THE SHORT-TERM OUTCOMES OF HIV-EXPOSED VERSUS HIV-UNEXPOSED VERY LOW BIRTH WEIGHT INFANTS

By

STUDENT: LINDA JANE RIEMER
RMRLIN001

SUBMITTED TO THE UNIVERSITY OF CAPE TOWN
In fullfillment of the requirements of the degree

MASTER OF MEDICINE (MMed) Paediatrics

FACULTY OF HEALTH SCIENCES
UNIVERSITY OF CAPE TOWN

Date of Submission: 02/06/2017

Supervisor: Dr Lloyd Tooke
Division of Neonatology
Groote Schuur Hospital

TABLE OF CONTENTS

TABLE OF CONTENTS **2**

DECLARATION **3**

ABSTRACT **4**

ACKNOWLEDGEMENTS **5**

LIST OF TABLES **6**

LIST OF FIGURES **7**

Abbreviations **8**

1. INTRODUCTION AND LITERATURE REVIEW **10**

BACKGROUND *10*

OBJECTIVES *11*

METHODS *11*

RESULTS *13*

DISCUSSION *16*

CONCLUSION *18*

REFERENCES *24*

2. METHODS **28**

AIMS AND OBJECTIVES *28*

STUDY DESIGN *28*

DATA CAPTURING *29*

ANALYSES *31*

3. RESULTS **33**

3.1 HIV EXPOSED VS HIV UNEXPOSED NEONATES *34*

3.2 MATERNAL ARV-EXPOSED VS. MATERNAL ARV UNDER-EXPOSED *41*

3.3 HIV-PCR POSITIVE VS. HIV-PCR NEGATIVE NEONATES *47*

4. DISCUSSION **54**

5. CONCLUSION **62**

6. RECOMMENDATIONS **63**

REFERENCES: **64**

Appendices **68**

A: Ethics Letter *68*

B: Definitions of Data Collection Points *69*

DECLARATION

I, *Linda Jane Riemer* 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.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature:

Date: 02 June 2017

ABSTRACT

Introduction: HIV exposed but uninfected infants have been shown to have a higher morbidity and mortality than unexposed infants. There is almost no literature comparing the short-term outcomes of HIV exposed versus unexposed VLBW neonates who are born prematurely.

Methods: A retrospective review of all VLBW neonates who were admitted at Groote Schuur Hospital nursery from 2012-2014. Data were obtained from the Vermont Oxford Database and the Prevention of Mother to Child register.

Results: A total of 1593 VLBW neonates were admitted during the 3 years of which it was possible to obtain maternal HIV status in 1579 babies. Of these 1579 babies, 316 (20%)were HIV exposed. Eleven of the 230 (4.8%)infant HIV tests were positive. There was no difference in mortality, birth weight, gestational age, length of stay, sepsis and delivery room outcomes for the HIV-exposed (HIVE), maternal ARV-exposed (mARVE) and HIV-positive neonates. Differences between HIV exposed and HIV unexposed neonates were noted in an increased risk of NEC [OR 1.83 (1.2-2.8)] and an increased need for ventilation [OR 1.35 (1.01-1.8)]. Maternal antiretroviral exposed neonates developed less NEC compared with maternal antiretroviral under-exposed neonates with a birth weight under 1000grams appearing to contribute in the development and outcome of NEC. Differences in HIV-positive neonates included more chronic lung disease [OR 5.49 (1.31-23)] and more necrotising enterocolitis [OR 4.12 (1.02-17.18)].

Conclusion: This study is the first to compare the short-term outcomes of HIV exposed and HIV unexposed very low birth weight infants and consider maternal ARV exposure. It demonstrated no difference in birth weight, gestational age, mortality or sepsis. Necrotising enterocolitis is increased in the HIV exposed neonates especially if they are under-exposed to maternal antiretrovirals. Adequate maternal antiretrovirals may have a protective effect on incidence of necrotising enterocolitis and respiratory outcomes.

ACKNOWLEDGEMENTS

It is virutally impossible to produce anything of much significance entirely on your own.

Thank-you:

To my supervisor who was incredibally patient, surprisingly gentle when he was disappointed and always managed to get me to come back with an even better version after I was convinced it had looked great.

To Ms Gabeba Abass, who sourced all the folders and saved me the trouble and time of having to negotiate the archives.

To my family and friends, who made cups of tea and coffee, tried to explain statistics to me, let me try and explain the complexities of some of the concepts, cheered from the sidelines and dragged me over the finish line.

Lastly to the babies, who create the opportunity to learn and understand so that we know better for next time.

This is for all of you.

LIST OF TABLES

Table 1.1: Summary of studies on mortality *19*
Table 1.2: Summary of studies on all-cause morbidity *20*
Table 1.3: Summary of studies on neonatal jaundice and respiratory distress syndrome *21*
Table 1.4: Summary of studies on sepsis *22*
Table 1.5: Summary of studies on necrotising enterocolitis *23*

Table 2.1: PMTCT regimens during time of study (2012 - 2014) *31*

Table 3.1.1: Ethnicity of cohort by HIV status *33*
Table 3.1.2: Maternal characteristics for HIVE and HIVU neonates. *34*
Table 3.1.3: Neonatal outcomes of HIVE and HIVU infants *37*
Table 3.1.4: Sub-group analysis of HIVE and HIVU neonates who required ventilation *38*
Table 3.1.5: Sub-group analysis of HIVE and HIVU neonates who developed NEC. *39*
Table 3.1.6: Feeding at discharge for HIVE and HIVU neonates. *40*
Table 3.2.1: Maternal characteristics of mARVE and mARVU neonates. *42*
Table 3.2.2: Neonatal outcomes for mARVE and mARVU infants *45*
Table 3.2.3: Sub-group analysis of mARVE and mARVU neonates who developed NEC *46*
Table 3.3.1: Maternal Characteristics of HIV-PCR positive and HIV-PCR negative neonates. *49*
Table 3.3.2: Neonatal outcomes of HIV-PCR positive and HIV-PCR negative neonates. *52*
Table 3.3.3: Subgroup analysis of HIV-PCR positive and HIV-PCR negative neonates who required non-invasive ventilation. *53*

LIST OF FIGURES

Figure 1.1: Flow diagram of article selection process……14

Figure 3.1.1: Distribution of the cohort based on HIV-exposure status.....33
Figure 3.1.2: Box plot distribution for birth weight of HIVE vs. HIVU neonates.....34
Figure 3.1.3: Box plot distribution for gestational age of HIVE and HIVU neonates.....35
Figure 3.1.4: Box plot distribution for length-of-stay for HIVE and HIVU neonates.....35
Figure 3.1.5: Box plot distribution of birth weights for HIVE vs. HIVU neonates who developed NEC 39

Figure 3.2.1: Distribution of maternal antiretroviral (ARV) exposure among the HIV-exposed cohort 41
Figure 3.2.2: Box plot distribution of gestational ages for mARVE and mARVU neonates 43
Figure 3.2.3: Box plot distribution of birth weight for mARVE and mARVU neonates. 43
Figure 3.2.4: Box plot distribution of length-of-stay for mARVE and mARVU neonates. 44
Figure 3.2.5: Box plot distribution of birth weight of mARVE and mARVU neonates who developed NEC.....46

Figure 3.3.1: Distribution of HIVE neonates based on HIV-PCR -testing and HIV-PCR -status.....47
Figure 3.3.2: Flowchart representation of infant disposition and timing of HIV-PCR testing 48
Figure 3.3.3: Box plot distribution of gestational age for HIV-PCR + and HIV-PCR - neonates.....50
Figure 3.3.4: Box plot distribution for birth weights of HIV-PCR + and HIV-PCR - neonates.....50
Figure 3.3.5: Box plot distribution for length-of-stay of HIV-PCR + and HIV-PCR - neonates.....51

Abbreviations

HIV	Human Immunodeficiency Virus
PMTCT	Prevention of Mother to Child Transmission
HIVEU	HIV Exposed but uninfected
HIV+	HIV infected
HIVU	HIV unexposed
HIVE	HIV exposed
LBW	Low birth weight
PIH	Pregnancy induced hypertension
PET	Pre-eclamptic Toxaemia
ELBW	Extreme low birth weight
ART	Antiretroviral therapy
ARV	Antiretroviral
ARVE	Antiretroviral exposed
ARVU	Antiretroviral under-exposed
VLBW	Very low birth weight
RDS	Respiratory distress syndrome
CLD	Chronic Lung Disease
BPD	Bronchopulmonary Dysplasia
PDA	Patent ductus arteriosus
IVH	Intraventricular Haemorrhage
NEC	Necrotising Enterocolitis
CMV	Cytomegalovirus
TB	Tuberculosis
GA	Gestational Age
HMD	Hyaline Membrane Disease
TTN	Transient tachypnoea of the newborn
GBS	Group B Streptococcus
EOS	Early Onset Sepsis
LOS	Late onset sepsis
CI	95% Confidence interval
HIV-PCR	Human Immunodeficiency Virus Polymerase chain reaction
SGA	Small for gestational age
ANC	Antenatal Care
CPAP	Continous positive airway pressure
VON	Vermont Oxford Network
AZT	Zidovudine
NVP	Nevirapine
HAART	Highly active antiretroviral therapy
NMR	Neonatal Mortality Rate
RR	Risk Ratio
OR	Odds Ratio
USA	United States of America
IRR	Incident Rate Ratio
LRTI	Lower Respiratory Tract Infection
aOR	Adjusted Odds Ratio
GSH	Groote Schuur Hospital
CSF	Cerebrospinal Fluid
ROP	Retinopathy of Prematurity
UCT	University of Cape Town
mARVE	Maternal Antiretroviral exposed
mARVU	Maternal Antiretroviral under-exposed

EBM	Exclusive Breast Milk
PVL	Periventricular Leukomalacia
NIV	Non-invasive Ventilation
UK	United Kingdom
RCT	Randomised Control Trial
WHO	World Health Organisation

1. INTRODUCTION AND LITERATURE REVIEW

BACKGROUND

Maternal HIV infection poses the documented risk of vertical transmission. Effective prevention-of-mother-to-child-transmission (PMTCT) programs have decreased this transmission in developing countries from as high as 48%[1] to less than 5%[2], with a resultant decrease in morbidity and mortality associated with paediatric HIV infection. With the decreasing incidence of perinatal HIV transmission, a growing number of infants and children are falling into the HIV-exposed but uninfected (HIVEU) population. These infants are gaining recognition as a group with their own unique risks in terms of morbidity and mortality, as compared to both HIV infected (HIV+) and HIV unexposed (HIVU) children.

Brennan et al have demonstrated an overall increased risk of mortality by 70%, in a meta-analysis on all-cause mortality between HIV-exposed (HIVE) and HIVU infants and children. This risk lessened after the widespread implementation of PMTCT programmes in 2002, but a significant difference in mortality still remained (<2002: RR 1.73, CI 1.22 – 2.46 and >2002 RR 1.46, CI 1.14 – 1.87) [3]. In a systematic review on mortality and morbidity, among HIVEU and HIVU infants and children under the age of 10 years in Sub-Saharan Africa, Le Roux et al also demonstrated increased overall mortality (RR 1.93, CI 1.17-3.17) [4]. Slogrove et al have evaluated the timing and type of infectious morbidity and mortality in HIVEU infants and children. They concluded firstly, that underlying causes of morbidity and mortality in these infants were the same as the common causes of under-5 mortality, but these tended however to be more severe in presentation. Secondly, that these infants are at an increased risk for invasive streptococcal infections. Thirdly, that the relative increase in morbidity and mortality is most evident between the ages of 2 and 6 months, with HIVEU neonates and HIVU neonates having equally increased risk of morbidity and mortality[5]. In a report from the 2016 annual workshop, established to review the growing evidence in support of this increased risk profile among HIVE infants and children, a summary of the postulated theories as to the yet unexplained aetiology of this increased risk was published. These include innate immune deficits by multiple proposed mechanisms i.e. exposure to antiretrovirals (ARV's) and HIV viral particles, as well as impaired maternal immunity. Additional factors may include nutritional deficiencies driven by both suboptimal breastfeeding practices and lower socioeconomic status, which often affects HIV-exposed households[6].

In addition to a possible impaired neonatal immune system, antenatal HIV-exposure has risks for infants with regards to higher rates of prematurity[7-9], low birth weight (LBW) [10] and intrauterine growth restriction[9,11,12]. These risks are of particular importance in mothers who have advanced HIV disease[13,14]. A meta-analysis published in 2015 by Xiao et al confirmed this increased risk of LBW (pooled OR 1.73, CI 1.64-1.82) and preterm birth (pooled OR 1.56, CI 1.49-1.63) [15].

An increased risk for pregnancy induced hypertension (PIH) including pre-eclampsia (PET) and eclampsia, has also been described in HIV-exposed pregnancies. The significance of this potential association is that it often results in the delivery of a premature and low birth weight infant. A systematic review of the evidence by Adams et al of HIV-positive mothers and ART with PIH, found the data to be conflicting[16]. Brown et al's meta-analysis found neither an associated risk with HIV or ART and PIH[17]. However, previously published data from the Groote Schuur Neonatal unit has showed a high prevalence of HIV-exposure among the Extreme Low Birth Weight (ELBW) neonates, most of whom were delivered for maternal hypertensive indications. Although there was no association between HIV and PET, there was a significant association between mothers who had been exposed to antiretroviral therapy (ART) for more than 4 weeks and PET[18].

On their own, very low birth weight (VLBW) neonates have an increased risk of morbidity and mortality. A Johannesburg based study found a survival rate of 86% in neonates with a birth weight of between 1001 and 1500 grams, and as low as 35% for neonates less than 1000grams[19]. Gestational age is also a key determinant of mortality and morbidity risk profiles for these infants, with an inverse relationship between the two[20,21]. Prematurity related complications such as respiratory distress syndrome (RDS) and subsequent bronchopulmonary dysplasia (BPD), patent ductus arteriosus, (PDA), intraventricular haemorrhages (IVH), necrotising enterocolits (NEC) and sepsis are common contributing factors[21].

The question we subsequently propose, is if all of these risk factors converge to produce an even more vulnerable population of HIVE, premature and very low birth weight neonates during the neonatal period?

OBJECTIVES

The objective of this literature review was to evaluate the available literature on short-term outcomes of HIV-exposed neonates in terms of mortality and morbidity, specifically with reference to those infants that are premature i.e. <37 weeks gestational age, or VLBW i.e. ≤1500g.

METHODS

A search was carried out using PubMed *(https://www.ncbi.nlm.nih.gov)* on 29.1.2017 using the terms HIV-EXPOSED, NEONATE, MORBIDITY in the following search string: (HIV-EXPOSED[All Fields] AND ("infant, newborn"[MeSH Terms] OR ("infant"[All Fields] AND "newborn"[All Fields]) OR "newborn infant"[All Fields] OR "neonate"[All Fields]) AND ("epidemiology"[Subheading] OR "epidemiology"[All Fields] OR "morbidity"[All Fields] OR "morbidity"[MeSH Terms])) AND "infant, newborn"[MeSH Terms].

This yielded 210 articles. 5 were immediately excluded because they were not published in English.

Primarily, articles were required to report outcomes of HIV-exposed and HIVEU neonates, either descriptively or by comparison with an unexposed cohort. Outcomes needed to specifically be focused on those relevant to short-term neonatal admission diagnosis. This included mortality and morbidity in the form of sepsis, jaundice, respiratory complications and NEC.

Articles were excluded if their title or abstract clearly did not meet inclusion criteria. When this was less clear the methods and results sections were scrutinised in the full text to evaluate for inclusion.

Studies were excluded if they looked at PMTCT outcomes, effects of ARV's or evidence of altered immune function based on haematological, serological or vaccine response studies. Studies were also excluded if they looked at long-term outcomes that are beyond the scope of the neonatal period, including growth and development, and outcomes based on feeding and nutritional interventions. Birth outcomes studies were reviewed separately and less formally for the introduction of this review and were also excluded. Studies that looked at congenital abnormalities and infections specifically Hepatitis B, Syphilis, CMV and TB were not included in our review. Although certainly relevant in terms of neonatal morbidity, their impact is too specific and beyond the scope of this review.

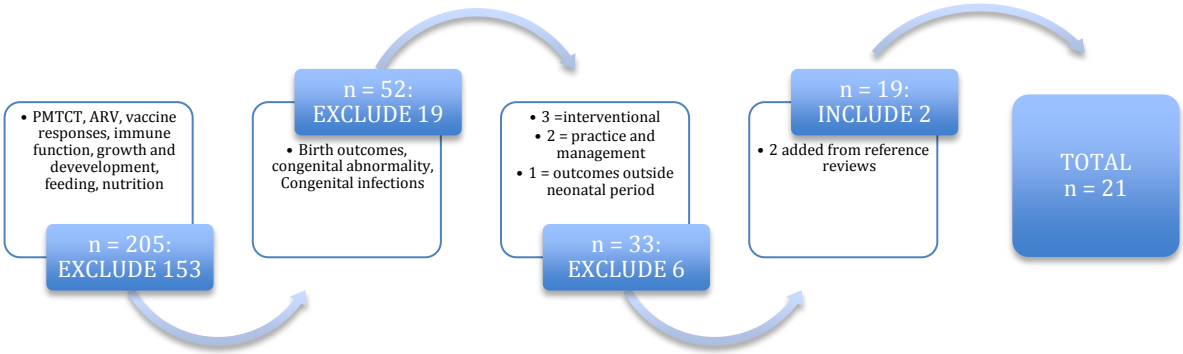
The remaining studies were excluded for the following reasons

- 3 reviewed outcomes based on specific interventions including, probiotics and Co-Trimoxazole prophylaxis.
- 2 were practice guidelines and management reviews

An additional 2 studies were identified as appropriate based on reference lists from the included studies.

The final 21 articles were reviewed in full text and findings were summarised in *Tables 1.1 – 1.5*.

Figure 1.1: Flow diagram of article selection process



RESULTS

The 21 articles were published between 1991 and 2015 with the majority of the literature (16/21) published after 2010.

Five reports came from outside of Africa: 4 of these from developed nations including the USA, France and Belgium[10,22-24] and 1 from Latin America[25]. The remaining 16 were published out of Sub-Saharan Africa with 7/16 coming from South Africa. The 2 studies from Zimbabwe reported findings from the same database (ZVITAMBO trial) [26] although Koyanagi et al reported morbidity data and Marinda et al reported on mortality[27,28].

Twelve out of 21 were prospective studies; 7 primary analyses [10,29-34] and 5 secondary[25,35-38]. The remaining 9 were retrospective; 7 primary[10,29,30,32-34,39] and 2 secondary analyses[27,28]. Four studies were case control cohorts[23,32,38,40]. Thirteen studies focused on mostly term babies, 6 on premature (<37 weeks) [23,34,40-42] and 2 did not report gestational age[29,36]. Thirteen studies reviewed mostly normal birth weight infants (>2500g) and 2 reviewed predominantly LBW between 1500g and 2500g[29,34]. VLBW infants i.e. 1000-1500g, were referenced as between 3% and 63% in 4 articles[10,29,30,34] and additionally represented the average weight of the 5 cohorts that evaluated NEC[23,40-43]. Only 1 study referenced ELBW infants (<1000g) as 8% of the cohort[34]. One study did not document birth weights[24].

MORTALITY

Seven studies reported on mortality *(Table 1.1)* [28,29,32,34-36,38]. All 7 were published out of Sub-Saharan Africa and sample sizes ranged from 70 to 13792. Five cohorts focused on mostly term and normal birth weight neonates[28,32,35,36,38]. Two looked at a largely preterm (median GA 34 weeks) and LBW infants[29,34].

