesitate to contact Mr Abia Sebaka at ,asebaka@gov.bw,Tel+267-3632754 and Mr.K.Motlhanka at kgmmotlhanka@gov.bw, Tel +267-3632751. Thank you for your cooperation and your commitment to the protection of human subjects in research.

Yours Sincerely

Signed by candidate

Mr Abia Sebaka
for/PERMANENT SECRETARY

MINISTRY OF HEALTH RESEARCH DIVISION
24 AUG 2022
P/BAG 0038, GABORONE
HEALTH POLICY RESEARCH & DEVELOPMENT

PRIVATE BAG 0038
GABORONE
BOTSWANA
REFERENCE:

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MINISTRY OF HEALTH AND WELLNESS

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TELEX: 2818 CARE BD

REFERENCE NO: HPDME 13/18/1 X1 **19 August 2021**

Health Research Development Committee

Principal Investigator **:** Gbolahan Ajibola

Notification of IRB Review: **Continuing Review**

Continuing Review: Effects of preterm birth on HIV acquisition risk and antiretroviral prophylaxis safety in HIV-exposed infants in Botswana. (PERHAPS STUDY)

Review Type: Expedited Review/ HRDD
Review Date: 19 August 2021
Approval Date: 19 August 2021
Effective Date: 25 August 2021
Expiration Date: 24 August 2022

This certifies that the continuing review request for the protocol above was reviewed under review procedures. Approval is valid for a period of 1 year.

__ Study is continuing.
____ The study has not been activated.
____ Open to Enrollment.
___ Accrual complete with treatment intervention and /or participant interviews/ surveys continuing.
X Open for analysis only.
____ Subject intervention/data collection ended on (date):
____ includes only collection of data from voice, video, digital, or image recordings made for research purposes.
___ Research on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior).

Attached:
1 HRDC continuing review application form dated August 02, 2021

If you have any questions please do not hesitate to contact Mrs D. Mgadla at dbabini@gov.bw, Tel +267-3632754 and Mr. K. Motlhanka at kgmmotlhanka@gov.bw, Tel +267-3632751. Thank you for your cooperation and your commitment to the protection of human subjects in research.

PRIVATE BAG 0038
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BOTSWANA
REFERENCE:

REPUBLIC OF BOTSWANA
MINISTRY OF HEALTH AND WELLNESS

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REFERENCE NO: HPDME 13/18/1 **25th August 2020**
Health Research & Development Committee

Principal Investigator: Gbolahan Ajibola
Notification of IRB Review: **Continuing Review**

Protocol Title: **EFFECTS OF PRETERM BIRTH ON HIV ACQUISITION RISK AND ANTIRETROVIRAL PROPHYLAXIS SAFETY IN HIV-EXPOSED INFANTS IN BOTSWANA (PERHAPS STUDY)**

Review Type: HRDC/Full Board
Review Date: 25 August 2020
Effective Date: 25 August 2020
Expiration Date: 24 August 2021

This certifies that the continuing review request for the protocol above was reviewed under review procedures. Approval is valid for a period of 1 year.

_X_ Study is continuing.
____ The study has not been activated.
____ Open to Enrollment.
____ Accrual complete with treatment intervention and /or participant interviews
____ Open for analysis only.
____ Research on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior).
____ Other, Please describe:
____ Research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies.

Attachments:

- HRDC Continuing review application form dated August 11, 2020

If you have any questions please do not hesitate to contact Mrs S. Mosweunyane at smosweunyane@gov.bw, Tel +267-3632754 and Mr. K. Motlhanka at kgmmotlhanka@gov.bw, Tel +267-3632751. Thank you for your cooperation and your commitment to the protection of human subjects in research.

Yours sincerely

Signed by candidate

Ms S. Mosweunyane
for /PERMANENT SECRETARY

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RESEARCH DIVISION
25 AUG 2020
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REFERENCE NO: HPDME 13/18/1 **02 October 2019**

Health Research Development Committee

Principal Investigator: Gbolahan Ajibola
Notification of IRB Review: **Continuing Review**

Protocol Title: **EFFECTS OF PRETERM BIRTH ON HIV ACQUISITION RISK AND ANTIRETROVIRAL PROPHYLAIXS SAFETY IN HIV-EXPOSED INFANTS IN BOTSWANA**

Review Type: Health Research Unit/Expedited
Review Date: 02 October 2019
Approval Date: 02 October 2019
Effective Date: 11 October 2019
Expiration Date: 10 October 2020

This certifies that the continuing review request for the protocol above was reviewed under review procedures. Approval is valid for a period of 1 year.