Ades et al published a Neonatal Mortality Rate (NMR) of 37/1000 live births[35] while Kourtis et al reported a rate of 0.056/person year[36]. Four of the 5 comparative studies did not find a difference in mortality between HIVE and HIVU infants[29,32,34,38].

Only Marinda et al reported a difference in mortality rates between HIV+, HIVEU and HIVU infants. They published a 30-day mortality proportion of 1.9% in HIVEU and 0.7% in HIVU infants, and statistically compared mortality at 8 weeks of age, demonstrating an increased risk in HIVEU compared to HIVU (RR 2.8 CI 2.1 – 3.9) [28].

Monebenimp et al found prematurity and LBW to be risk factors for mortality but in both HIVE and HIVU infants[32]. Kourtis et al found only LBW to be associated with neonatal death in their cohort[36]. Ades et al found multiple associations with neonatal mortality in their cohort (n=351), but only prematurity was associated with mortality on multivariate analysis *(Table 1.1)* [29,35].

ALL CAUSE MORBIDITY

Only 3 articles looked at all cause morbidity *(Table1. 2)* [27,29,31]. All 3 were published from Sub-Saharan Africa between 2005 and 2014. Sample sizes ranged from 70 – 12937. One study compared HIVE vs. HIVU[29] and two studies compared HIVEU vs. HIVU[27,39]. Two studies focused on predominantly term and normal birth weight infants[27,31]. Adhikari et al did not report gestational age for their cohort but most babies were less than 2.5kg (90% and 87% for HIVE and HIVU respectively) with a large proportion of them falling into the VLBW category (63% and 45%). In addition the most common admission diagnosis was hyaline membrane disease (HMD), so we can postulate that the cohort was predominantly premature[29].

Only the Zimbabwe study reported a difference in all-cause morbidity. Incidentally this was also the earliest study, with data captured between 1997 and 2000. They reported more all-cause sick visits between HIVEU and HIVU infants, incident rate ratio (IRR) 1.2, CI 1.1-1.4. Presentation with a lower respiratory tract infection (LRTI) was the only specific clinical cause to explain this difference with an IRR of 1.6, CI 1.1-2.3[27]. The remaining 2 studies reported no difference in all cause morbidity[29,31].

SPECIFIC MORBIDITY

The remaining 12 articles looked at specific morbidity causes.

One article looked at jaundice requiring phototherapy in a small numbered (n = 149) prospective study comparing incidence, and found that HIVE was protective in terms of developing supra-treatment jaundice. This study was poorly powered however and is limited in terms of its application *(Table 1.3)* [33].

Two studies looked at Respiratory Distress Syndrome (RDS) [10,25]. One was published in 1997 out of the USA and one in 2011 from Latin America. They both looked at prospective cohorts of largely term and normal birth weight infants, and they both only had cohorts of HIVE infants thus do not have good comparative data. The later Latin American study quantified respiratory morbidity as part of the secondary outcomes from within a larger study, which compared respiratory outcomes based on mode of delivery in an HIVE cohort. They reported a morbidity of 7.5%, mostly due to RDS and transient tachypnoea of the newborn (TTN) [25]. The earlier USA based study was more helpful by comparing their numbers to expected values for the population, but observed no difference in respiratory morbidity among HIVE neonates *(Table 1.3)* [10].

Four studies on sepsis were published between 2010 and 2015 *(Table 1.4)* [22,24,30,37]. Two of these were published out of the same clinical research unit in Belgium and with some overlap of the time period. The first looked at incidence of Group B Streptococcal (GBS) infection and the second at all-cause severe infections, albeit in children under 1 year of age[22,24]. The remaining 2 reports were published out of Baragwanath Hospital in Soweto, also with overlap in the data collection years. Again one report focused on all-cause sepsis, however restricted to the neonatal period, and the second looked at evidence for increased GBS disease[30,37]. Sample sizes ranged between 21 and 4108, and all 4 studies looked at mostly term infants.

Only one study, Cutland et al , had appropriate data for all-cause sepsis morbidity in the neonatal period[37], as the Belgium study only reported incidence at the end of the first year of life[22]. The Johannesburg based study, reflective of a large cohort of 4108 neonates, prospective in nature and well matched in terms of maternal and neonatal characteristics, reported a borderline increased incidence of early onset culture-positive sepsis in HIVE compared to HIVU neonates (p=0.05). No difference was detected between clinically suspected early onset sepsis (EOS) or overall EOS. Further analysis of confirmed HIVEU vs. HIVU neonates (n=2432) actually showed a decreased incidence of clinically suspected and overall EOS, which is contrary to what is hypothesised. No difference between either the HIVE or HIVEU groups compared to HIVU neonates was found with regards to incidence of late onset sepsis (LOS) i.e. after day 3 of life[37].

Three studies found evidence that HIVE babies get more GBS sepsis. Specifically looking at HIVEU babies, Epalza et al showed an overall RR of 19.6 (CI 7.5 – 51.7) and specifically an increased risk for late onset disease (RR 125.2; CI 26.3-620.2) [24]. This evidence is limited by very small numbers and not well powered (n = 21). Adler et al also looked at HIVEU infants and showed a 13-times increased incidence in GBS sepsis by comparing their findings to the reported population incidence[22]. Cutland et al found both increased overall risk (RR 2.25, CI 1.84 – 2.76) as well as specific increased risk for late onset GBS disease i.e. HIVE 58.1% vs. HIVU 41.9%, p=0.004. This data reports essentially HIVE babies as opposed to HIVEU as PCR results were only available for 28% of the sample[30].

Risk factors reported for sepsis in the neonatal period included prematurity[22,24] and small for gestational age (SGA) [24]. Cutland et al reported an increased case fatality rate for VLBW babies and a GA <33 weeks[30]. Epalza et al reported a trend for increased severity of disease (meningitis and septic shock) among HIVEU babies, as did Cutland et al (meningitis and bacteraemia) [24,30].

Five of the studies looked at NEC *(Table 1.5)*. Only one study was published from a developed nation i.e. France and was also the earliest study from 2005[23]. The remaining 4 studies, published between 2010 and 2014, were all from South Africa (1 from Johannesburg, 3 from Cape Town) [40-43]. All of the cohorts comprised of premature (30–31 weeks) and VLBW neonates (1.2 – 1.6kg). All of the studies were retrospective and small in size ranging from n=37 to n=337. Two of the studies compared incidence of NEC between HIVE and HIVU infants[23,40]. Angura et al found no difference but Desfrere et al found an increased risk (aOR 6.63, CI 1.26 – 35). When comparing methodology and cohorts, significant differences included the time period during which data was

captured (late 1990's/early 2000's for Desfrere and mid-2000's for Angura), the prevalence of HIV in the population (low for Desfrere and high for Angura) and feeding [Desfrere babies first fed slightly later than Angura i.e. day 4 and day 2 respectively, but Desfrere babies received more breast milk than Angura (88% vs. 58%)].

None of the four studies that looked at clinical outcome or extent of disease found any differences between HIVE and HIVU[40-43], but Arnold et al did report an increase in post-operative sepsis in HIVE neonates compared to HIVU (41% vs. 16%, p=0.03) [41]. Four studies reported on mortality with 2 reporting no difference[41] while Karpelowsky reported increased mortality (OR 4.8, p =0.05) [43], as did Chokoe (20% vs. 83%, p = 0.0002) [42]. Both of these studies were limited by the fact that they were retrospective reviews, did not adjust outcomes for other confounding factors and had small sample sizes (n =70 and 37 respectively). Comparing the evidence of the studies that reported no difference, Arnold et al and Angura et al, the same limitations applied with the exception of Angura who had the largest cohort, n = 330[40,41].

DISCUSSION

Data on outcomes of HIVE neonates is limited. The literature is comprised of prospective and retrospective reviews made up of relatively small number cohorts, with the best level of evidence being a single prospective matched case-control study[38]. In addition the literature predominantly reflects cohorts of term and normal birth weight babies. This probably stems from the majority of the work being published out of Sub-Saharan Africa. Despite the high prevalence of HIV in this region, the mortality of premature and VLBW infants is significant, which makes it difficult to find adequate numbers of these babies to sample. Outcomes relating to NEC - a pathology almost exclusively seen in premature infants - is the exception with regards to birth weight and gestational age sampling, however the cohort numbers are small.

Prematurity is reported as a risk factor for mortality and sepsis among HIVE neonates in the reports we reviewed. On closer scrutinisation of this literature, Monebenimp et al's[32] findings on increased mortality risk relates to both HIVE as well as HIVU neonates and Ades et al[35] is descriptive data from a cohort of only HIVE infants. It is therefore difficult to determine if this risk does not reflect the known risk of prematurity on its own. Regarding birth weight, Cutland et al's[30] observation of HIV-exposed VLBW neonates having increased mortality is limited to those who developed GBS sepsis. The only other study that commented on LBW as a risk factor for mortality among HIVE neonates was Kourtis et al[36], but being a descriptive study, it possibly again reflects the known increased risk of the LBW population.

The quality and relevance of the evidence regarding neonatal jaundice and RDS limits our ability to draw any valuable conclusions. *(Table 1.3)* Only one study suggests no increased morbidity related to RDS, but this is only relevant to term neonates[10].

Ades et al's[35] reported neonatal mortality rate of 37/1000 live births is much higher than the local Ugandan NMR (23.1 – 20.4/1000) for the time period between 2009 and

2012 during which the data was captured[44]. Marinda et al also reported an increased mortality rate[28]. Their study, although well designed and with impressive numbers, only allows us to assume a significant 28-day mortality based on the statistics at 8 weeks of life. A second consideration is that the data reflects HIV-exposure prior to the widespread use of maternal ARV's and PMTCT programmes and may now be outdated[28]. We have seen from Le Roux et al's[4] postulations from their systematic review, that better maternal health may minimise the risk of mortality to their infants. This is supported by Brennan et al's[3] findings of decreased mortality in the post-ARV era. Four out of the 5[29,32,34,38] comparative studies did not show an increased mortality, with at least 2 of the 4 studies reflective of a largely preterm cohort[29,34]. The evidence as such seems to favour no difference in mortality *(Table 1.1)*.

Two out of 3 studies on all-cause morbidity found no difference including one that reflects an assumed cohort of preterm infants[29,31]. Koyanagi's et al's study[27] which did report an increased morbidity represents the same large Zimbabwe cohort as Marinda et al and so the pre-ARV era considerations of expected poorer outcomes apply equally *(Table 1.2)*.

Three out of the 4 studies on sepsis reported increased risk of sepsis in HIVE neonates, but all three specifically looked at GBS. Two of the studies convincingly excluded infections in HIV positive infants but one is poorly powered (n = 21) [24] and the second is a descriptive study comparing an incidence rate with that of the general population[22]. The 2015 study from Baragwanath[30] has HIV PCR data available for only 28% of their HIVE neonates, thus is limited by it's inability to accurately distinguish HIV-infected and HIVEU infants. This is especially relevant given that an earlier study from this unit found a clear increase in overall sepsis in HIV positive neonates[37]. A second limitation of this data is that the overall incidence of GBS was increased in the study (2.72 cases/1000 live births) compared to global (0.53 cases/1000) and African (1.21 cases/1000) estimates, reported in a meta-analysis on GBS sepsis, between 2000 – 2011[45]. Consequently, the increased GBS incidence reflected may be prone to population bias. Two studies[24,30] show this increased GBS sepsis risk is reflected in the late onset period and although Cutland et al[30] shows both EOS and LOS have increased risk; it is quantitatively higher in late disease (RR for EOS 1.69, CI 1.28 – 2.24, RR for LOS 3.18, CI 2.34 – 4.36, *Table 1.4*).

There was 1 study on all-cause sepsis and that refuted the hypothesis of an increased risk in HIVEU neonates. It reported a decreased incidence of clinically suspected early onset sepsis between HIVEU neonates compared to HIVU (20.6 vs. 33.7 per 1000 births; p = .046), and no difference in late onset disease (5.8 vs. 4.1; p = 0.563). However, in the same cohort, when looking at the HIVE neonates i.e. disregarding neonatal HIV status, they reported an increased incidence of culture positive EOS among HIVE neonates compared to HIVU[37]. Some key issues to note in this review include;

- a very small number of neonates actually had positive cultures (0.3%), which may affect the validity of this outcome.
- Significantly more of the HIV+ neonates had EOS compared to the HIVU neonates (134 vs. 21.5 per 1000 births, p = 0.046), which might account for the increased sepsis in the HIVE cohort.

- The neonates in the "clinical sepsis" group were possibly oversampled based on excessively broad criteria for sepsis.
- This cohort was at risk of sample bias in favour of less sepsis, as only 4% of the cohort was premature, a number well below the incidence of the unit. We did not review the inclusion and exclusion criteria of the original maternal cohort for the primary analysis that may have predisposed to this selection bias[37]. *(Table 1.4)*

Despite the literature on NEC being relevant to premature and VLBW infants it is limited by small sample sizes (n = 37 -330) and retrospective data. The trend for similar presentation of disease between HIVE and HIVU infants seems constant in the articles, and one study showed an increased risk for post-operative sepsis [41]. Regarding incidence of disease and mortality it is divided *(Table 1.5)*.

CONCLUSION

There is good evidence that older HIVEU infants and children have increased morbidity and mortality as compared to their HIVU counterparts, however it remains unclear as to the underlying aetiology of this increased risk. The literature on HIVE neonates is limited to cohort studies, with poor representation of high risk VLBW and premature neonates. Regarding specific outcomes, there is only 1 comparative study on respiratory outcomes and it focuses on term neonates. The evidence pertaining to increased sepsis in HIVE neonates is almost exclusively related to GBS infections. While the NEC literature is pertinent to premature and VLBW neonates, it is limited in terms of study design and small sample sizes. Overall however, the available evidence does not favour a hypothesis of increased mortality and all-cause morbidity between HIVE and HIVU neonates.

There is a need for well-powered evidence comparing HIVE and HIVU premature and LBW infants looking at short-term neonatal outcomes. Of interest is mortality, differences in gestation, birth weight and growth restriction as well as morbidity due to respiratory distress, sepsis and NEC.

Table 1.1: Summary of studies on mortality

#	FIRST AUTHOR	TITLE	PUBLISH DATE	STUDY DESIGN	LOCATION	MAIN OUTCOME	SAMPLE SIZE	GA	BIRTH WEIGHT	MORTALITY OUTCOME	MORBIDITY OUTCOME	OTHER
39.	Zash RM	Risk factors for mortality among HIV-exposed and HIV unexposed infants admitted to a neonatal care unit in Botswana	2014	Prospective. Secondary analysis. Comparative.	Botswana: 2008/2009	Risk factors for neonatal mortality	**n = 449**. HIVE n=128 (29%) HIVU n=272 (60%) unknown status n = 49 (11%)	Median GA 34 weeks	LBW 32% VLBW 23% ELBW 8%	No difference between HIVE (27%) and HIVU (20%) p = 0.19	Discharge diagnoses the same. No difference in sepsis. (p =0.92)	
35.	Marinda E	Child mortality according to maternal and infant HIV status in Zimbabwe	2007	Retrospective. Secondary analysis of ZVITAMBO trial data. Comparative.	Zimbabwe: 1997-2000	Compare mortality rates for HIV+/HIVEU/HIVU infants in the first 2 years	**n = 13 792**. HIVEU: 3135 HIVU: 9510 HIV+: 1147	Mean GA 39 weeks.	LBW HIVEU 15% HIVU 12% *<1500G excluded*	30day deaths: HIVEU 1.9% HIVU 0.7% 8-week risk of mortality HIVEU vs. HIVU RR 2.8 (2.1 - 3.9)		
40.	Ades V	Neonatal mortality in HIV-exposed infants born to women receiving combination antiretroviral therapy in rural Uganda	2013	Prospective. Secondary analysis of PMTCT study. Descriptive.	Uganda: 2009-2012	Assess risk factors for neonatal morbidity in HIVE infants	**n = 351**	Median GA 39 weeks	LBW 20%	Neonatal mortality rate 37/1000 live births		ASSOCIATIONS WITH MORTALITY Univariate analysis: low GA, LBW and shorter duration of maternal ART. Multivariate analysis = low GA.
41.	Kourtis AP	Health outcomes in HIV-exposed uninfected infants	2013	Prospective. Secondary analysis of BAN study (Breastfeeding, Antiretrovirals and Nutrition). Descriptive.	Malawi: 2004-2010	Evaluate effect of prognostic factors on infant morbidity and mortality	**n = 2250**	not documented	LBW 7% *<2000g excluded*	Neonatal mortality rate of 0.056/ person year		Only association with death was LBW (<2500g)
37.	Monebenimp F	HIV exposure related newborn morbidity and mortality in the University Teaching Hospital of Yaounde, Cameroon	2011	Prospective. Primary analysis. Case-control	Cameroon 2006/2007	HIVE related to neonatal morbidity and mortality	**n = 240.** HIVE 80 HIVU 160	preterm 24%	LBW 21% Mean bwt 2.9kg	No difference p = 0.52		On univariate, analysis prematurity and LBW associated with HIVE, not on multivariate analysis.
42.	Lepage P	Perinatal transmission of HIV-1: lack of impact of maternal HIV infection on characteristics of live births and on neonatal mortality in Kigali, Rwanda	1991	Prospective. Primary analysis. Matched case-control.	Rwanda: 1988-1989	Uncertain - Only abstract available: Part of a perinatal transmission study	**n = 436.** HIVE 218 HIVU 218	Median GA 39 weeks	LBW 20%	No difference		
29.	Adhikari M	The HIV-1 exposed neonate: outcome of intensive care management in the first week of life	2005	Prospective. Primary analysis. Comparative.	Durban: 2000/2001	Compare short and medium-term prognosis between HIVE and HIVU neonates *(excluded if mom received nevirapine)*	**n=70.** HIVE 30 HIVU 40	not reported	HIVE: LBW 90% VLBW 63% HIVU: LBW 87% VLBW 45%	No difference p = 0.42	No clinical difference between the two groups	Majority of babies were VLBW and admitted with HMD so probably a mostly preterm cohort.

GA = gestational age, HIVE = HIV exposed, HIVU = HIV unexposed, LBW = low birth weight (< 2500g), VLBW = very low birth weight (1000g – 1500g), ELBW = extremely low birth weight (<1000g), HIVEU = HIV exposed but uninfected, ART = antiretroviral therapy, BWT = birth weight

Table 1.2: Summary of studies on all-cause morbidity

#	FIRST AUTHOR	TITLE	DATE PUBLISH	STUDY DESIGN	LOCATION	MAIN OUTCOME	SAMPLE SIZE	GA	BIRTH WEIGHT	MORTALITY OUTCOME	MORBIDITY OUTCOME	OTHER
34.	Koyanagi A	Morbidity among HIV exposed but uninfected, HIV infected and HIV unexposed infants in Zimbabwe before availability of HAART	2011	Retrospective. Secondary analysis of ZVITAMBO study. Comparative.	Zimbabwe: 1997 - 2000	Evaluate rates of total and cause specific sick clinic visits or hospitalisations up to 2 years age.	**n= 12 937** HIVEU 2661 HIVU 9207 HIV+ 1069	Preterm 6.7%	Mean BWT 3000g <*1500g excluded*		Increased neonatal all cause sick clinic visits IRR 1.2 (1.1-1.4) LRTI IRR 1.6 (1.1 - 2.3)	Increased all cause admissions IRR 1.5 (1.2 - 2.0); LRTI significant IRR 2.7 (1.6 - 4.7)
36.	Moraleda C	Impact of HIV exposure on health outcomes in HIV-negative infants born to HIV-positive mothers	2014	Prospective. Primary analysis. Comparative.	Mozambique: 2008 - 2009	Evaluate impact of maternal HIV status on child health.	**n=318.** HIVEU 158 HIVU 160	Median GA 39 weeks	LBW 5.5% Median BWT 3000g		No difference in OPD visits or hospital admissions in first month between HIVE and HIVEU.	
29.	Adhikari M	The HIV-1 exposed neonate: outcome of intensive care management in the first week of life	2005	Prospective. Primary analysis. Comparative.	Durban: 2000/2001	Compare short and medium-term prognosis between HIVE and HIVU neonates *(excluded if mom received nevirapine)*	**n=70.** HIVE 30 HIVU 40	not reported	HIVE: LBW 90% VLBW 63% HIVU: LBW 87% VLBW 45%	No difference	No clinical difference between the two groups	Majority of babies were VLBW and admitted with HMD so probably a mostly preterm cohort.