_X_ Continuing
___ Accrual complete with treatment intervention and /or participant interviews/ surveys continuing.
____ Subject interventions/data collection ended on (date):_____
___ (Closed to Enrolment) Open for analysis only. Expected end date: _____
____ Competed (including all analysis). Dated completed:_____
____ Cooperative Review
___ Other, Please describe:
____ The study has not been activated.

Attachment:

- HRDC continuing review application form dated August 19, 2019
- Effects of preterm birth on HIV acquisition risk and antiretroviral prophylaxis safety in HIV-exposed infants in Botswana. Version 1.0, August 3, 2018.

If you have any questions please do not hesitate to contact Mr. K. Motlhanka at kgmmotlhanka@gov.bw, Tel +267-3632751. Thank you for your cooperation and your commitment to the protection of human subjects in research.

Yours sincerely

Signed by candidate

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MINISTRY OF HEALTH AND WELLNESS

REFERENCE NO: HPDME 13/18/1 VI (29) **12 November 2018**

Health Research and Development Division

Notification of IRB Review: **New application**

Gbolahan Ajibola
Botswana Harvard Partnership
Private Bag BO 320
Gaborone

Dear Gbolahan Ajibola

Protocol Title: **EFFECTS OF PRETERM BIRTH ON HIV ACQUISITION RISK AND ANTIREROVIRAL PROPHYLAXIS SAFETY IN HIV-EXPOSED INFANTS IN BOTSWANA (PERHAPS) VERSION 1.0 NOVEMBER 05, 2018. HRDC #00847**

Review Type: HRDC/Full Board
Review Date: 11 October 2018
Approval Date: 11 October 2018
Effective Date: 12 November 2018
Expiration Date: 10 October 2019
Risk Determination: Minimal risk

Thank you for submitting new application for the above referenced protocol. The permission is granted to conduct the study.

Attachments:

- HRDC Initial application form
- Study protocol Version 1.0 dated November 5, 2018
- Manuscript from Mpepu
- CV for PI and other co-investigators

This permit does not however give you authority to collect data from the selected sites without prior approval from the management. Consent from the identified individuals should be obtained at all times.

The research should be conducted as outlined in the approved proposal. Any changes to the approved proposal must be submitted to the Health Research and Development Division in the Ministry of Health for consideration and approval.

Appendix 4: South African Journal of HIV Medicine submission guidelines

Overview

The author guidelines include information about the types of articles received for publication and preparing a manuscript for submission. Other relevant information about the journal's policies and the reviewing process can be found under the about section. The **compulsory cover letter** forms part of a submission and must be submitted together with all the required **forms.** All forms need to be completed in English.

Original Research Article

An original article provides an overview of innovative research in a particular field within or related to the focus and scope of the journal, presented according to a clear and well-structured format.

Submission status	open
Word limit	3500-5000 words (excluding the abstract, tables, figures, graphs, and references)
Abstract	maximum: 250 words requires structural headings: Background, Objectives, Method, Results and Conclusion
Callout	maximum: 50 words requires structural heading: What this study adds
Main text	requires structural headings, refer to the full structure 'Ethical considerations' is a sub-section in the manuscript and must include: Name of the ethical review committee Study approval number Manner of consent (written, oral) for human participants Description of measures taken to maintain the confidentiality of data If the study was not human or animal research or the study was determined to be non-human subjects research or exempt, the authors must provide a statement with those details in this section.
References	60 or less, adhere to the **Vancouver referencing style**
Tables, figures and graphs	7 or less, adhere to the Illustrations requirements found in the AOSIS House style guide
Formatting requirements	apply the guidelines located on the **Formatting requirements page** and the **AOSIS house style guide**
Compulsory supplementary file(s)	the **Authorship, disclosure statements, copyright, and license agreement form**, **Ethical Clearance/Waiver Documentation** and any other relevant form applicable to your submission
Ethical clearance/waiver documentation	evidence of ethical clearance for the study, such as the study approval letter or certificate from the Institutional Review Board (IRB), a waiver from the IRB et cetera

Title: The article's full title should contain a maximum of 95 characters (including spaces).

Abstract: The abstract, written in English, should be no longer than 250 words and must be written in the past tense. The abstract should give a succinct account of the objectives, methods, results and significance of the matter. The structured abstract for an Original Research article should consist of six paragraphs labelled Background, Objectives, Method, Results and Conclusion.
Background: *Why do we care about the problem?* State the context and purpose of the study. (What practical, scientific or theoretical gap is your research filling?)
Objectives: *What problem are you trying to solve*? What is the scope of your work (e.g. is it a generalised approach or for a specific situation)? Be careful not to use too much jargon.
Method: *How did you go about solving or making progress on the problem*? State how the study was performed and which statistical tests were used. (What did you actually do to get the results?) Clearly express the basic design of the study; name or briefly describe the basic methodology used without going into excessive detail. Be sure to indicate the key techniques used.
Results: *What is the answer*? Present the main findings (that is, as a result of completing the procedure or study, state what you have learnt, invented or created). Identify trends, relative changes or differences on answers to questions.
Conclusion: *What are the implications of your answer*? Briefly summarise any potential implications. (What are the larger implications of your findings, especially for the problem or gap identified in your motivation?)
Do not cite references and do not use abbreviations excessively in the abstract.

What this study adds: What key insights into the research results and its future function are revealed? How do these insights link to the focus and scope of the journal? It should be a concise statement of the primary contribution of the manuscript; and how it fits within the scope of the journal.

Introduction: The introduction must contain your argument for the social and scientific value of the study, as well as the aim and objectives:
Social value: The first part of the introduction should make a clear and logical argument for the importance or relevance of the study. Your argument should be supported by the use of evidence from the literature.
Scientific value: The second part of the introduction should make a clear and logical argument for the originality of the study. This should include a summary of what is already known about the research question or specific topic and should clarify the knowledge gap that this study will address. Your argument should be supported by the use of evidence from the literature.
Conceptual framework: In some research articles it will also be important to describe the underlying theoretical basis for the research and how these theories are linked together in a conceptual framework. The theoretical evidence used to construct the conceptual framework should be referenced from the literature.
Aim and objectives: The introduction should conclude with a clear summary of the aim and objectives of this study.
Research methods and design: This must address the following:
Study design: An outline of the type of study design.
Setting: A description of the setting for the study; for example, the type of community from which the participants came or the nature of the health system and services in which the study is conducted.
Study population and sampling strategy: Describe the study population and any inclusion or exclusion criteria. Describe the intended sample size and your sample size calculation or

justification. Describe the sampling strategy used. Describe in practical terms how this was implemented.
Intervention (if appropriate): If there were intervention and comparison groups, describe the intervention in detail and what happened to the comparison groups.
Data collection: Define the data collection tools that were used and their validity. Describe in practical terms how data were collected and any key issues involved, e.g. language barriers.
Data analysis: Describe how data were captured, checked and cleaned. Describe the analysis process, for example, the statistical tests used or steps followed in qualitative data analysis.
Ethical considerations: Approval must have been obtained for all studies from the author's institution or other relevant ethics committee and the institution's name and permit numbers should be stated here.
Results: Present the results of your study in a logical sequence that addresses the aim and objectives of your study. Use tables and figures as required to present your findings. Use quotations as required to establish your interpretation of qualitative data. All units should conform to the **SI convention** and be abbreviated accordingly. Metric units and their international symbols are used throughout, as is the decimal point (not the decimal comma).

Discussion: The discussion section should address the following four elements:
Key findings: Summarise the key findings without reiterating details of the results.
Discussion of key findings: Explain how the key findings relate to previous research or to existing knowledge, practice or policy.
Strengths and limitations: Describe the strengths and limitations of your methods and what the reader should take into account when interpreting your results.
Implications or recommendations: State the implications of your study or recommendations for future research (questions that remain unanswered), policy or practice. Make sure that the recommendations flow directly from your findings.
Conclusion: Provide a brief conclusion that summarises the results and their meaning or significance in relation to each objective of the study.

Acknowledgements: Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution. Authors are responsible for ensuring that anyone named in the Acknowledgments agrees to be named. Refer to the acknowledgement structure guide on our *Formatting Requirements* page.

Also provide the following, each under their own heading:
Competing interests: This section should list specific competing interests associated with any of the authors. If authors declare that no competing interests exist, the article will include a statement to this effect: *The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.* Read our **policy on competing interests**.
Author contributions: All authors must meet the criteria for authorship as outlined in the **authorship** policy and **author contribution** statement policies.
Funding: Provide information on funding if relevant
Data availability: All research articles are encouraged to have a data availability statement.
Disclaimer: A statement that the views expressed in the submitted article are his or her own and not an official position of the institution or funder.
References: Authors should provide direct references to original research sources whenever possible. References should not be used by authors, editors, or peer reviewers to promote self-interests. Refer to the journal referencing style downloadable on our *Formatting Requirements* page.