GA = gestational age, HIVEU = HIV exposed yet uninfected, HIVU = HIV unexposed, HIV+ = HIV positive, BWT = birth weight, IRR = incidence risk ratio, LRTI = lower respiratory tract infection, LBW = low birth weight, OPD = outpatients' department, HIVE = HIV exposed, VLBW = very low birth weight, HMD = hyaline membrane disease.

Table 1.3: Summary of studies on neonatal jaundice and respiratory distress syndrome

#	FIRST AUTHOR	TITLE	DATE PUBLISH	STUDY DESIGN	LOCATION	MAIN OUTCOME	SAMPLE SIZE	GA	BIRTH WEIGHT	MORTALITY OUTCOME	MORBIDITY OUTCOME	OTHER
38.	Nakanga W	Supra-treatment threshold neonatal jaundice: Incidence in HIV-exposed compared to non-exposed neonates at Queen Elizabeth Central Hospital in Blantyre, Malawi.	2015	Prospective. Primary analysis. Comparative.	Malawi: 4 weeks Sept/Oct 2013	Test hypothesis that HIVE neonates have higher incidence of neonatal jaundice	**n=149.** HIVE 17 HIVU 132	Premature 31%	LBW 43%		HIVU = more jaundice. (81% vs. 47%, p <0.0001).	
25.	Kreitchmann R	Mode of delivery and neonatal respiratory morbidity among HIV-exposed newborns in Latin America and the Caribbean: NISDI perinatal-LILAC studies.	2011	Prospective. Secondary analysis. Descriptive.	Latin America/ Caribbean: 2002- 2009	Assess effect of mode of delivery on neonatal respiratory distress in HIVE infants	**n = 1443**	Premature 9.7%	LBW 10%		Incidence 7.5% mostly due to RDS and TTN	
10.	Martin R	Incidence of premature birth and neonatal respiratory disease in infants of HIV positive mothers	1997	Prospective. Primary analysis. Descriptive (compared to general population)	USA: 1990 - 1994	1. Characterize rates of prematurity and LBW in cohort of HIVE infants. 2. Determine spectrum, incidence and severity of respiratory disease	**n = 600**	Premature 19%	LBW 18% VLBW 3%		Respiratory outcomes as expected for the general population	

GA = gestational age, HIVE = HIV exposed, HIVU = HIV unexposed, LBW = low birth weight, HIVU = HIV unexposed, RDS = respiratory distress syndrome, TTN = transient tachypnoea of the newborn, VLBW = very low birth weight.

Table 1.4: Summary of studies on sepsis

#	FIRST AUTHOR	TITLE	DATE PUBLISH	STUDY DESIGN	LOCATION	MAIN OUTCOME	SAMPLE SIZE	GA	BIRTH WEIGHT	MORTALITY OUTCOME	MORBIDITY OUTCOME	OTHER
24.	Epalza C	High incidence of invasive group B streptococcal infections in HIV-exposed uninfected infants	2010	Retrospective. Primary analysis. Comparative.	Brussels: 2001 - 2008	Characterise and compare GBS infections in HIVEU infants with HIVU infants (recruited up to 90days life)	**n=21.** HIVEU 5 HIVU 16	PT 37%	not documented		Increased risk in HIVEU – RR for GBS overall 19.6 (7.5 - 51.7) EOD not statistically different. LOD = 125.2 (26.3 - 620.2)	More PT and SGA in the HIVE group. Tended towards more severe in HIVE.
22.	Adler C	Severe Infections in HIV-Exposed Uninfected Infants Born in a European Country.	2015	Retrospective. Primary analysis. Descriptive (compared to general population)	Brussels: 1985 - 2006	Examine incidence and risk factors for all cause severe infection in HIVEU cohort (data representative of first year of life)	**n=537**	PT 11.6%	mean weight 3037g 15% LBW		GBS infection 13x higher than general population. PTB = risk factor in the neonatal period. (aOR 21.34; 7.13 - 63.93)	
30.	Cutland CL	Increased risk for GBS sepsis in young infants exposed to HIV, SOWETO, SOUTH AFRICA.	2015	Prospective. Primary analysis. Comparative.	Soweto, JHB: 2004 - 2008	Examine clinical and epidemiological characteristics of GBS sepsis in a population with high HIV prevalence. (recruited up to 90 days)	**n = 327**	PT 27%; 70% < 33 weeks	Median weight 2800g LBW 36% VLBW 11%		Overall RR 2.25 (1.84 - 2.76) times increased invasive GBS in HIVE vs. HIVU. EOD: RR 1.69, CI 1.28 – 2.24 LOD: RR 3.18, CI 2.34 – 4.36 Bacteraemia and meningitis increased in HIVE infants. VLBW and <33 weeks = increased case fatality rate. HIVE more likely to have LOD than EOD.	
26.	Cutland CL	Maternal HIV infection and vertical transmission of pathogenic bacteria	2012	Prospective. Secondary analysis. Comparative.	Soweto, JHB: 2004 - 2007	Examine effect of maternal HIV infection on prevalence of pathogens in mothers and subsequent transmission and sepsis rates in the neonates	**n = 2432 - 4108**	4% PT. Significant difference in HIVE (6%) vs. HIVU (3%)	Median weight 3130g		Borderline significant increased culture confirmed EOD in HIVE (p=0.05) HIVEU had lower incidence compared to HIVU (p=0.045). LOD: no difference	

GA = gestational age, GBS = group B streptococcus, HIVEU = HIV exposed uninfected, HIVU = HIV unexposed, PT = preterm, RR = risk ratio, EOD = early onset disease, LOD = late onset disease, SGA = small for gestational age, HIVE = HIV exposed, PTB = preterm birth, aOR = adjusted odds ratio, JHB = Johannesburg, LBW = low birth weight, VLBW = very low birth weight,

Table 1.5: Summary of studies on necrotising enterocolitis

#	FIRST AUTHOR	TITLE	DATE PUBLISH	STUDY DESIGN	LOCATION	MAIN OUTCOME	SAMPLE SIZE	GA	BIRTH WEIGHT	MORTALITY OUTCOME	MORBIDITY OUTCOME	OTHER
31.	Karpelowsky JS	Effect of maternal human immunodeficiency virus status on the outcome of neonates with necrotizing enterocolitis	2010	Retrospective. Primary analysis. Comparative.	Cape Town: 1998 - 2008	Assess the effect of HIV exposure on survival and extent of NEC disease in patients requiring surgery	**n = 70**	Mean GA 31.6 weeks	Mean weight 1417g	Increased mortality in HIVE OR 4.8, p = 0.05	No difference in extent of disease	
27.	Angura P	Risk factors for necrotising enterocolitis in an HIV-endemic region.	2014	Retrospective. Primary analysis. Case control: NEC vs. non-NEC.	JHB: 2005 - 2008	Comparative study to assess risk factors for NEC in premature infants with grade 2 or 3 NEC.	**n = 330**	Median GA 31 weeks	Median weight 1370g	HIVE not a risk factor. Clinical presentation and mortality similar		
28.	Arnold M	HIV exposure does not worsen outcome in stage III necrotizing enterocolitis with current treatment protocols	2012	Retrospective. Primary analysis. Comparative.	Cape Town. 4 years not specified	To evaluate the disease presentation, progression and outcome of stage III NEC among HIV-exposed compared with unexposed infants	**n = 87** HIVE 17 HIVU 70	Mean GA 30 weeks	Mean weight 1300g	No difference in mortality	No difference in presentation. Increased post-op sepsis 41% vs. 16%, p = 0.03	
23.	Desfrere L	Increased incidence of necrotizing enterocolitis in premature infants born to HIV-positive mothers	2005	Retrospective. Primary analysis. Case control.	Paris: 1995 - 2003	Evaluate if premature birth and HIV-exposure is an independent risk factor for NEC	**n=237.** 79 NEC Controls 158	Mean PMA 30 weeks	Mean weight 1300g		Multivariate analysis = HIVE OR 6.63 (1.26 - 35) for NEC	
32.	Chokoe MJ	Necrotizing Enterocolitis in HIV-Exposed and Nonexposed Infants: Clinical Presentation and Histopathological Features	2012	Retrospective. Primary analysis. Comparative.	Tygerberg: 1992 - 2008	Compare clinical and histopathological features between HIVE and HIVU infants with NEC	**n=37.** HIVE = 10	Mean GA 31 weeks	Mean weight HIVE 1200g HIVU 1600g	Increased mortality in HIVE 80% mortality vs. 15%, p = 0.0002	No clinical or histopathological difference	

GA = gestational age, NEC = necrotising enterocolitis, HIVE = HIV exposed, OR = odds ratio, JHB = Johannesburg, HIVU = HIV unexposed, PMA = post-menstrual age.

REFERENCES

(1) Bobat R, Coovadia H, Coutsoudis A, Moodley D. **Determinants of mother-to-child transmission of human immunodeficiency virus type 1 infection in a cohort from Durban, South Africa.** *The Pediatric Infectious Disease Journal* 1996 Jul;15(7):604-610.

(2) Geddes R, Knight S, Reid S, Giddy J, Esterhuizen T, Roberts C. **Prevention of mother-to-child transmission of HIV programme: Low vertical transmission in KwaZulu-Natal, South Africa.** *South African Medical Journal/Suid-Afrikaanse Mediese Tydskrift* 2008 Jun 1,;98(6):458-462.

(3) Brennan AT, Bonawitz R, Gill CJ, Thea DM, Kleinman M, Useem J, et al. **A meta-analysis assessing all-cause mortality in HIV-exposed uninfected compared with HIV-unexposed uninfected infants and children.** *AIDS* 2016 September 24;30(15):2351-2360.

(4) le Roux SM, Abrams EJ, Nguyen K, Myer L. **Clinical outcomes of HIV-exposed, HIV-uninfected children in sub-Saharan Africa.** *Trop Med Int Health* 2016 July 01;21(7):829-845.

(5) Slogrove AL, Goetghebuer T, Cotton MF, Singer J, Bettinger JA. **Pattern of Infectious Morbidity in HIV-Exposed Uninfected Infants and Children.** *Front Immunol* 2016 May 06;7:164.

(6) Slogrove AL, Archary M, Cotton MF. **Optimizing Research Methods to Understand HIV-Exposed Uninfected Infant and Child Morbidity: Report of the Second HEU Infant and Child Workshop.** *Frontiers in Immunology* 2016 Dec 6,;7.

(7) Lorenzi P, Spicher VM, Laubereau B, Hirschel B, Kind C, Rudin C, et al. **Antiretroviral therapies in pregnancy: maternal, fetal and neonatal effects.** *AIDS* 1998 Dec 24,;12(18):F247.

(8) Abrams ET, Danny A. Milner, Jesse Kwiek, Victor Mwapasa, Deborah D. Kamwendo, Donglin Zeng, et al. **Risk Factors and Mechanisms of Preterm Delivery in Malawi.** *American Journal of Reproductive Immunology* 2004 Aug;52(2):174-183.

(9) Parekh N, Ribaudo H, Souda S, Chen J, Mmalane M, Powis K, et al. **Risk factors for very preterm delivery and delivery of very-small-for-gestational-age infants among HIV-exposed and HIV-unexposed infants in Botswana.** *International Journal of Gynecology and Obstetrics* 2011;115(1):20-25.

(10) Martin R, Boyer P, Hammill H, Peavy H, Platzker A, Settlage R, et al. **Incidence of premature birth and neonatal respiratory disease in infants of HIV-positive mothers. The Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted Human Immunodeficiency Virus Infection Study Group.** *J Pediatr* 1997 Dec;131(6):851-856.

(11) Thea DM, Grimm K, Bateman D, Kaul A, Rogers M, Abrams EJ, et al. **Neonatal predictors of infection status and early death among 332 infants at risk of HIV-1 infection monitored prospectively from birth.** *Pediatrics* 1995 Sep 1,;96(3):451.

(12) Sofeu CL, Warszawski J, Ndongo FA, Penda IC, Ndiang ST, Guemkam G, et al. **Low Birth Weight in Perinatally HIV-Exposed Uninfected Infants: Observations in Urban Settings in Cameroon.** *PLoS One* 2014 Apr 1,;9(4).

(13) van der Merwe K, Hoffman R, Black V, Chersich M, Coovadia A, Rees H. **Birth outcomes in South African women receiving highly active antiretroviral therapy: a retrospective observational study.** *Journal of the International AIDS Society* 2011;14(1):42.

(14) Schulte J, Dominguez K, Sukalac T, Bohannon B, Fowler MG, Pediatric Spectrum of HIV Disease Consortium. **Declines in Low Birth Weight and Preterm Birth Among Infants Who Were**

Born to HIV-Infected Women During an Era of Increased Use of Maternal Antiretroviral Drugs: Pediatric Spectrum of HIV Disease, 1989-2004. *Pediatrics* 2007 Apr 1,;119(4):e906.

(15) Xiao PL, Zhou YB, Chen Y, Yang MX, Song XX, Shi Y, et al. **Association between maternal HIV infection and low birth weight and prematurity: a meta-analysis of cohort studies.** *BMC Pregnancy Childbirth* 2015 Oct 8;15:z.

(16) Adams JW, Watts DH, Phelps BR. **A systematic review of the effect of HIV infection and antiretroviral therapy on the risk of pre-eclampsia.** *International Journal of Gynecology and Obstetrics* 2016 Apr;133(1):17-21.

(17) Browne JL, Schrier VJ, Grobbee DE, Peters SA, Klipstein-Grobusch K. **HIV, Antiretroviral Therapy, and Hypertensive Disorders in Pregnancy: A Systematic Review and Meta-analysis.** *JAIDS Journal of Acquired Immune Deficiency Syndromes* 2015 Sep 1,;70(1):91.

(18) Tooke L, Riemer L, Matjila M, Harrison M. **Antiretrovirals causing severe pre-eclampsia.** *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health* 2016 Oct;6(4):266-268.

(19) Ballot DE, Chirwa TF, Cooper PA. **Determinants of survival in very low birth weight neonates in a public sector hospital in Johannesburg.** *BMC pediatrics* 2010;10(1):30.

(20) Kalimba EM, Ballot D. **Survival of extremely low-birth-weight infants.** *South African Journal of Child Health* 2013 Feb 1,;7(1):13.

(21) Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, et al. **Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network.** *Pediatrics* 2010 Sep;126(3):443-456.

(22) Adler C, Haelterman E, Barlow P, Marchant A, Levy J, Goetghebuer T. **Severe Infections in HIV-Exposed Uninfected Infants Born in a European Country.** *PLoS One* 2015 August 18;10(8):e0135375.

(23) Desfrere L, de Oliveira I, Goffinet F, El Ayoubi M, Firtion G, Bavoux F, et al. **Increased incidence of necrotizing enterocolitis in premature infants born to HIV-positive mothers.** *AIDS* 2005 Sep 23;19(14):1487-1493.

(24) Epalza C, Goetghebuer T, Hainaut M, Prayez F, Barlow P, Dediste A, et al. **High incidence of invasive group B streptococcal infections in HIV-exposed uninfected infants.** *Pediatrics* 2010 September 01;126(3):631.

(25) Kreitchmann R, Cohen RA, Stoszek SK, Pinto JA, Losso M, Pierre R, et al. **Mode of delivery and neonatal respiratory morbidity among HIV-exposed newborns in Latin America and the Caribbean: NISDI Perinatal–LILAC Studies.** *International Journal of Gynecology and Obstetrics* 2011;114(2):91-96.

(26) Humphrey JH, Iliff PJ, Marinda ET, Mutasa K, Moulton LH, Chidawanyika H, et al. **Effects of a Single Large Dose of Vitamin A, Given during the Postpartum Period to HIV-Positive Women and Their Infants, on Child HIV Infection, HIV-Free Survival, and Mortality.** *The Journal of Infectious Diseases* 2006 Mar 15,;193(6):860-871.

(27) Koyanagi A, Humphrey JH, Ntozini R, Nathoo K, Moulton LH, Iliff P, et al. **Morbidity among human immunodeficiency virus-exposed but uninfected, human immunodeficiency virus-infected, and human immunodeficiency virus-unexposed infants in Zimbabwe before availability of highly active antiretroviral therapy.** *Pediatr Infect Dis J* 2011 January 01;30(1):45-51.

(28) Marinda E, Humphrey JH, Iliff PJ, Mutasa K, Nathoo KJ, Piwoz EG, et al. **Child mortality according to maternal and infant HIV status in Zimbabwe.** *Pediatr Infect Dis J* 2007 June 01;26(6):519-526.

(29) Adhikari M, Jeena P, Pillay T, Moodley A, Kieliela P, Cassol S. **The HIV-1 exposed neonate: outcome of intensive care management in the first week of life.** *Indian Pediatr* 2005 December 01;42(12):1215-1219.

(30) Cutland CL, Schrag SJ, Thigpen MC, Velaphi SC, Wadula J, Adrian PV, et al. **Increased risk for group B Streptococcus sepsis in young infants exposed to HIV, Soweto, South Africa, 2004-2008(1).** *Emerg Infect Dis* 2015 April 01;21(4):638-645.

(31) Moraleda C, de Deus N, Serna-Bolea C, Renom M, Quinto L, Macete E, et al. **Impact of HIV exposure on health outcomes in HIV-negative infants born to HIV-positive mothers in Sub-Saharan Africa.** *J Acquir Immune Defic Syndr* 2014 Feb 1;65(2):182-189.

(32) Monebenimp F, Nga-Essono DE, Zoung-Kany Bissek AC, Chelo D, Tetanye E. **HIV exposure and related newborn morbidity and mortality in the University Teaching Hospital of Yaounde, Cameroon.** *Pan Afr Med J* 2011;8:43.

(33) Nakanga W, Patel P, Panjwani S, Kennedy N, Kawaza K. **Supra-treatment threshold neonatal jaundice: Incidence in HIV-exposed compared to non-exposed neonates at Queen Elizabeth Central Hospital in Blantyre, Malawi.** *Malawi medical journal : the journal of Medical Association of Malawi* 2015 Sep;27(3):104.

(34) Zash RM, Ajose-Popoola O, Stordal K, Souda S, Ogwu A, Dryden-Peterson S, et al. **Risk factors for mortality among human immunodeficiency virus-exposed and unexposed infants admitted to a neonatal intensive care unit in Botswana.** *J Paediatr Child Health* 2014 Mar;50(3):189-195.

(35) Ades V, Mwesigwa J, Natureeba P, Clark TD, Plenty A, Charlebois E, et al. **Neonatal mortality in HIV-exposed infants born to women receiving combination antiretroviral therapy in Rural Uganda.** *J Trop Pediatr* 2013 December 01;59(6):441-446.

(36) Kourtis AP, Wiener J, Kayira D, Chasela C, Ellington SR, Hyde L, et al. **Health outcomes of HIV-exposed uninfected African infants.** *AIDS* 2013 March 13;27(5):749-759.

(37) Cutland CL, Schrag SJ, Zell ER, Kuwanda L, Buchmann E, Velaphi SC, et al. **Maternal HIV infection and vertical transmission of pathogenic bacteria.** *Pediatrics* 2012 Sep;130(3):e590.

(38) Lepage P, Dabis F, Hitimana D, Msellati P, van Goethem C, Stevens A, et al. **Perinatal transmission of HIV-1: Lack of impact of maternal HIV infection on characteristics of livebirths and on neonatal mortality in Kigali, Rwanda.** *AIDS* 1991 Jan 1,;5(3):295-300.

(39) Moraleda C, de Deus N, Serna-Bolea C, Renom M, Quinto L, Macete E, et al. **Impact of HIV exposure on health outcomes in HIV-negative infants born to HIV-positive mothers in Sub-Saharan Africa.** *J Acquir Immune Defic Syndr* 2014 February 01;65(2):182-189.

(40) Angura P, Velaphi S. **Risk factors for necrotising enterocolitis in an HIV-endemic region.** *Paediatrics and International Child Health* 2014 Aug 1,;34(3):208-215.

(41) Arnold M, Moore SW. **HIV exposure does not worsen outcome in stage III necrotizing enterocolitis with current treatment protocols.** *J Pediatr Surg* 2012 Apr;47(4):665-672.

(42) Chokoe MJ, Wright CA, Bezuidenhout J, Moore SW, Smith J. **Necrotising enterocolitis in HIV-exposed and nonexposed infants: Clinical presentation and histopathological features.** *Pediatric and Developmental Pathology* 2012 April 6;15:293-297.

(43) Karpelowsky JS, van Mil S, Numanoglu A, Leva E, Millar AJ. **Effect of maternal human immunodeficiency virus status on the outcome of neonates with necrotizing enterocolitis.** *J Pediatr Surg* 2010 Feb;45(2):8; discussion 318.

(44) The World Bank - Mortality rate, Neonatal. Available at: data.worldbank.org/indicator/SH.DYN.NMRT.

(45) Edmond KM, Kortsalioudaki C, Scott S, Schrag SJ, Zaidi AK, Cousens S, et al. **Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis.** *The Lancet* 2012 Feb 17,;379(9815):547-556.

2. METHODS

AIMS AND OBJECTIVES

The aim of the study is to determine if there is an increased morbidity profile or mortality among premature, HIV exposed, very low birth weight neonates (VLBW).

To do this, a comparison between a cohort of HIV exposed and HIV unexposed VLBW neonates was done focusing on the following outcomes:

1. PRIMARY OBJECTIVE:

   To determine if there are any differences between the two groups in terms of:

   i. Anthropometry and gestational age
   ii. Maternal factors including antenatal care and receipt of steroids, maternal morbidity including hypertension and chorioamnionitis, and lastly the delivery method i.e. vaginal vs. caesarean section
   iii. Mortality before discharge home
   iv. Morbidity before discharge including
      - Respiratory support and subsequent development of chronic lung disease
      - Necrotising Enterocolitis (NEC)
      - Intraventricular haemorrhage (IVH) and Periventricular Leukomalacia (PVL)
      - Sepsis
   v. Length of Stay
   vi. Feeding method at discharge

2. SECONDARY OBJECTIVE

   i. To evaluate the impact of adequate maternal antiretroviral therapy (defined as > 8 weeks of therapy prior to delivery) on the primary outcomes in HIV exposed neonates.
   ii. To determine the HIV transmission rate in the exposed group and to compare the primary outcomes of those neonates who tested HIV-positive to those who were exposed but uninfected.

STUDY DESIGN

A retrospective review of infants born weighing ≤1500g (VLBW) admitted to the neonatal unit at Groote Schuur Hospital (GSH) in Cape Town, South Africa between January 2012 and December 2014 was conducted. Based on the latest antenatal surveillance reports, the HIV prevalence within this area - the Western Cape Metro - is 21.7%,[1] which is higher than the GSH prevalence of 16%.[2] GSH is a tertiary level facility attached to the University of Cape Town (UCT) Faculty of Health Sciences. It is a

government funded public healthcare facility that provides specialist services to patients within the Western Metro municipal district of Cape Town. It is the preferred delivery and admission facility for neonates with an expected birth weight of ≤1200g. The neonatal unit admits approximately 2000 infants per year of which roughly 500 are VLBW.

DATA CAPTURING

Maternal demographics and neonatal outcomes are recorded for all VLBW infants admitted to the unit for the Vermont Oxford Network (VON) Database. This is an international collaborative neonatal database with over a thousand units from 30 countries that contribute. Neonatal outcomes reflect events that occurred from the time of admission until the date of death or discharge home, including events during transfer to another facility. Patient data submitted to the database are de-identified and confidential. [3]

Definitions of these data points were taken from the Vermont Manual of Operations[4] and are explained in more detail in appendix B.

Maternal demographics included:

- Ethnicity:
- Antenatal care (ANC):
- Chorioamnionitis
- Antenatal steroids
- Maternal hypertension
- Type of delivery

Neonatal outcomes included:

- Birth Weight
- Gestational age
- Length of stay
- Death
- Delivery room resuscitation
- Respiratory support
- Culture proven sepsis
- NEC
- Surgery
- Intraventricular haemorrhage and cystic periventricular leukomalacia

We also included the type of feeding at discharge i.e. exclusive breast milk, formula feeding or mixed. Analysis of these neonates was restricted to only include the neonates who were discharged home directly from our unit. We excluded neonates who died (as most of them were not fed in the 24 hours prior to their death) and those who were transferred out. Neonates are often transferred to a step-down unit for weight gain prior to discharge and are still receiving breast milk fortification with FM85. This may have skewed the analysis in favour of mixed feeding if this data had not been appropriately updated when the neonate was discharged home.

HIV related information, which is not captured on the VON database, was obtained from folder review of neonates identified as being HIV-exposed from the Maternity Centre Prevention of Mother to Child Transmission (PMTCT) register.

These variables included

- Maternal ARV exposure including duration and type i.e. AZT/NVP prophylaxis or HAART.
- Neonatal HIV HIV-PCR test results

During the 3 years that the data reflects, the PMTCT protocol was changed in March 2013 from World Health Organisation (WHO) Option A, to Option B. [5] The major difference involved a switch from prophylaxis for women with a CD4 count >350 to HAART for all HIV+ pregnant or breastfeeding women regardless of CD4 count. *(Table 2.1)*

OPTION/YEAR	MATERNAL	LABOUR AND DELIVERY	NEONATES
A: **2010 – 2013***	CD4 ≤ 350, stage 3 or 4 HIV, TB/HIV co-infection: HAART (TDF +3TC/FTC + NVP)	* Continue HAART	• 6 weeks of NVP • 6 week HIV-PCR test • Breastfeeding encouraged BUT free formula provided for 6

	CD4 > 350: AZT from 14 weeks gestation	• sdNVP at onset of labour • 3 hourly AZT during labour • sdTDF +FDC post-delivery	months if formula feeding chosen.
B: **2013 – 2015****	All pregnant and breastfeeding women eligible for HAART	If on HAART – continue. If no antenatal HAART • sdNVP at onset of labour • 3 hourly AZT during labour • Evaluate post delivery for HAART	If ≥ 8 weeks of maternal HAART: 4 weeks of NVP If < 8 weeks of maternal HAART: 12 weeks of NVP Birth HIV-PCR based on discretion of attending doctor based on clinical suspicion OR high risk neonate. All babies = 6 week HIV-PCR testing Feeding as above

Table 2.1: PMTCT regimens during time of study (2012 - 2014)

HAART = Highly active antiretroviral therapy, TDF = Tenofovir, 3TC = Lamivudine, FTC = Emtricitabine, NVP = Nevirapine, HIV = Human Immunodeficiency Virus, TB = Tuberculosis, HIV-PCR = Polymerase Chain Reaction, AZT = Zidovudine, sd = single dose

- National PMTCT guidelines[6]
- ** Western Cape PMTCT guidelines[7]

A database of this information was collected in a Microsoft Excel spread sheet.

Ethical approval for this study was obtained from the UCT Faculty of Health Sciences Research Ethics committee (HREC603/2015).

ANALYSES

Continuous variables i.e. birth weight, gestational age and length-of-stay were assessed for normal distribution by testing for normality using the Shapiro-Wilk test online calculator at http://contchart.com/goodness-of-fit.aspx.

Only the data for the HIV-PCR + babies tested as being normally distributed. However, given the small sample size, which predisposes to testing positive for normal distribution, the lack of correlation on visual inspection of histogram analysis (using StatPlus:mac, Analystsoft Inc.) and because the comparative group of HIV-PCR - babies were not normally distributed, non-parametric testing was used to test all continuous variables including the HIV-PCR +/HIV-PCR - data. A comparison of medians was done using the Vassarstats online Mann-Whitney U test calculator available at http://www.vassarstats.net/utest.html.

Univariate analysis of categorical variables was done using Medcalc online statistical software (https://www.medcalc.org/calc/) using Chi-square and Pearson tests as well

as Fisher-exact tests to determine odds ratios and p-value significance. We used two-tail p-values and a α-value of 0.05 to determine significance.

Analysis was done in three categories:

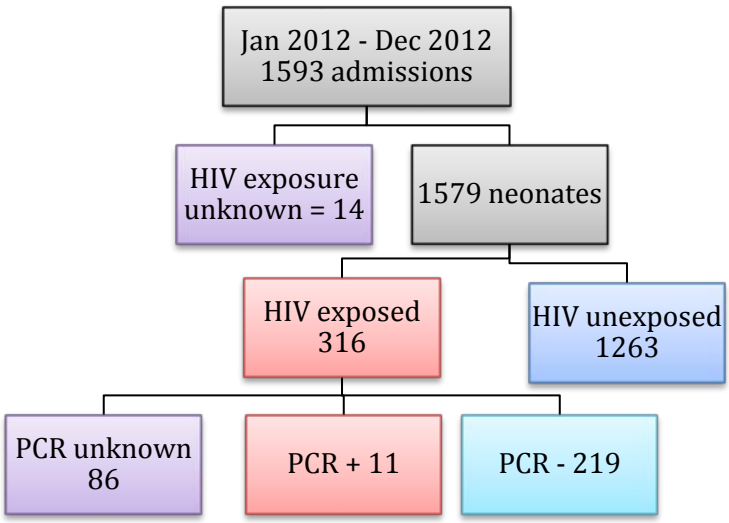
i. HIV-exposed vs. HIV-unexposed neonates
ii. Neonates with adequate maternal ARV exposure vs. maternal ARV-underexposed neonates
iii. HIV-PCR positive vs. HIV-PCR negative neonates

3. RESULTS

Between January 2012 and December 2014, 1593 VLBW neonates were admitted to the neonatal unit.

Of these, 14 had no HIV exposure status documented and were subsequently excluded. Of the remaining 1579, 316 (20%) were HIV-exposed and 1263 (79%) were unexposed.

Figure 3.1.1: Distribution of the cohort based on HIV-exposure status



The ethnicity of the study population is broken down in Table 3.1.1. African babies made up the largest proportion of the cohort (58%), followed by Coloured (41%). 1% of the cohort was attributed to 11 White babies and 4 who were Asian.

Table 3.1.1: Ethnicity of cohort by HIV status

Ethnicity n (%)	**HIVE (%) n = 316**	**HIVU n = 1263**
Black n = 919 (58)	272 (30)	647 (70)
Coloured n = 646 (41)	42 (6)	604 (94)
White n = 11 (0.7)	0	11 (100)
Asian n = 4 (0.3)	3 (75)	1 (25)

HIVE = HIV exposed, HIVU = HIV unexposed

3.1 HIV EXPOSED VS HIV UNEXPOSED NEONATES

MATERNAL CHARACTERISTICS.

There was no significant difference between any of the maternal variables with regards to HIV exposure although there was a trend to less chorioamnionitis in the HIVE neonates. These are summarized in table 3.1.2.

Table 3.1.2: Maternal characteristics for HIVE and HIVU neonates.

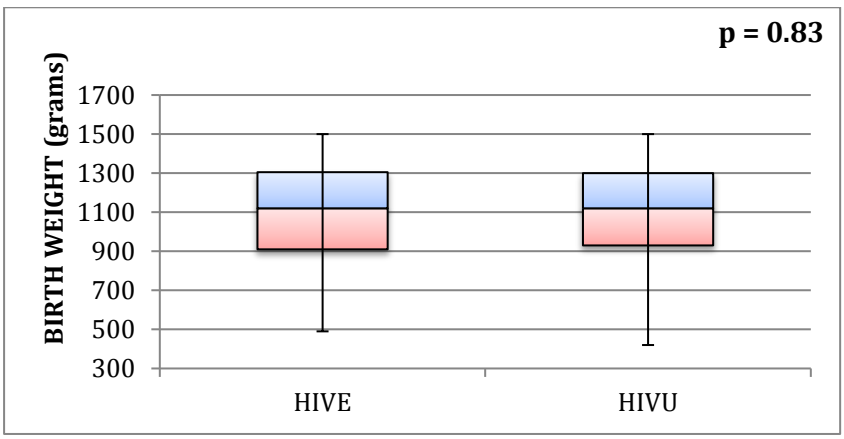
	HIVE n 316(%)	HIVU n 1263(%)	p value	OR (CI)
ANC	267 (85)	1053 (83)	0.63	1.1 (0.77 -1.5)
CHORIOAMNIONITIS	8 (3)	65 (5)	0.051	0.48 (0.23 -1.0)
HT	156 (49)	585 (46)	0.33	1.1 (0.88-1.45)
ANTENATAL STEROIDS	203 (64)	778 (62)	0.39	1.12 (0.87-1.45)
NVD	103 (33)	433 (34)	0.57	0.93 (0.71-1.21)

HIVE = HIV-exposed, HIVU = HIV-unexposed, OR = odds ratio, CI = 95% confidence interval, ANC = antenatal care, HT = hypertension, NVD = normal vaginal delivery

NEONATAL CHARACTERISTICS

As depicted in the figures 3.1.2-3.1.4, there was no difference in the median birth weight, gestational age or length-of-stay between the HIVE and HIVU groups. Median birth weight was 1120g for both groups (p = 0.83), median gestational ages 30 weeks for both groups (p = 0.54) and median length of stay 34 days and 37 days (p = 0.16).

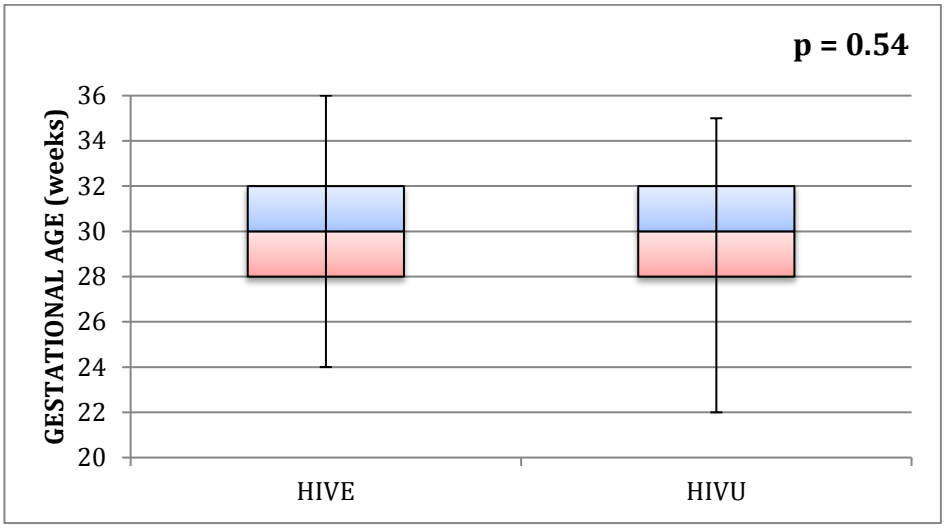
Figure 3.1.2: Box plot distribution for birth weight of HIVE vs. HIVU



MEDIAN	**1120g**	**1120g**
IQR	910g – 1305g	930g – 1300g

HIVE = HIV-exposed, HIVU = HIV-unexposed, IQR = interquartile range

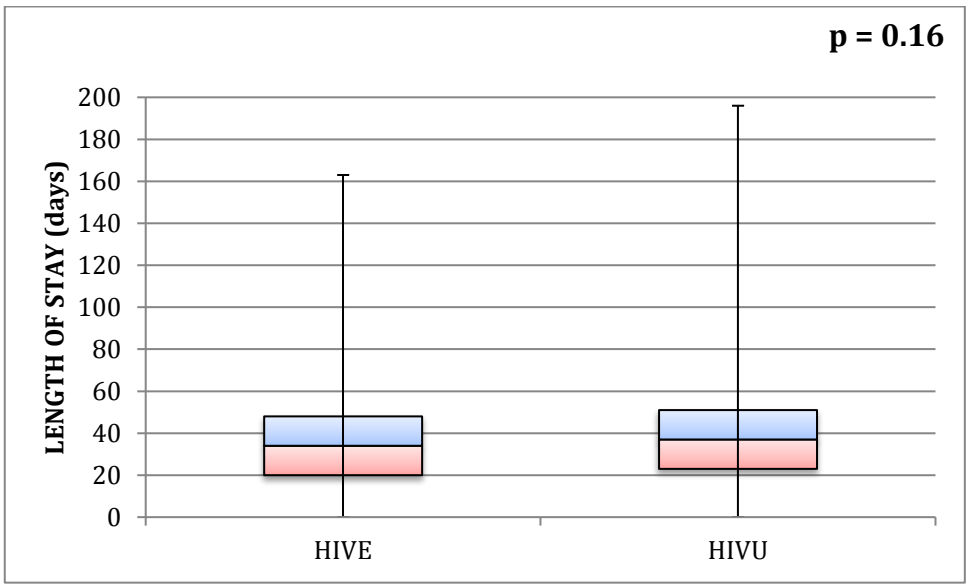
Figure 3.1.3: Box plot distribution for gestational age of HIVE and HIVU neonates.



MEDIAN	30 WEEKS	30WEEKS
IQR	28 -32	28 -32

HIVE = HIV-exposed, HIVU = HIV-unexposed, IQR = interquartile range

Figure 3.1.4: Box plot distribution for length-of-stay for HIVE and HIVU neonates



MEDIAN	34 days	37 days
IQR	20 - 48	23 - 51

HIVE = HIV-exposed, HIVU = HIV-unexposed, IQR = interquartile range

The remaining neonatal outcomes are summarised in Table 3.1.3.

Table 3.1.3: Neonatal outcomes of HIVE and HIVU infants

OUTCOME		HIVE n = 316 (%)	HIVU n = 1263 (%)	p VALUE	OR (CI)
	MALE	148 (47)	591 (47)	0.99	
	SGA	170 (54)	668 (53)	0.77	1.04 (0.81-1.3)
	DEATH	73 (23)	242 (19)	0.12	1.27 (0.94-1.71)
Delivery Room Resuscitation	FACEMASK VENTILATION	167 (53)	678 (54)	0.8	0.76 (0.76 – 1.24)
	CHEST COMPRESSIONS	39 (12)	196 (15)	0.16	0.77 (0.53-1.12)
	INTUBATION	29 (9)	117 (9)	0.96	0.99 (0.65 – 1.52)
	DRUGS	12 (4)	34 (3)	0.3	1.43 (0.73-2.79)
Respiratory Support	**VENTILATION**	**83 (26)**	**264 (21)**	**0.04**	**1.35 (1.01 – 1.8)**
	NIV	255 (81)	992 (78)	0.4	1.14 (0.84 -1.56)
	NPO2	268 (85)	1052 (84)	0.51	1.12 (0.8 – 1.57)
	SURFACTANT	94 (30)	384 (30)	0.82	0.97 (0.74 – 1.27)
	CLD	17 (5)	53 (4)	0.36	1.3 (0.74 -2.27)
Sepsis	EARLY SEPSIS	6 (2)	20 (2)	0.69	1.2 (0.48 - 3.02)
	LATE SEPSIS	31 (10)	103 (8)	0.35	1.23 (0.8 -1.87)
	NOSOCOMIAL	32 (10)	113 (9)	0.51	1.15 (0.76 – 1.74)
	FUNGAL	1 (0.3)	7 (2)	0.6	0.57 (0.07 – 4.65)
NEC	**NEC**	**34 (11)**	**78 (6)**	**0.005**	**1.83 (1.2 – 2.8)**
	NEC SURGERY	5/34 (22)	16/78 (20)	0.47	0.67 (0.22 – 2.0)
	NEC DEATH	21/34 (62)	41/78 (53)	0.37	1.46 (0.64 – 3.3)
	ANY SURGERY	13 (4)	37 (3)	0.28	1.42 (0.75 – 2.71)
Neurological	ANY IVH	68/275 (25)	316/1088 (29)	0.16	0.8 (0.59 – 1.1)
	HIGH GRADE IVH (grade III or IV)	20/275 (7)	57/1088 (5)	0.19	1.4 (0.84 -2.4)
	PVL	6/275 (2)	26/1088 (2)	0.84	0.91 (0.37 – 2.24)

HIVEU = HIV-exposed but uninfected, HIVU = HIV-unexposed, OR = odds ratio, CI = 95% confidence interval, SGA = small for gestational age, NIV = non-invasive ventilation, NPO2 = nasal cannula oxygen, CLD = chronic lung disease, NEC = necrotising enterocolitis, IVH = Intraventricular haemorrhage, PVL = periventricular leukomalacia

i. VENTILATION

HIVE neonates were ventilated more often (OR 1.35, CI 1.01 -1.8, p=0.04).

As summarized in Table 3.1.4, within this subgroup of HIVE and HIVU neonates, there was no difference between factors that may have predisposed to a need for ventilation. These included SGA, maternal ANC, chorioamnionitis, antenatal steroids, type of delivery, administration of surfactant, sepsis or incidence of NEC. There was also no difference in median birth weight (both 1100 grams, p =0.65) or median gestational age (30 weeks and 29 weeks respectively, p=0.19).

Table 3.1.4: Sub-group analysis of HIVE and HIVU neonates who required ventilation

	HIVE n=83 (%)	**HIVU n=264 (%)**	**p VALUE**
MEDIAN BIRTH WEIGHT	1100g IQR (930 -1278g)	1100g IQR (960g – 1240g)	0.65
MEDIAN GESTATIONAL AGE	30 weeks IQR (28 – 31)	29 weeks IQR (28 – 31)	0.19
SGA	34 (41)	88 (33)	0.21
ANC	71 (86)	205 (78)	0.12
CHORIOAMNIONITIS	6 (8)	14/254 (6)	0.57
ANTENATAL STEROIDS	39 (47)	119 (45)	0.76
NVD	29 (35)	107 (41)	0.36
NEC	15 (18)	43 (16)	0.7
EARLY SEPSIS	4 (5)	12 (5)	0.92
LATE SEPSIS	11 (13)	51 (20)	0.21
SURFACTANT	59 (71)	204 (77)	0.25

HIVE = HIV-exposed, HIVU = HIV-unexposed, IQR = interquartile range, SGA = small for gestational age, ANC = antenatal care, NVD = normal vertex delivery, NEC = necrotising enterocolitis,

ii. NEC

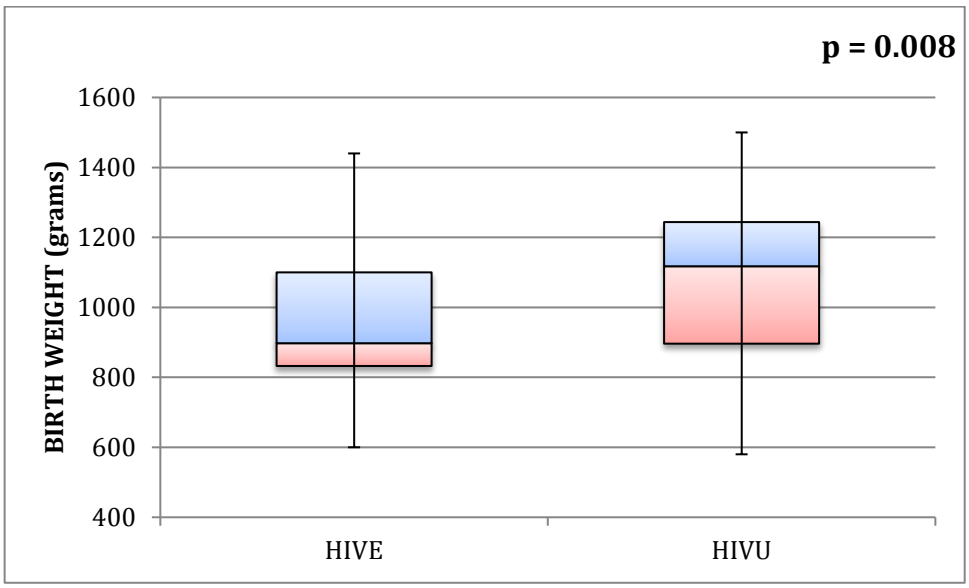
More HIVE neonates were diagnosed with NEC (OR 1.83, CI 1.2 – 2.8, p=0.005), however of those who developed NEC, there was no difference in outcome with regards to surgery (p=0.47) or death (p = 0.37, *Table 3.1.3).* Sub-group analysis of SGA, maternal ANC, antenatal steroids, and gestational age revealed no difference between the groups but a significant difference in birth weight was noted. HIVE babies, with a median birth weight of 898g compared to 1118g in the HIVU group, weighed less (p=0.008). *Table 3.1.5, Figure 3.1.5.*

Table 3.1.5: Sub-group analysis of HIVE and HIVU neonates who developed NEC.

	HIVE n = 34 (%)	HIVU n = 78 (%)	p VALUE
MEDIAN BIRTH WEIGHT	FIGURE 3.1.5		
MEDIAN GESTATIONAL AGE	29 weeks IQR (28 – 30)	29 weeks IQR (28 -31)	0.57
SGA	18 (60)	35 (45)	0.16
ANC	23 (77)	65 (84)	0.35
ANTENATAL STEROIDS	19 (63)	45 (58)	0.59

HIVE = HIV-exposed, HIVU = HIV-unexposed, SGA = small for gestational age, ANC = antenatal care

Figure 3.1.5: Box plot distribution of birth weights for HIVE vs. HIVU neonates who developed NEC



MEDIAN	898g	1118g
IQR	833g – 1100g	896g – 1244g

HIVE = HIV-exposed, HIVU = HIV-unexposed, IQR = interquartile range, NEC = necrotising enterocolitis

FEEDING AT DISCHARGE

Feeding at discharge was documented for 178 (56%) of the HIVE and 721 (57%) of the HIVU neonates. In both groups, the majority of the babies (49% and 76% respectively) were discharged on exclusive breastfeeding. *(Table 3.1.6)*

Less HIVE babies were discharged on EBM (0.3, CI 0.22 – 0.43, p <0.0001) and mixed feeding (OR 0.56, CI 0.33 – 0.93, p = 0.026) compared to HIVU babies. More HIVE infants were however discharged on exclusive formula feeding (OR 9.97, CI 6.5 – 15.2, p = <0.0001).

Table 3.1.6: Feeding at discharge for HIVE and HIVU neonates.

	HIVE n =178 (%)	**HIVU n = 721 (%)**	**p-value**	**OR (CI)**
EBM	87 (49)	547 (76)	<0.0001	0.3 (0.22 – 0.43)
FORMULA	72 (40)	46 (6)	<0.0001	9.97 (6.5 – 15.2)
MIXED	19 (11)	127 (18)	0.026	0.56 (0.33 – 0.93)

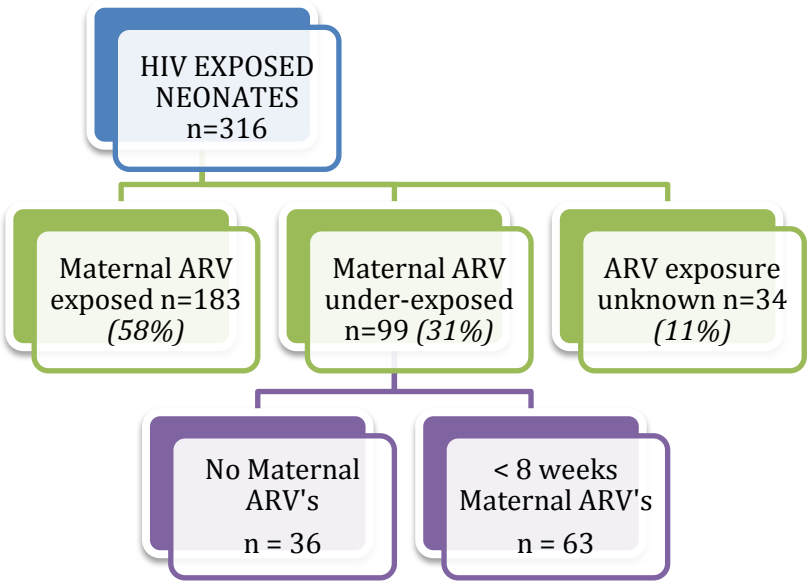
HIVE = HIV-exposed, HIVU = HIV-unexposed, OR = odds ratio, CI = 95% confidence interval, EBM = exclusive breast milk

3.2 MATERNAL ARV-EXPOSED VS. MATERNAL ARV UNDER-EXPOSED

Adequate maternal ARV-exposure (mARVE) was defined as those mothers who were documented as having antenatal exposure to ARV's for ≥ 8 weeks at the time of delivery, or had a documented suppressed viral load at the time of delivery when ARV-exposure was uncertain. The maternal ARV under-exposed (mARVU) group included those babies whose mothers had less than 8 weeks, or no exposure to ARV's prior to delivery.

Of the 316 HIV-positive mothers, 58% were exposed to adequate ARV's and 31% were classified as ARV under-exposed. Within these 99 mothers, 36 received no ARV's, and 63 received <8 weeks of ARV's. The remaining 11% had incomplete records. These 34 infants were subsequently excluded from this analysis. *(Figure 3.2.1)*

Figure 3.2.1: Distribution of maternal antiretroviral (ARV) exposure among the HIV-exposed cohort



MATERNAL CHARACTERISTICS

Adequate maternal ARV exposure was associated with more ANC (OR 3.0, CI 1.5 – 6.0, p=0.002), but no other difference was noted. *(Table 3.2.1)*

Table 3.2.1: Maternal characteristics of mARVE and mARVU neonates.

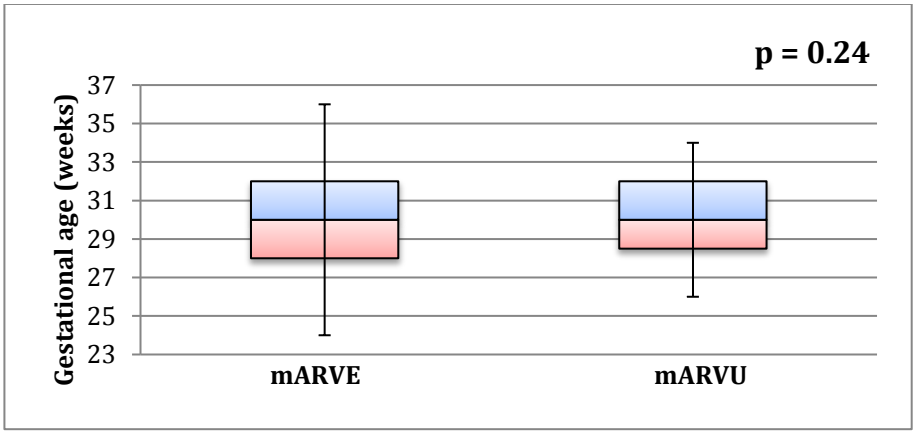
	mARVE n = 183 (%)	**mARVU n = 99 (%)**	**p-value**	**OR (CI)**
ANC	**167 (91)**	**77 (77)**	**0.002**	**3.0 (1.5 – 6.0)**
CHORIOAMNIONITIS	4 (2)	4 (4)	0.37	0.53 (0.13 – 2.1)
HYPERTENSION	96 (52)	45 (45)	0.26	1.3 (0.81 – 2.2)
ANTENATAL STEROIDS	125 (68)	58 (58)	0.1	1.45(0.92 – 2.53)
VAGINAL DELIVERY	52 (28)	36 (36)	0.17	0.69 (0.4 – 1.17)

ARV = antiretroviral, mARVE = maternal ARV exposed, mARVU = maternal ARV under-exposed, OR = odds ratio, CI = 95% confidence interval, ANC = antenatal care

NEONATAL CHARACHTERISTICS

There was no significant difference in gestational age, birth weight and length-of-stay for mARVE and mARVU neonates. Median gestational ages for both groups were 30 weeks (p = 0.24, *figure 3.2.2*) and birth weight medians were 1140g and 1100g respectively (p = 0.52, *figure 3.2.3*). There was a trend towards a longer admission in the mARVU group with a median length-of-stay of 38 days in comparison with the mARVE group's 30 days (p=0.054, *figure 3.2.4*).

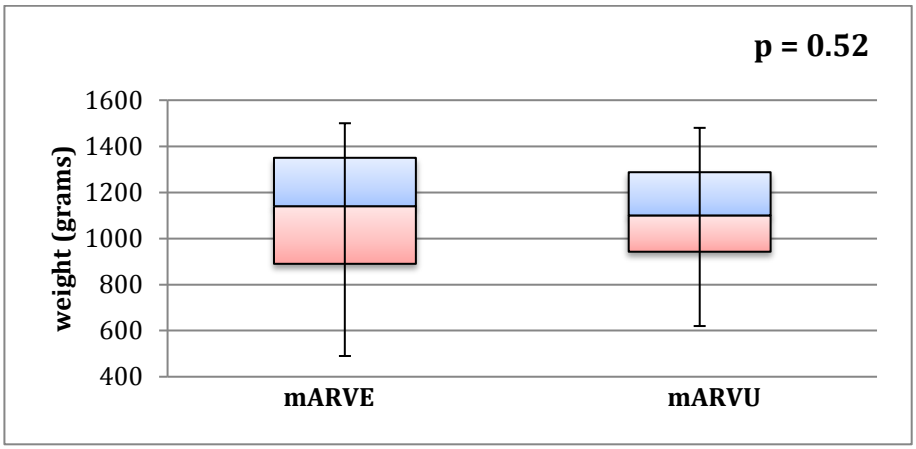
Figure 3.2.2: Box plot distribution of gestational ages for mARVE and mARVU neonates



MEDIAN	**30 weeks**	**30 weeks**
IQR	28 -32 weeks	29 – 32 weeks

ARV = antiretroviral, mARVE =maternal ARV-exposed, mARVU = maternal ARV under-exposed, IQR = interquartile range

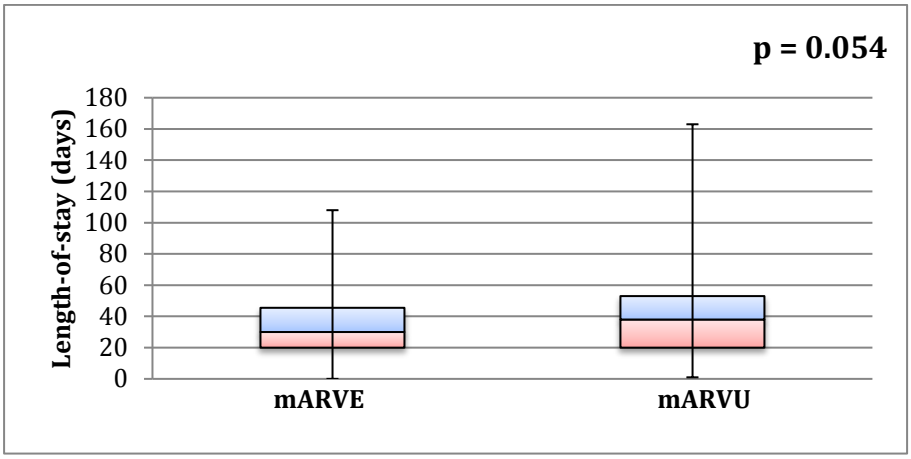
Figure 3.2.3: Box plot distribution of birth weight for mARVE and mARVU neonates.



MEDIAN	**1140g**	**1100g**
IQR	890g – 1350g	943g -1288g

ARV = antiretroviral, mARVE = maternal ARV-exposed, mARVU = maternal ARV under-exposed, IQR = interquartile range

Figure 3.2.4: Box plot distribution of length-of-stay for mARVE and mARVU neonates.



	MEDIAN	30 days	38 days
	IQR	20 – 46 days	20 -53 days

ARV = antiretroviral, mARVE = ARV-exposed, mARVU = ARV-unexposed, IQR = interquartile range

Table 3.2.2: Neonatal outcomes for mARVE and mARVU infants

OUTCOMES		mARVE n = 183 (%)	mARVU n = 99 (%)	p-value	OR (CI)
	MALE	76 (42)	54 (55)	0.037	
	SGA	101 (55)	51 (51)	0.55	1.2 (0.71 – 1.9)
	DEATH	41 (22)	25 (25)	0.6	0.85 (0.48 – 1.5)
Delivery Room outcomes	FACE MASK VENTILATION	102 (56)	55 (56)	0.98	1 (0.62 – 1.65)
	CHEST COMPRESSIONS	24 (13)	12 (12)	0.81	1.1 (0.52 – 2.3)
	INTUBATION	17 (9)	10 (10)	0.83	0.9 (0.4 – 2.1)
	DRUGS	8 (4)	3 (3)	0.58	1.46 (0.4 – 5.6)
Respiratory outcomes	VENTILATION	38 (21)	31 (31)	0.051	0.57 (0.33 – 1.0)
	NIV	141 (77)	85 (86)	0.08	0.55 (0.3 – 1.1)
	NPO2	154 (84)	85 (86)	0.7	0.87 (0.4 – 1.74)
	SURFACTANT	49 (27)	29 (29)	0.65	0.88 (0.5 – 1.52)
	CLD	9 (5)	6 (6)	0.68	0.8 (0.3 – 2.3)
Sepsis	EARLY SEPSIS	2 (1)	4 (4)	0.13	0.26 (0.05 – 1.46)
	LATE SEPSIS	15 (8)	15 (15)	0.07	0.5 (0.23 – 1.1)
	NOSOCOMIAL	15 (8)	15 (15)	0.07	0.5 (0.23 – 1.1)
	FUNGAL	0	0	0.76	0.54 (0.01 – 27.3)
NEC	**NEC**	**13 (7)**	**16 (16)**	**0.02**	**0.4 (0.18 – 0.86)**
	NEC SURGERY	3/13 (23)	2/16 (13)	0.46	2.1 (0.3 – 15)
	NEC DEATH	**11/13 (85)**	**5/16 (31)**	**0.008**	**12 (1.9 – 76)**
	ANY SURGERY	8 (4)	5 (5)	0.8	0.86 (0.27 – 2.7)
Neurological	ALL IVH	34/160 (21)	26/84 (26)	0.096	0.6 (0.3 – 1.09)
	HIGH GRADE IVH (Grade 3 or 4)	10/160 (6)	7/84 (7)	0.55	0.73 (0.27 – 2.00)
	PVL	4 /160(2)	1/84 (1)	0.5	2.1 (0.23 – 19)

ARV = antiretroviral, mARVE = maternal ARV exposed, mARVU = maternal ARV under-exposed, OR = odds ratio, CI = 95% confidence interval, SGA = small for gestational age, NIV = non-invasive ventilation, NPO2 = nasal cannula oxygen, CLD = chronic lung disease, NEC = necrotising enterocolitis, IVH = Intraventricular haemorrhage, PVL = periventricular leukomalacia

The remaining neonatal outcomes are summarized in table 3.2.2.

A trend to less need for invasive ventilation was seen in the mARVE group (p=0.051).

The incidence of NEC was again significant. Adequate maternal ARV's were associated with less NEC in the neonates compared to mARVU neonates (OR 0.4, CI 0.18 – 0.86, p = 0.02). In the mARVE neonates who developed NEC however, mortality was significantly higher (OR 12, CI 1.9 – 76, p = 0.008).

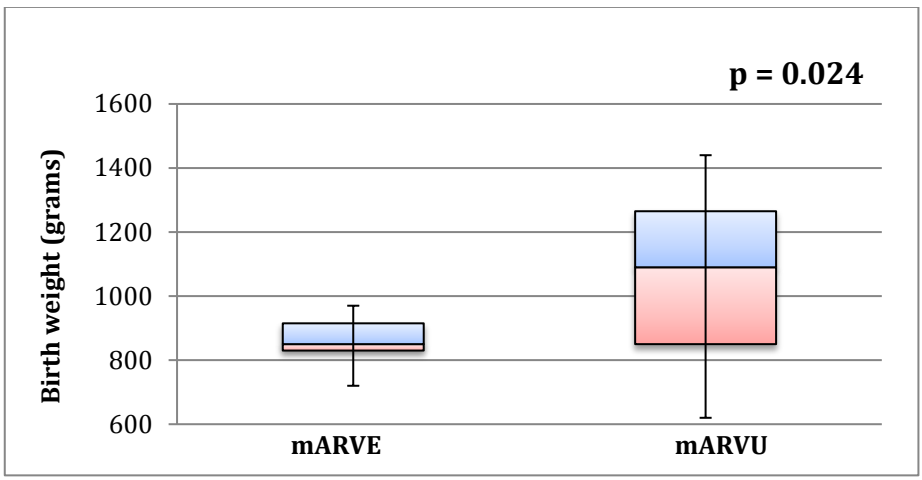
Sub-group analysis of the mARVE NEC cohort *(Table 3.2.3)* showed no difference in ANC (p = 0.06), antenatal steroids (p = 0.96), gender (p =0.54) or gestational age (p = 0.11), however birth weight was again significantly different between the mARVE and mARVU groups. Birth weight was significantly less among mARVE neonates compared to the mARVU neonates who contracted NEC (p=0.024). *Figure 3.2.5*

Table 3.2.3: Sub-group analysis of mARVE and mARVU neonates who developed NEC

	mARVE n = 13 (%)	**mARVU n = 16 (%)**	**p-value**
MEDIAN GESTATIONAL AGE	28 weeks IQR 27 – 30 weeks	30 weeks IQR 29 – 31 weeks	0.11
MALE	5 (38)	8 (50)	0.54
SGA	6 (46)	9 (56)	0.47
ANC	13 (100)	10 (63)	0.06
ANTENATAL STEROIDS	8 (62)	10 (62)	0.96

Figure 3.2.5: Box plot distribution of birth weight of mARVE and mARVU neonates who developed NEC

ARV = antiretroviral, mARVE =maternal ARV-exposed, mARVU = maternal ARV under-exposed, SGA = small for gestational age, ANC = antenatal care



MEDIAN	**850g**	**1090g**
INTERQUARTILE RANGE	830 – 915g	850 -1265g

ARV = antiretroviral, mARVE = maternal ARV-exposed, mARVU = maternal ARV under-exposed, NEC = necrotising enterocolitis

3.3 HIV-PCR POSITIVE VS. HIV-PCR NEGATIVE NEONATES

As shown in Figure 3.3.1, of the 316 HIVE babies, 86 had no retrievable HIV-PCR test result. Eleven out of 230 HIV-PCR tested neonates were HIV-PCR positive (4.8%) and 219 were HIV-PCR negative (95.2%). Fifty-three out of the 230 (23%) who had documented testing, had early HIV-PCR testing i.e. prior to 6 weeks. All 11 HIV-PCR positive infants were from this early testing group. Most of this testing was done in the first week of life, (median day of testing = day 1 of life, IQR 1 -5 days). Only 2 early HIV-PCR tests were done in 2012, 11 were done in 2013 and the remaining 40 were done in 2014. Among the 177 neonates who had a HIV-PCR test done at or after 6 weeks, all tests were negative. Of the 86 untested neonates, 52 (60%) died in the first 6 weeks of life and most of these were in the first 2 weeks (median LOS 4 days, IQR 3 – 11 days). Thirty of the 34 neonates with no retrievable HIV-PCR test were discharged prior to 6 weeks of age and 4 were discharged after. *(Figure 3.3.2)*

Figure 3.3.1: Distribution of HIVE neonates based on HIV-PCR -testing and HIV-PCR -status

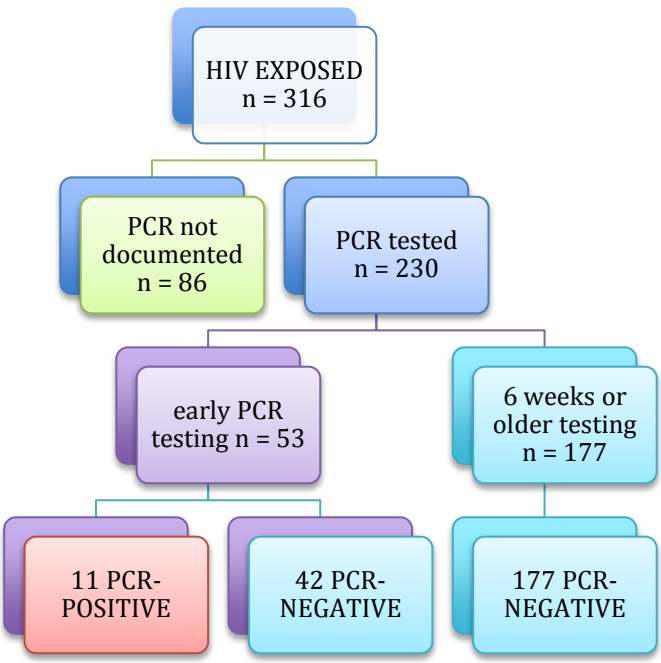
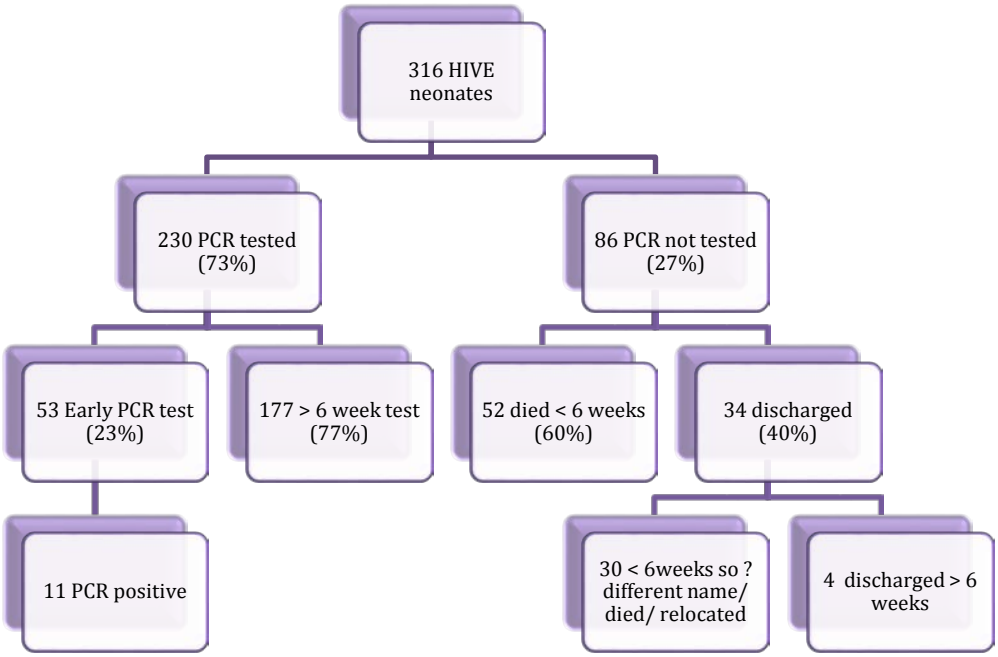


Figure 3.3.2: Flowchart representation of infant disposition and timing of HIV-PCR testing



MATERNAL CHARACTERISTICS

The only statistically different maternal characteristic between the HIV-PCR positive neonates and HIV-PCR negative was related to maternal ARV exposure. HIV-PCR positive neonates were significantly less likely to have a mother on ARV's for >2 months at the time of delivery (OR 0.12, CI 0.02 – 0.58, p = 0.008). *Table 3.3.1*

Table 3.3.1: Maternal Characteristics of HIV-PCR positive and HIV-PCR negative neonates.

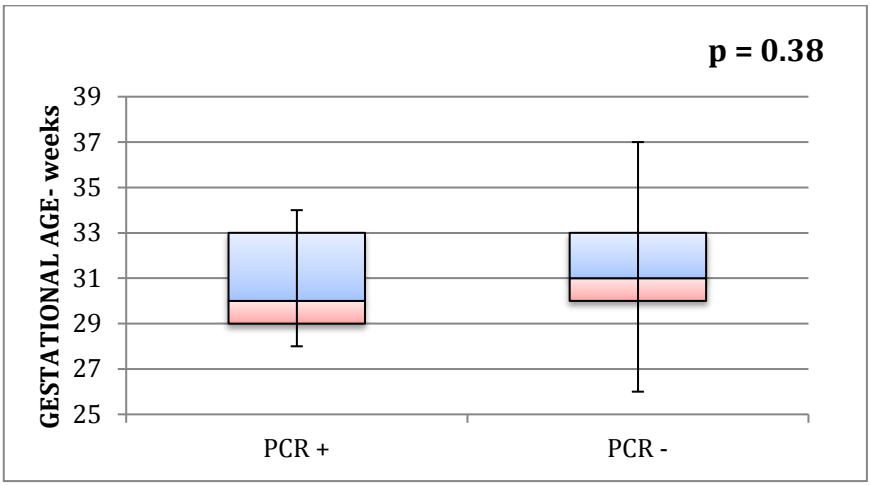
	HIV-PCR + n = 11 (%)	**HIV-PCR - n = 219 (%)**	**p-value**	**OR (CI)**
ANC	8 (72)	182 (83)	0.27	0.46 (0.12 – 1.81)
CHORIOAMNIONITIS	0	5 (2)	0.8	1.5 (0.08 – 26.7)
HYPERTENSION	5 (45)	102 (47)	0.82	0.9 (0.26 – 2.9)
ANTENATAL STEROIDS	5 (45)	147 (67)	0.21	0.46 (0.14 – 1.54)
VAGINAL DELIVERY	6 (54)	67 (31)	0.13	2.5 (0.75 – 8.5)
mARVE	**2/10 (20)**	**133/196 (68)**	**0.008**	**0.12 (0.02 – 0.58)**

HIV-PCR = polymerase chain reaction, HIV-PCR + = HIV-PCR -positive, HIV-PCR - = HIV-PCR -negative, OR = odds ratio, CI = 95% confidence interval, ANC = antenatal care, mARVE = >2 months of maternal antiretroviral exposure.

NEONATAL CHARACTERISTICS

There was no difference between the median gestational ages of the two groups (*Figure 3.3.3,* p = 0.38), birth weight (*Figure 3.3.4*, p = 0.61) or length-of-stay (*Figure 3.3.5*, p = 0.41).

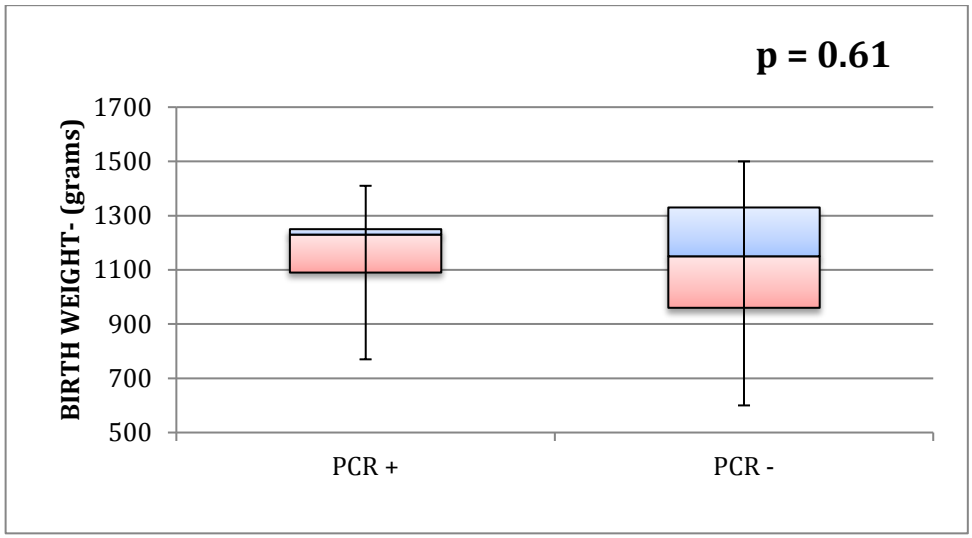
Figure 3.3.3: Box plot distribution of gestational age for HIV-PCR positive and HIV-PCR negative neonates.



Median	**30 weeks**	**30 weeks**
IQR	29 – 33 weeks	29 – 32 weeks

HIV-PCR = polymerase chain reaction, HIV-PCR + = HIV-PCR positive, HIV-PCR - =negative, IQR = interquartile range

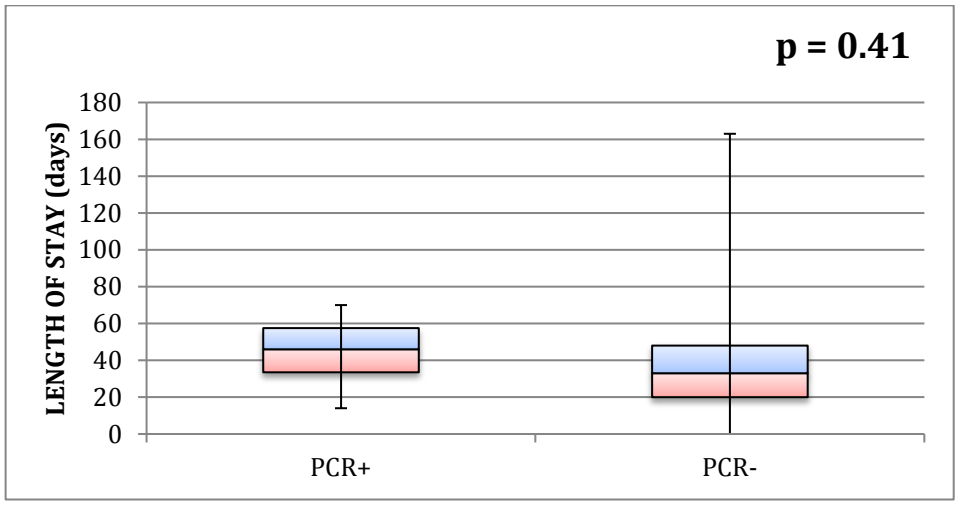
Figure 3.3.4: Box plot distribution for birth weights of HIV-PCR positive and HIV-PCR -negative neonates



MEDIAN	1230g	1150g
IQR	1090g – 1250g	960g – 1330g

HIV-PCR = polymerase chain reaction, HIV-PCR + = HIV-PCR positive, HIV-PCR - = HIV-PCR negative, IQR = interquartile range

Figure 3.3.5: Box plot distribution for length-of-stay of HIV-PCR positive and HIV-PCR negative neonates.



MEDIAN	46 days	39 days
IQR	34 – 58 days	27 – 52 days

HIV-PCR = polymerase chain reaction, HIV-PCR + = HIV-PCR positive, HIV-PCR - = HIV-PCR negative, IQR = interquartile range

Of the remaining neonatal outcomes *(Table 3.3.2)*, differences emerged regarding non-invasive ventilation, CLD and NEC.

Table 3.3.2: Neonatal outcomes of HIV-PCR positive and HIV-PCR negative neonates.

OUTCOMES		HIV-PCR + n=11	HIV-PCR - n=219	p Value	OR (CI)
	MALE	6 (55)	97 (44)	0.47	
	SGA	7 (63)	115 (53)	0.4	1.72 (0.49-6.04)
	DEATH	2 (18)	22 (10)	0.40	1.99 (0.40-9.80)
Delivery Room outcomes	FACE MASK VENTILATION	7 (64)	106 (48)	0.33	1.87 (0.53-6.56)
	CHEST COMPRESSIONS	1 (9)	22 (10)	0.92	0.90 (0.11-7.33)
	INTUBATION	1 (9)	18 (8)	0.92	1.12 (0.14-9.22)
	DRUGS	0	5 (2)	0.73	1.70 (0.09-32.57)
Respiratory support	VENTILATION	2 (18)	52 (24)	0.67	0.71 (0.15-3.41)
	NIV	**6 (55)**	**177 (81)**	**0.046**	**0.28 (0.08-0.98)**
	NPO2	8 (72)	184 (84)	0.33	0.51 (0.13-2.01)
	SURFACTANT	1 (9)	65 (30)	0.17	0.24 (0.03-1.89)
	CLD	**3 (27)**	**14 (6)**	**0.02**	**5.49 (1.31-23.02)**
Sepsis	EARLY SEPSIS	1 (9)	2 (1)	0.06	10.8 (0.91-129.92)
	LATE SEPSIS	0	21 (10)	0.53	0.4 (0.02-7.05)
	NOSOCOMIAL	0	22 (10)	0.51	0.38 (0.02–6.7)
	FUNGAL	0	1 (0.5)	0.27	6.3 (0.24-164.18)
NEC	**NEC**	**3 (27)**	**18 (8)**	**0.047**	**4.12 (1.02-17.18)**
	NEC SURGERY	0/3	4/18(22)	0.63	0.46 (0.02-10.70)
	NEC DEATH	1/3(33)	8 /18(44)	0.72	0.63 (0.05-8.20)
	ANY SURGERY	0	12 (5)	0.83	0.72 (0.04-12.97)
Neurological ***10 HIV-PCR + 203 HIV-PCR -***	ANY IVH	2 (20)	46 (23)	0.84	0.85 (0.18-4.16)
	HIGH GRADE IVH (grade III and IV)	0	14 (7)	0.75	0.62 (0.03-11.2)
	PVL	0	5 (2)	0.72	1.72 (0.09-33.20)

HIV-PCR = polymerase chain reaction, HIV-PCR + = HIV-PCR positive, HIV-PCR - = HIV-PCR negative, OR = odds ratio, CI = 95% confidence
interval SGA = small for gestational age, NIV = non-invasive ventilation, NPO2 = nasal cannula oxygen, CLD = chronic lung disease, NEC = necrotising enterocolitis, IVH = Intraventricular haemorrhage, PVL = periventricular leukomalacia.

i. Non-invasive ventilation

55% of the HIV-PCR -positive neonates required non-invasive ventilation, which was significantly less than the 81% of the HIV-PCR negative neonates (OR 0.28, CI 0.08 – 0.98, p = 0.046). There was no difference when correcting for median birth weights (p = 0.165), gestational ages (p = 0.165), SGA (p = 0.37), antenatal steroids (p = 0.55), antenatal care (p = 0.07) or surfactant use (p = 0.35). *(Table 3.3.3)*

Table 3.3.3: Subgroup analysis of HIV-PCR positive and HIV-PCR negative neonates who required non-invasive ventilation.

	HIV-PCR + n = 6 (%)	**HIV-PCR - n = 177 (%)**	**p-value**
MEDIAN BIRTH WEIGHT	1090grams IQR (979 – 1160)	1100grams IQR (910 – 1270)	0.165
MEDIAN GESTATIONAL AGE	30 weeks IQR (29 – 32wks)	30 weeks IQR (29 – 32wks)	0.165
SGA	4 (67)	84 (47)	0.37
ANC	3 (50)	145 (82)	0.07
ANTENATAL STEROIDS	3 (50)	110 (62)	0.55
VAGINAL DELIVERY	2 (33)	60 (34)	0.49
SURFACTANT	1 (17)	64 (36)	0.35

HIV-PCR = polymerase chain reaction, HIV-PCR + = HIV-PCR positive, HIV-PCR - = HIV-PCR negative, SGA = small for gestational age, ANC = antenatal care, IQR = interquartile range

ii. Chronic Lung Disease

Despite needing less non-invasive ventilation, significantly more HIV-PCR positive babies developed CLD compared to the HIV-PCR negative babies (OR 5.49, CI 1.31 – 23, p = 0.02). *(Table 3.3.2)*

iii. NEC

HIV-PCR positive babies had an increased risk of developing NEC (OR 4.12, CI 1.02 – 17.18; p=0.047). *(Table 3.3.2)*

Sub-group analysis was not carried out on these two variables as the numbers affected were considered too small to yield any statistically reliable results.

4. DISCUSSION

Worldwide, as care of HIV mothers has improved and effective PMTCT regimens have been implemented, drastically reduced perinatal transmission of HIV has resulted in the unmasking of a vulnerable population of infants and children who are HIV exposed yet uninfected. A growing body of literature is finding these children to be at increased risk of infections and death despite the absence of HIV. The mechanism is likely multifactorial and some proposed aetiologies include an innate immunodeficiency driven by antenatal exposure to ARV's, in-utero exposure to HIV viral particles or impaired maternal immune function. Additionally these children are at risk of nutritional deficiencies due to suboptimal breastfeeding practices and lower socioeconomic status often associated with HIV exposed households. [8] HIV exposed pregnancies have also been associated with more premature and LBW birth outcomes then HIV unexposed pregnancies. [9] This may be directly linked to poor maternal health and indirectly related to HIV associated socioeconomic factors or in-utero exposure to maternal ART. The literature to date about premature HIVE neonates is limited by the small numbers of neonates who survive prematurity in resource-poor yet high HIV prevalence areas such as Sub-Saharan Africa. Consequently, many clinicians have a perception that HIVE neonates are often premature, smaller and sicker, with increased mortality.

The Groote Schuur unit is well placed for this research as it is situated in a high prevalence HIV area that is able to provide NCPAP and surfactant therapy to these VLBW neonates which improves their survival. [1,10] This study provides the first piece of literature that examines a cohort of HIVE, premature and VLBW neonates with a comparative cohort of HIVU neonates. It is the first to report on several comparative short-term neonatal outcomes in HIVE, antenatal ARV-exposed and HIV-PCR positive VLBW neonates. These include birth weight, gestational age, SGA, length-of-stay, IVH, respiratory and delivery room resuscitation outcomes. The data on NEC is also the first to report on outcomes related to antenatal ARV-exposure and infant HIV-PCR status.

HIV-EXPOSED VERSUS HIV-UNEXPOSED NEONATES

There was no difference in maternal characteristics that may have influenced outcomes in these neonates including antenatal care exposure to antenatal steroids or type of delivery. There was no increased hypertension among HIV-positive mothers compared to HIV-negative mothers, which is consistent with the meta-analysis findings of Brown et al, who showed no increased PIH in relation to maternal HIV[11]. There was a trend towards less chorioamnionitis among the HIV-positive mothers (OR 0.48, CI 0.23 – 1.0, p = 0.051) which is surprising as chorioamnionitis has been shown to be of equal or increased prevalence between HIV positive and HIV negative mothers [12,13]. Due to the small numbers of documented cases however, i.e. 8/316 (3%) in the HIV-positive mothers and 65/1263 (5%) among those who are HIV-negative, the question is whether cases of chorioamnionitis were being properly identified during admission. VON data

capturing does not offer specific clinical criteria for chorioamnionitis and at GSH, placentas are not routinely sent off for histological analysis. The validity of this finding is thus questionable.

There was no difference between mortality of HIVE neonates (23%) and HIVU neonates (19%). This correlates with most of the current literature on neonatal mortality that has been compared between HIVE and HIVU neonates in 5 previous studies [14-18]. Two of these studies focused on premature and LBW infants and both reported no difference in mortality. The first, a small South African based cohort of 70 neonates, found no difference in mortality between HIVE and HIVU neonates[18]. The second study from Botswana, with a cohort of 449 neonates and a median gestational age of 34 weeks, also showed no significant difference between the 27% of the HIVE neonates who died compared to 20% of the HIVU neonates (p = 0.19) [14].

There was no difference between HIVE and HIVU neonates with regards to median birth weight, gestational age and length-of-stay. This differs from the meta-analysis findings published by Xiao et al in 2015 which showed both an increased risk of LBW (pooled OR 1.73, CI 1.64 – 1.82) and prematurity (pooled OR 1.56, CI 1.49 – 1.63) among HIVE neonates[9]. In previous studies, Parekh et al have found an increased risk for neonates to be very-small-for-gestational-age among HIVE infants in Botswana (aOR 1.9, CI 1.41 – 2.55) and Sofeu et al from Cameroon have demonstrated less SGA among HIVU neonates compared to HIVEU neonates (aOR 0.5, 95% CI 0.4 – 0.7) [19,20]. There was however no difference in SGA among this cohort of GSH HIVE neonates compared to those who are HIVU. This could be because of better maternal health among the mothers in the Western Cape as compared to Botswana and Cameroon for either socio-economic reasons or as a result of better access to ARV's. Alternatively, since a large proportion of the deliveries at GSH are related to pregnancy related hypertensive disorders, it could be that HIVU neonates were significantly growth restricted for this reason [21].

The incidence of sepsis among the HIVE neonates is of interest due to the increased risk of sepsis that has recently come to the fore among HIV-exposed but uninfected older infants and children[22,23]. The literature pertinent to neonates has previously been reviewed by Slogrove et al and concluded that the neonatal period is an equally high-risk period for sepsis for both HIVE and HIVU neonates[24]. Most of the literature on neonatal sepsis in HIVE neonates relates to Group B Streptococcus (GBS) and so is difficult to compare to this study data as VON does not keep a record of specific organisms cultured. Only Cutland et al have compared all-cause sepsis morbidity in a large Johannesburg based cohort (n = 4108) of HIVE and HIVU neonates[25]. They demonstrated a marginally increased risk of culture positive early onset sepsis among HIVE neonates and no difference in late onset sepsis. The findings in our GSH study demonstrate no significant difference between risk of either early onset sepsis or late onset sepsis. Previous studies from GSH have demonstrated that most of the neonates are delivered by caesarean section for complications related to maternal hypertension [2,21]. This iatrogenic delivery, in the absence of active labour suggests that less GSH neonatal admissions are at risk of septic exposure perinatally. This may underlie the observation of an equal incidence of early onset sepsis.

Significant differences that occurred between HIVE and HIVU neonates included need for ventilation and increased risk of NEC.

The literature available on respiratory morbidity in HIVE neonates is limited to 2 reports on largely term and normal birth weight infants. Kreitchmann et al reported a morbidity of 7.5% and mostly related to TTN and RDS but with no comparative cohort [26]. Martin et al found no difference in respiratory outcomes compared to general population data [27]. Multiple mechanisms have been postulated to impact on lung growth and function of HIVE neonates, including in-utero exposure to a pro-inflammatory environment, HIV viral particles and maternal co-infections [28].

This study demonstrated 35% more invasive ventilation among HIVE compared to HIVU neonates (OR 1.35, CI 1.01 – 1.8, p = 0.04), and on secondary analysis no clear association with potential confounders could be identified (*Table 3.1.4*). These included birth weight, gestational age, and antenatal steroids. Since there was no difference in administration of surfactant it can be assumed that the increased need for ventilatory support was not related to lung immaturity or HMD. This data is limited in that it does not reflect when during admission, for what reason or for how long the baby was ventilated. There was however no difference in NEC, early onset sepsis or late onset sepsis between HIVE and HIVU neonates to suggest a possible reason for ventilation.

NEC outcomes are divided into incidence, clinical presentation and mortality. Almost twice as much NEC was demonstrated among HIVE compared to HIVU babies (OR 1.83, CI 1.2 – 2.8, p = 0.005). This is consistent with the findings of Desfrere et al who also demonstrated an increased risk in their 2005 case-control cohort of 237 infants in France (OR 6.63, 95% CI 1.26 – 35) [29]. There is only one other study on NEC incidence in HIVE babies and from that Johannesburg based cohort of 330 babies, HIV exposure was not an associated risk factor [30].

Two studies have previously demonstrated an increased mortality among HIVE neonates who developed NEC. Karpelowsky et al demonstrated an almost 5-fold increased mortality (OR 4.8, 95% CI 1.7 – 14.2) and Chokoe et al found an 80% mortality in HIVE infants versus 15% in HIVU (p = 0.0002) [31,32]. However Angura et al and Arnold et al did not demonstrate a difference in mortality among HIVE neonates in their studies[30,33]. Similarly, this GSH study did not demonstrate a difference in mortality in the infants who developed NEC. The incidence of surgery was also no different between the HIVE and HIVU neonates who developed NEC. This correlates with the available literature comparing clinical presentation and histology between HIVE and HIVU neonates that has showed no differences [30,31,33,34].

The pathophysiology of NEC excluding HIV-exposure is poorly understood and is likely related to multiple triggers [35]. Differences in risk factors that are associated with NEC were considered in a secondary analysis of the HIVE neonates who developed NEC (*Table 3.1.5*). There was no difference in gestational age or antenatal steroid administration in comparison with the HIVU NEC neonates. There was a significantly lower birth weight in the HIVE neonates who developed NEC compared to the HIVU neonates, with median birth weights of 898g and 1118g respectively (p = 0.008, *figure 3.1.5*). Although not a statistically significant proportion, more (60%) of the HIVE

neonates who developed NEC were SGA compared to the HIVU neonates (45%, p = 0.16). The lower birth weight and relatively higher proportion of growth restriction is a significant finding with regards to NEC risk in HIVE neonates. The VON database does not include blood transfusion and inpatient feeding records, which limits the interpretation of potential associations for this increased risk of NEC among the HIVE neonates.

Feeding at discharge was documented for half of the cohort (57%). The remainder of the cohort was excluded based on death or transfer. Neonates who died were excluded, as many of them were not fed in the 24 hours prior to death. Neonates who are transferred to a step-down unit for weight gain are almost always still receiving breast milk fortification with FM85, which may skew the analysis in favour of mixed feeding. Within the HIVE cohort the neonates were 10-times more likely to be discharged on formula feeding (OR 9.97, CI 6.5 – 15.2, p = <0.0001), 70% less likely to be discharged on exclusive breast feeds (OR 0.3, CI 0.22 – 0.43, p = <0.0001,) but also 50% less likely to be discharged on mixed feeding (OR 0.56, CI 0.33 – 0.93, p = 0.026). This goes against the current WHO guidelines on infant feeding which recommends exclusive breastfeeding with maternal ART support for PMTCT in developing nations [36]. At the time of our study PMTCT policy encouraged exclusive breastfeeding for the first 6 months. Free infant formula was provided however for mothers who chose to avoid the transmission risk associated with breast milk[6,7]. It is possible that their feeding decision was likely swayed in the direction of formula, based on this incentive. Subsequent to this, the PMTCT policy has been revised in 2015 to withdraw the provision of free formula and increase breastfeeding support in order to promote exclusive breastfeeding of HIVE infants.

ADEQUATE MATERNAL ARV-EXPOSURE VS. MATERNAL ARV-UNDER-EXPOSED NEONATES

The PMTCT regimens in use at the time of our study are discussed in detail in the methods section of this paper and summarised in Table 2.1.

The most recent South African PMTCT guidelines have subsequently been updated in 2015 and have increased the duration of adequate ART exposure from 8 weeks to 12 weeks[37]. Consequently, our decision to use 2 months of ARV-exposure as a cut off for adequate viral suppression in the mothers, may be disputed. There is evidence to encourage 3 months of ART from a UK based study that found HAART initiated after 20 weeks unlikely to reduce viral load to <50 copies/ml by the time of delivery [38]. Aziz et al disputed this however by showing a reduction to <400 copies/ml in a median of 25 days in 2013 and the 2009 Cochrane review had reported there are no RCT's or observational studies to determine the optimal timing of ARV's [39,40].

ARVE mothers were 3-times more likely to receive antenatal care compared to those mothers who were ARVU (OR 3.0, CI 1.5 – 6.0, p = 0.002). HIV testing at initial antenatal booking and during pregnancy is an essential component of basic antenatal care in the South African health system. Lack of maternal ART is virtually synonymous with a mother who has not accessed antenatal care. Despite this, there were no other differences in terms of maternal characteristics, including antenatal steroid administration and maternal hypertension. This differs from the finding of a previous study in this unit that demonstrated an association with maternal ART for more than 4 weeks and PIH (p = 0.007) [21]. It is consistent with the findings of Brown et al however in their meta-analysis, which found no association with either HIV or ART and PIH [11].

Although ARVE mothers received more ANC compared to the ARVU mothers, there was no difference in gestational age, birth weight or length-of-stay of the mARVE and mARVU neonates. There was also no difference in SGA proportions or delivery room outcomes. There were less male infants in the mARVE cohort as opposed to mARVU babies. This finding cannot readily be explained but it does not appear to have had any influence on the remaining neonatal outcomes. Similar to the HIVE infants, there was no difference in mortality between mARVE (22%) and mARVU (25%) neonates.

There was a trend among the mARVE neonates for less invasive ventilation compared to the mARVU neonates (OR 0.57, CI 0.33 – 1.0, p = 0.051). This differs from HIVE infants who required more invasive ventilation compared to HIVU neonates (OR 1.35, CI 1.01 – 1.8). In this observation we find that the mARVE neonates demonstrate an outcome that is the same as HIVU neonates (21% required invasive ventilation in mARVE and HIVU groups). This could suggest that adequate maternal ART has a beneficial effect for neonatal lung health indirectly through better maternal health or less exposure to maternal co-infections. The similar use of surfactant between mARVE and mARVU neonates, as in the HIVE and HIVU group, suggests that RDS due to lung immaturity is not the underlying reason for this difference.

There was no difference in early onset sepsis between the mARVE and mARVU neonates. Although there was no difference in late onset sepsis, there was a trend of less sepsis in the mARVE cohort (8%) compared to in the mARVU group (15%). Incidentally, the incidence of late onset sepsis in the mARVE neonates is the same as the incidence in HIVU neonates (both 8%). These two observations may suggest a neonatal immune system functionally like HIVU neonates with adequate exposure of maternal ART.

There was a difference in the incidence of NEC with mARVE neonates having 60% less NEC than mARVU neonates (OR 0.4, CI 0.18 – 0.86, p = 0.02). It is again interesting to note that the incidence of NEC in mARVE neonates (7%) is very similar to the incidence of NEC in HIVU neonates (6%). The mortality among the 13 mARVE neonates who developed NEC however, was 12-times greater than mARVU neonates with NEC (OR 12, CI 1.9 – 76, p = 0.008). Secondary analysis of potential confounders (*Table 3.2.5*) revealed no difference in gender, gestational age, SGA, or antenatal steroids. There was a trend to better antenatal care among the mARVE neonates that may influence the decreased incidence in NEC but would not explain the higher mortality. The mARVE neonates had a significantly smaller birth weight (median 850g) compared to mARVU neonates (1090g, p = 0.024). In summary, mARVE neonates were less likely to get NEC. Those who did were smaller (all had birth weights under 1000grams), and were more likely to die. It is unclear if this difference in mortality reflects the birth weights of these neonates or the maternal ARV's.

HIV-PCR POSITIVE AND NEGATIVE NEONATES

The data for the 11 HIV-PCR positive babies in our study has been included and evaluated comparatively to the known HIV-PCR negative patients. The small numbers as well as factors mentioned in the limitations section below, limit the validity of these observations.

The transmission rate of 4.8% (11/230) is above the reported national average of between 2.1 and 1.6% last reported between 2013 and 2014 [41]. This may reflect an increased risk of vertical transmission in both the premature and LBW infants that has previously been proposed[42].

Unlike the maternal component of PMTCT, Option A and B (*Table 2.1*) both offer Nevirapine prophylaxis for all HIV-exposed infants. An extended period of cover is incorporated into Option B however if the mothers had inadequate antenatal exposure to ARV's. Option B additionally suggests HIV-PCR testing earlier than 6 weeks of age based on clinician discretion for a high-risk neonate. The majority of the neonates (177/230, 77%) had HIV-PCR testing done at 6 weeks of age, however all the babies identified as HIV-PCR positive were diagnosed on a HIV-PCR test done before 6 weeks. We have not captured in our data the reasons for the earlier testing, but it would have been either because the infant was unwell with sepsis, NEC, features suggestive of a congenital infection, or because they were identified as having a high transmission risk due to antenatal risk factors. Of our HIVE neonates 86/316 (27%) had no HIV-PCR

testing documented. This was either because of the babies dying prior to reaching the 6-week testing point (52/86, 60%) or being discharged prior to 6 weeks of age. While it was possible to trace a HIV-PCR test for many of these infants with a combination of their surname, date of birth or hospital folder number, if the infants were subsequently registered under a different name, relocated provinces or died we had no means of tracing them. As of 2015, South Africa has adopted birth HIV-PCR as a routine practice in PMTCT regimens and identifying these infants should become easier.

The only difference in maternal characteristics among mothers of HIV-PCR positive babies was 90% less exposure to maternal ARV's (OR 0.12, CI 0.02 – 0.58, p = 0.008). This is not unexpected as an unsuppressed maternal viral load at delivery is a known risk factor for antenatal as well as perinatal transmission [42].

Birth characteristics were similar. Gestational age medians were both 30 weeks for HIV-PCR positive and HIV-PCR negative infants. Birth weights were similar respectively as well as length-of-stay. There was no difference in proportion of SGA infants, mortality, early onset or late onset sepsis and IVH .

While there was no difference in invasive ventilation and surfactant administration, HIV-PCR positive infants needed 72% less non-invasive ventilation (OR 0.28, CI 0.08 – 0.98, p = 0.046). HIV-PCR positive infants however developed 5-times more chronic lung disease (OR 5.49, CI 1.31 – 23.02, p = 0.02) i.e. still oxygen dependant at 36 weeks post-menstrual age. No further analysis was conducted on the chronic lung disease (CLD) neonates, as the numbers represented are small with only 3/11 HIV-PCR positive babies developing CLD. A sub-group analysis however of the 6 HIV-PCR positive neonates, who received NIV compared to the 177 HIV-PCR negative neonates, did not yield any potential answers (*Table 3.3.3*). Birth weight and gestational age were similar. There was also no difference in SGA, receipt of antenatal steroids or surfactant. Factors that HIV-exposure introduces in the development of foetal lungs have previously been mentioned, including a pro-inflammatory in utero environment, which may accelerate lung maturity and explain the decreased need for respiratory support. Additionally, exposure to maternal co-infections and a more vulnerable immune system than HIV-PCR negative neonates may explain the increased incidence of CLD in the HIV-PCR positive neonates.

A 4-fold increased risk of NEC among HIV-PCR positive neonates compared to HIV-PCR negative neonates (OR 4.12, CI 1.02 – 17.18, p = 0.047) was demonstrated. There was no difference in surgery and mortality among these neonates however. The small numbers (3/11 HIV-PCR positive neonates) make further analysis difficult and raise questions around the validity of the result due to inadequate power.

LIMITATIONS

A major limitation of the study is that not all neonates received HIV-PCR testing. The deficit in HIV-PCR testing was mostly as a result of PMTCT guidelines during the study, which allowed early testing only for those babies deemed at high risk for infection due to antenatal factors. Of the 53 neonates who received early testing, 11 were HIV-PCR

positive while none of the neonates who were considered lower risk and tested later became HIV-PCR positive. This finding is repeated in later studies that were performed [43]. HIV-PCR positive neonates represent a distinct group with an independent risk profile and including them in the analysis may skew the results. It could be argued that an analysis of HIV exposed but uninfected (HIVEU) neonates would be superior. It was not possible to do this however for the following reasons:

i. We could not disregard the HIV-PCR positives, as it is possible that there were other neonates who were HIV infected in the 'HIV-PCR untested' group. It is likely however, that the incidence of HIV-PCR positive neonates would be low in this group, as most high-risk transmissions would have been identified and tested early.
ii. We could not disregard the 'HIV-PCR unknown' group as a far higher proportion of the HIV-PCR -untested neonates died (53/86; 60%) compared to the HIV-PCR tested group (21/230; 9%). The high mortality rate in the untested group represents a significant proportion of mortality and morbidity data. A consequence of removing the HIV-PCR unknown group would be that HIVE neonates would have a statistically higher chance of surviving than the HIVU neonates. Morbidity outcomes would also be affected in a similar way.

It is clear from the above that we had to include both the HIV-PCR positive and the HIV-PCR unknown group in our analysis. Including the HIV-PCR positive group is unlikely to change any findings however due to both the small numbers and the statistical similarities to the HIV-PCR negative neonates.

Overall, the data is limited by its retrospective nature. There is also a lack of maternal variables including CD4 counts, cigarette, alcohol and recreational drug use information, socioeconomic factors and nutritional assessments. These factors have important implications for maternal overall health which impacts on neonatal outcomes.

STRENGTHS

Overall, this study provides evidence from a large cohort of VLBW neonates with reasonably powered data regarding outcomes of HIVE versus HIVU neonates. Being the first of it's kind it provides a good foundation from which to focus further research on the topic in similar units in Sub-Saharan Africa.

5. CONCLUSION

This is one of the first studies to report on several short-term neonatal outcomes in HIVE, maternal ARV-exposed and HIV-PCR positive VLBW neonates.

For the most part, we did not demonstrate worse short-term neonatal outcomes between HIVE VLBW babies compared to their HIVU counterparts. Similarities included anthropometry and gestation, length-of-stay, sepsis, intraventricular haemorrhage and mortality. The HIVE neonates did demonstrate more need for respiratory support, which does not appear to be related to prematurity. In addition, there was an increased incidence of NEC, which was associated with a lower birth weight.

Adequate maternal ART is associated with improved neonatal outcomes in the areas of respiratory support, NEC and possibly late onset sepsis. In these areas, the neonates behaved similarly to those who were HIV unexposed. The higher mortality rate from NEC in neonates whose mothers received adequate antenatal ART was associated with a birth weight of under 1000grams.

There was a higher HIV-transmission rate compared to the national average that could be expected in our premature, VLBW cohort and was associated with inadequate maternal ART. The data analysis reported based on HIV-PCR -status of the neonates is limited by small numbers and so it is difficult to draw any relevant conclusions.

6. RECOMMENDATIONS

1. Further evaluation of HIVE, premature and VLBW neonates in other settings is needed to validate our findings and early HIV-PCR testing is encouraged.
2. Key outcome areas that need further study include respiratory morbidity and NEC.
3. Further study on the increased incidence of NEC should include inpatient breastfeeding/nutrition data to inform the correlation with necrotizing enterocolitis and possibly evaluate for evidence of effect modification.
4. There is a need for evaluation of a larger cohort of HIV-PCR positive infants. Given that the transmission rate is <5%, multi-centre analysis may be the best way to power this research.
5. Maternal ART seems to have some beneficial neonatal outcomes however ongoing surveillance is needed especially considering the possible increased mortality in those who developed NEC.
6. Neonatal care needs to incorporate more promotion of breastfeeding through counselling, breastfeeding support and optimizing maternal health with antiretrovirals.

REFERENCES:

(1) **The National Antenatal Sentinel HIV prevalence Survey, South Africa.** *National Department of Health* 2013.

(2) Tooke L, Horn A, Harrison M. **HIV Transmission to Extremely Low Birth Weight Infants.** *The Pediatric Infectious Disease Journal* 2013 Jan;32(1):36-38.

(3) Vermont Oxford Network. Available at: public.vtoxford.org.

(4) Vermont Oxford Network. **2016 Vermont Oxford Network Manual of Operations: Part 2. Data definitions and infant data forms. Release 20.0.** 2015 October.

(5) World Health Organisation. **Use of antiretroviral drugs for treating pregnant women and preventing HIV infection in infants. Recommendations for a public health approach - 2010 version.** France: World Health Organisation.

(6) National Department of Health South Africa, South African National AIDS Council. **Clinical Guidelines : PMTCT (Prevention of Mother to Child Transmission) ** 2010.

(7) Western Cape Department of Health. **PMTCT Clinical Guidelines Update 2013.** 2013 May.

(8) Slogrove AL, Archary M, Cotton MF. **Optimizing Research Methods to Understand HIV-Exposed Uninfected Infant and Child Morbidity: Report of the Second HEU Infant and Child Workshop.** *Frontiers in Immunology* 2016 Dec 6,;7.

(9) Xiao PL, Zhou YB, Chen Y, Yang MX, Song XX, Shi Y, et al. **Association between maternal HIV infection and low birth weight and prematurity: a meta-analysis of cohort studies.** *BMC Pregnancy Childbirth* 2015 Oct 8;15:z.

(10) Gerhardus Kirsten, Cheryl Kirsten, Philippus Henning, Johan Smith, Sandi Holgate, Adrie Bekker, et al. **The Outcome of ELBW Infants Treated With NCPAP and InSurE in a Resource-Limited Institution.** *Pediatrics* 2012 Apr 1,;129(4):e959.

(11) Browne JL, Schrier VJ, Grobbee DE, Peters SA, Klipstein-Grobusch K. **HIV, Antiretroviral Therapy, and Hypertensive Disorders in Pregnancy: A Systematic Review and Meta-analysis.** *JAIDS Journal of Acquired Immune Deficiency Syndromes* 2015 Sep 1,;70(1):91.

(12) Ocheke AN, Agaba PA, Imade GE, Silas OA, Ajetunmobi OI, Echejoh G, et al. **Chorioamnionitis in pregnancy: a comparative study of HIV-positive and HIV-negative parturients.** *International Journal of STD & AIDS* 2016 Mar;27(4):296-304.

(13) David A. Schwartz, Suthi Sungkarat, Nathan Shaffer, Jirasak Laosakkitiboran, Wendy Supapol, Pichai Charoenpanich, et al. **Placental Abnormalities Associated with Human Immunodeficiency Virus Type 1 Infection and Perinatal Transmission in Bangkok, Thailand.** *The Journal of Infectious Diseases* 2000 Dec 1,;182(6):1652-1657.

(14) Zash RM, Ajose-Popoola O, Stordal K, Souda S, Ogwu A, Dryden-Peterson S, et al. **Risk factors for mortality among human immunodeficiency virus-exposed and unexposed infants admitted to a neonatal intensive care unit in Botswana.** *J Paediatr Child Health* 2014 Mar;50(3):189-195.

(15) Monebenimp F, Nga-Essono DE, Zoung-Kany Bissek AC, Chelo D, Tetanye E. **HIV exposure and related newborn morbidity and mortality in the University Teaching Hospital of Yaounde, Cameroon.** *Pan Afr Med J* 2011;8:43.

(16) Marinda E, Humphrey JH, Iliff PJ, Mutasa K, Nathoo KJ, Piwoz EG, et al. **Child mortality according to maternal and infant HIV status in Zimbabwe.** *Pediatr Infect Dis J* 2007 June 01;26(6):519-526.

(17) Lepage P, Dabis F, Hitimana D, Msellati P, van Goethem C, Stevens A, et al. **Perinatal transmission of HIV-1: Lack of impact of maternal HIV infection on characteristics of livebirths and on neonatal mortality in Kigali, Rwanda.** *AIDS* 1991 Jan 1,;5(3):295-300.

(18) Adhikari M, Jeena P, Pillay T, Moodley A, Kieliela P, Cassol S. **The HIV-1 exposed neonate: outcome of intensive care management in the first week of life.** *Indian Pediatr* 2005 December 01;42(12):1215-1219.

(19) Sofeu CL, Warszawski J, Ndongo FA, Penda IC, Ndiang ST, Guemkam G, et al. **Low Birth Weight in Perinatally HIV-Exposed Uninfected Infants: Observations in Urban Settings in Cameroon.** *PLoS One* 2014 Apr 1,;9(4).

(20) Parekh N, Ribaudo H, Souda S, Chen J, Mmalane M, Powis K, et al. **Risk factors for very preterm delivery and delivery of very-small-for-gestational-age infants among HIV-exposed and HIV-unexposed infants in Botswana.** *International Journal of Gynecology and Obstetrics* 2011;115(1):20-25.

(21) Tooke L, Riemer L, Matjila M, Harrison M. **Antiretrovirals causing severe pre-eclampsia.** *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health* 2016 Oct;6(4):266-268.

(22) Brennan AT, Bonawitz R, Gill CJ, Thea DM, Kleinman M, Useem J, et al. **A meta-analysis assessing all-cause mortality in HIV-exposed uninfected compared with HIV-unexposed uninfected infants and children.** *AIDS* 2016 September 24;30(15):2351-2360.

(23) le Roux SM, Abrams EJ, Nguyen K, Myer L. **Clinical outcomes of HIV-exposed, HIV-uninfected children in sub-Saharan Africa.** *Trop Med Int Health* 2016 July 01;21(7):829-845.

(24) Slogrove AL, Goetghebuer T, Cotton MF, Singer J, Bettinger JA. **Pattern of Infectious Morbidity in HIV-Exposed Uninfected Infants and Children.** *Front Immunol* 2016 May 06;7:164.

(25) Cutland CL, Schrag SJ, Zell ER, Kuwanda L, Buchmann E, Velaphi SC, et al. **Maternal HIV infection and vertical transmission of pathogenic bacteria.** *Pediatrics* 2012 Sep;130(3):e590.

(26) Kreitchmann R, Cohen RA, Stoszek SK, Pinto JA, Losso M, Pierre R, et al. **Mode of delivery and neonatal respiratory morbidity among HIV-exposed newborns in Latin America and the Caribbean: NISDI Perinatal–LILAC Studies.** *International Journal of Gynecology and Obstetrics* 2011;114(2):91-96.

(27) Martin R, Boyer P, Hammill H, Peavy H, Platzker A, Settlage R, et al. **Incidence of premature birth and neonatal respiratory disease in infants of HIV-positive mothers. The Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted Human Immunodeficiency Virus Infection Study Group.** *J Pediatr* 1997 Dec;131(6):851-856.

(28) Slogrove AL, Frigati L, Gray DM. **Maternal HIV and Paediatric Lung Health.** *Paediatric Respiratory Reviews* 2017 Jan;21:47-53.

(29) Desfrere L, de Oliveira I, Goffinet F, El Ayoubi M, Firtion G, Bavoux F, et al. **Increased incidence of necrotizing enterocolitis in premature infants born to HIV-positive mothers.** *AIDS* 2005 Sep 23;19(14):1487-1493.

(30) Angura P, Velaphi S. **Risk factors for necrotising enterocolitis in an HIV-endemic region.** *Paediatrics and International Child Health* 2014 Aug 1,;34(3):208-215.

(31) Chokoe MJ, Wright CA, Bezuidenhout J, Moore SW, Smith J. **Necrotising enterocolitis in HIV-exposed and nonexposed infants: Clinical presentation and histopathological features.** *Pediatric and Developmental Pathology* 2012 April 6;15:293-297.

(32) Karpelowsky JS, van Mil S, Numanoglu A, Leva E, Millar AJ. **Effect of maternal human immunodeficiency virus status on the outcome of neonates with necrotizing enterocolitis.** *J Pediatr Surg* 2010 Feb;45(2):8; discussion 318.

(33) Arnold M, Moore SW. **HIV exposure does not worsen outcome in stage III necrotizing enterocolitis with current treatment protocols.** *J Pediatr Surg* 2012 Apr;47(4):665-672.

(34) Karpelowsky JS, van Mil S, Numanoglu A, Leva E, Millar AJ. **Effect of maternal human immunodeficiency virus status on the outcome of neonates with necrotizing enterocolitis.** *J Pediatr Surg* 2010 Feb;45(2):8; discussion 318.

(35) Thompson AM, Bizzarro MJ. **Necrotizing Enterocolitis in Newborns: Pathogenesis, Prevention and Management.** *Drugs* 2008;68(9):1227-1238.

(36) **World Health Organization, United Nations Children's Fund. Guideline: updates on HIV and infant feeding: the duration of breastfeeding, and support from health services to improve feeding practices among mothers living with HIV.** *World Health Organisation* 2016.

(37) National Department of Health South Africa. **National Consolidated Guidelines for the Prevention of Mother to Child Transmission (PMTCT) and the management of HIV in Children and Adolescents and Adults.** 2015 April.

(38) Read PJ, Mandalia S, Khan P, Harrisson U, Naftalin C, Gilleece Y, et al. **When should HAART be initiated in pregnancy to achieve an undetectable HIV viral load by delivery?** *AIDS* 2012 Jan 1,;26(9):1095-1103.

(39) Aziz N, Sokoloff A, Kornak J, Leva N, Mendiola M, Levison J, et al. **Time to viral load suppression in antiretroviral‐naive and ‐experienced HIV‐infected pregnant women on highly active antiretroviral therapy: implications for pregnant women presenting late in gestation.** *BJOG: An International Journal of Obstetrics & Gynaecology* 2013 Nov;120(12):1534-1547.

(40) Sturt AS, Dokubo EK, Sint TT. **Antiretroviral therapy (ART) for treating HIV infection in ART-eligible pregnant women.** *The Cochrane database of systematic reviews* 2010(3):CD008440.

(41) **Millennium Development Goals: Country Report 2015/ Statistics South Africa.** *Statistics South Africa* 2015.

(42) Kroon M. **Recognising and managing increased HIV transmission risk in newborns.** *Southern African Journal of HIV Medicine* 2015 Apr 1,;16(1):e7.

(43) Levin C, Le Roux D, Harrison M, Tooke L. **HIV transmission to premature very low birth weight infants.** *The Pediatric Infectious Disease Journal* 2017 April 13,;Epub ahead of Print.

(44) Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R. **New Ballard Score, expanded to include extremely premature infants.** *The Journal of Pediatrics* 1991;119(3):417-423.

Appendices

A: Ethics Letter

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

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

01 September 2015

HREC REF: 603/2015

Dr L Tooke
Division of Neonatology
GSH

Dear Dr Tooke

PROJECT TITLE: THE SHORT-TERM OUTCOMES OF HIV-EXPOSED VERSUS HIV-UNEXPOSED VERY LOW BIRTH WEIGHT INFANTS (MMeD-candidate-L Riemer)

Thank you for your response letter addressing the issues raised by the Human Research Ethics Committee (HREC).

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

Approval is granted for one year until the 30th September 2016.

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)

We acknowledge that the following student: - Dr Linda Riemer is also involved in this project.

Please quote the HREC reference no in all your correspondence.

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

Yours sincerely

Signed by candidate

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research

Hrec/ref:603/2015

B: Definitions of Data Collection Points

Maternal demographics

- Ethnicity:
  Restricted to African, White, Asian or Other, which in this population predominantly reflected the Cape Malay community, colloquially referred to as Coloured.
- Antenatal care (ANC):
  Defined as any antenatal care and not restricted in terms of number of visits or time prior to admission.
- Chorioamnionitis
  Not limited in terms of specific clinical criteria. Coded if documented as such in the maternal or neonatal folder.
- Antenatal steroids:
  Intramuscular or intravenous administration of betamethasone, dexamethasone or hydrocortisone given at any time prior to delivery to the mother and not restricted to exclude partial treatment doses.
- Maternal hypertension:
  Chronic and pregnancy-induced hypertension. PET and eclampsia were included in the pregnancy-induced hypertension definition.
- Type of delivery
  Vaginal delivery vs. Caesarean section.

Neonatal outcomes:

- Gestational age
  Due to limited access to ultrasound facilities in our referral system these were largely determined based on clinical Ballard scores at the time of delivery (± 70%) and the remainder based on ultrasound gestation determined prior to 20 weeks.[44]
- Length of stay – duration up until discharge home or death, including if transferred out to other facilities.
- Delivery room resuscitation:
  - Face-mask ventilation
  - Chest compressions
  - Intubation
  - Adrenaline administration – given via either the endotracheal or intravenous route
- Respiratory support at any time during admission
  - Invasive ventilation:
    - High Frequency Oscillation
    - Intermittent Positive Pressure Ventilation)
  - Non-invasive ventilation:
    - CPAP
    - High-Flow Oxygen therapy)
  - Nasal cannulae oxygen
  - Surfactant administration

- Chronic lung disease:
  Any infant still requiring supplemental oxygen at 36 weeks post menstrual age.

- Sepsis (based on culture of significant organisms as dictated by prescribed pathogen lists from the Vermont Oxford Network)
  - Early sepsis:
    A positive blood or CSF culture on day 1, 2 or 3 of life.
  - Late sepsis:
    Any positive blood or CSF culture after day 3 of life including bacteria and fungi.
  - Nosocomial sepsis:
    Any positive blood or CSF bacterial culture after day 3 of life.
  - Fungal sepsis:
    Any positive blood or CSF fungal culture after day 3.
  - NEC:
    Infants needed at least 1 of the prescribed clinical criteria and 1 radiological feature to meet the definition of NEC.
    CLINICAL
    - Bilious vomits or gastric aspirates,
    - Abdominal distension
    - Blood in the stools, either occult or visible

    RADIOLOGICAL
    - Pneumatosis Intestinalis
    - Hepatobiliary gas
    - Pneumoperitoneum

- Surgery
  Major surgery defined by a prescribed list of procedures including NEC, ROP and PDA ligation. Coded in the database as
    - Any surgery
    - NEC
    - ROP
    - Cardiac
    - Neurosurgical
    - Other

- Intraventricular haemorrhage and cystic periventricular leukomalacia
  Documented as the worst grade IVH seen on cranial ultrasound in the first 28 days of life and graded as follows –
    - Grade 1: Subependymal germinal matrix haemorrhage only
    - Grade 2: Intraventricular blood, no ventricular dilation
    - Grade 3: Intraventricular blood, ventricular dilation
    - Grade 4: Intraparenchymal haemorrhage
    - Cystic periventricular leukomalacia with multiple small periventricular cysts, excluding echogenicity without cysts or a porencephalic cyst in the area of previously identified Intraparenchymal haemorrhage.

- Feeding at discharge

Documented based on enteral feeding in the 24 hours prior to discharge classified as follows

- EBM (Exclusive Breast Milk): receiving only human milk either breast-fed or expressed breast milk.
- Formula: receiving only formula milk.
- Mixed: receiving human milk, plus human milk fortifier and/or formula milk.